CARACTERIZACION MOLECULAR Y EPIDEMIOLOGIA DEL VIRUS INFLUENZA TIPO A EN PORCINOS DE COLOMBIA

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RESUMEN

Esta tesis se compone de varios capítulos. El primer capítulo describe los objetivos y el racional científico para la realización de este trabajo de investigación. El segundo capítulo comprende una revisión bibliográfica del virus de influenza tipo A (IAV), su impacto e importancia para la salud animal y la salud pública. Este capítulo permite también comprender la importancia de la vigilancia de enfermedades en el sector porcino. El tercer capítulo se detallan los resultados de un estudio observacional sobre el uso de fluidos orales porcinos para la detección molecular y monitoreo de IAV en grupos de cerdos en granjas comerciales basada en una técnica de muestreo a nivel poblacional de fácil aplicación, costo efectiva y eficiente para la vigilancia de enfermedades virales en cerdos. El cuarto capítulo corresponde a un análisis de la estructura, medidas de bioseguridad (MB) y prácticas de manejo (PM) en granjas porcinas y su relación con infección por IAV. Esta sección muestra los resultados de diferentes análisis estadísticos multivariados aplicados en datos de una encuesta obtenidos de 176 granjas porcinas con inventario mayor a 100 hembras de cría, para identificar patrones de agrupamiento de estas granjas acorde a sus características y establecer su asociación con la detección de IAV. En el quinto capítulo, se muestran los resultados de la caracterización filogenética a nivel poblacional de un grupo seleccionado de 132 aislados virales obtenidos de este trabajo. Estos análisis se realizaron con el fin de esclarecer un poco más la epidemiologia molecular y diversidad genética de los IAV en porcinos de Colombia En el último capítulo (sexto) se resaltan los principales hallazgos de este trabajo de investigación y se relaciona la información suplementaria y anexos.

Palabras clave: Cerdos, Influenza Porcina, Bioseguridad Porcina, Factores de riesgo, Filogenia de IAV, Diversidad genética de IAV, Detección molecular, Epidemiología de IAV.

ABSTRACT

This thesis is made up of several chapters. The first chapter describes the objectives and the scientific rationale for carrying out this research work. The second chapter includes a bibliographic review of the influenza virus type A (IAV), its impact and importance for animal health and public health. This second chapter also provides an understanding of the importance of disease surveillance in the pig sector. The third chapter details the results of an observational study on the use of porcine oral fluids for the molecular detection and monitoring of IAV in groups of pigs in commercial farms based on an easy-to-use, cost-effective and efficient population-level sampling technique for surveillance of viral diseases in pigs. The fourth chapter corresponds to an analysis of the structure, biosecurity measures (MB) and management practices (PM) in pig farms and their relationship with IAV infection. This section shows the results of different multivariable statistical analyzes applied to survey data obtained from 176 farms by inventories over 100 sows, to identify clustering patterns of these farms according to their characteristics and establish their association with the detection of IAV. In the fifth chapter, are shown the results of the phylogenetic characterization at the population level of a selected group of 132 viral isolates obtained from this work. These analyzes were carried out in order to clarify a little more the molecular epidemiology and genetic diversity of IAV in Colombian pigs. In the last chapter (sixth) the main findings of this research work are highlighted and the supplementary information and annexes are listed.

Keywords: Pigs, Swine Influenza, Swine Biosecurity, Risk Factors, IAV Phylogeny, IAV Viral Genetic Diversity, Molecular Detection, IAV Epidemiology.

TABLA DE CONTENIDOS

Página

LISTA DE FIGURAS

Capítulo II

Capítulo III

Capítulo IV

Capítulo V

LISTA DE TABLAS

Capítulo II

Capítulo VI

Capítulo V

LISTA DE SÍMBOLOS Y ABREVIATURAS

Símbolo o Abreviatura		Descripción
µg		Microgramo
µL		Microlitro
µM		Micromolar
ADN		Ácido desoxirribonucleico
ARN		Ácido ribonucleico
gr		Gramo
HA		Proteína Hemaglutinina
IAV		Virus influenza A
IP		Influenza Porcina
Kg		Kilogramo
Km		Kilometro
L		Litro
M		Molar
m		Metro
M		Proteína Matriz
MB		Medidas de bioseguridad
mg		Miligramo
mL		Mililitro
NA		Proteína Neuraminidasa
NS		Proteína No Estructural
pdm		Virus Pandémico
PM		Prácticas de manejo
RT-PCR		Transcripción reversa y Reacción en cadena de la polimerasa
Rx		Reacción
TM		Marca registrada
Vol		Volumen

CAPÍTULO I. INTRODUCCIÓN, ANTECEDENTES, RACIOCINIO CIENTÍFICO Y OBJETIVOS.

En este capítulo se proporciona una descripción general del virus influenza. En particular, la atención se centra en los virus influenza que afectan a los cerdos, su genética molecular y evolución, así como la amenaza que representa para la salud de estos y otros animales y para los humanos.

1.1. Introducción

Los virus influenza tipo A (IAV) se distribuyen mundialmente y afectan a diversas especies animales incluidos los cerdos y los humanos (Zhuang et al., 2019). Sin embargo, no todos los IAV son capaces de cruzar la barrera inter-especie. Estos virus son considerados patógenos prioritarios a nivel global por su potencial zoonótico y pandémico (Tomley y Shirley, 2009). Los cerdos se consideran un huésped importante en la ecología de estos virus, ya que en determinadas circunstancias pueden infectarse no solo con cepas IAV de origen porcino sino también de origen humano y aviar (Wenjun Ma et al., 2009), generando una condición de riesgo para la aparición de nuevas variantes virales producto del reordenamiento genético de múltiples cepas de IAV (Brown, 2001). Por las características genéticas del IAV pandémico del 2009, se determinó que los cerdos facilitaron la transmisión del virus a otras especies como los humanos y las aves (Mena et al., 2016). Sin embargo, múltiples estudios filogenéticos han demostrado que existen más virus influenza de origen humano que se han adaptado a las poblaciones porcinas en comparación con virus de origen porcino adaptados a las poblaciones humanas (Wenjun Ma et al., 2009).

La epidemiologia de los IAV en poblaciones porcinas y sus características moleculares no están del todo definidas, además existen múltiples factores que afectan la dinámica de infección y transmisión de estos virus en cerdos. Se sabe que estos virus se transmiten fácilmente entre los animales por contacto directo, aerosoles y fómites. Adicionalmente, se han descrito múltiples grupos filogenéticos de virus influenza que circulan endémicamente en determinadas regiones geográficas del mundo. Sin embargo, en Colombia la información que existe sobre influenza porcina es muy limitada. Para entender la dinámica de transmisión de los virus influenza en los cerdos colombianos y poder identificar los determinantes

moleculares que permiten o restringen su transmisión es importante estudiar dichos virus en poblaciones porcinas endémicamente infectadas. La presencia de los virus influenza tipo A en cerdos colombianos ha estado documentada por años, no obstante, es muy poco lo que se conoce sobre su diversidad genética y distribución. Jiménez y colaboradores (Jiménez et al., 2014) identificaron la presencia del virus pandémico en 7 granjas ubicadas en los departamentos de Antioquia y el Valle del Cauca en cerdos destetos y de engorde, lo cual coincide con los hallazgos de otros investigadores, quienes además de aislar cepas de influenza pandémica también identificaron el IAV porcino clásico en diferentes granjas del país (Ramirez-Nieto et al., 2012). De igual forma trabajos llevados a cabo por Karlsson et. al. en el 2013, reportaron la presencia del virus pandémico H1N1 en diferentes granjas porcinas del departamento del Meta (Karlsson et al., 2013).

Teniendo en cuenta el impacto que tiene el IAV en la salud y producción animal, sumado al riesgo para la salud pública por su potencial zoonótico, se hace necesario entender la prevalencia y composición genética de estos virus en las poblaciones porcinas colombianas, que permita cerrar brechas de conocimiento para entender mejor su origen, evolución y distribución. Esta información es clave puesto que facilita el diseño de mejores estrategias de prevención y control de las infecciones por IAV en cerdos, así como la implementación de estrategias que permitan minimizar el riesgo de transmisión del virus de los cerdos a otras especies, incluido el humano.

1.2. Antecedentes y planteamiento del problema

La influenza porcina (IP) es una infección respiratoria altamente contagiosa en los cerdos (Kothalawala et al., 2006). La enfermedad se caracteriza por problemas respiratorios agudos, fiebre, pérdida de apetito y letargo. Un brote de IP en una granja tiene un impacto económico alto, debido a los costos de los medicamentos y al retraso en el crecimiento de los animales; especialmente, cuando la IP se presenta como parte del complejo de enfermedades respiratorias porcinas (Opriessnig et al., 2011) o como una infección persistente a nivel poblacional (Rose et al., 2013). En la última década, la industria porcina Colombiana se ha fortalecido enormemente y es uno de los principales contribuyentes al PIB del país (González, 2019). Esta industria concentra la mayoría de sus explotaciones en cuatro

regiones geográficas de Colombia. En el año 2015, se reporta que el país tenía aproximadamente un total de 4.6 millones de cerdos y más de la mitad de ellos se encontraban en tres departamentos: Antioquia (1,6 millones), Cundinamarca (0,5 millones) y Valle del Cauca (0,4 millones). Para esa época, un tercio de los cerdos era mantenido en sistemas de traspatio y dos tercios en granjas tecnificadas (van Haandel, 2016).

En Colombia existen pocos estudios de la caracterización y epidemiologia del IAV en producciones porcinas y la mayoría de estos estudios fueron realizados hace más de 10 años. El primer reporte sobre la detección del virus de influenza en cerdos fue en 1977 (Hanssen et al., 1977). Aunque actualmente se reconoce una gran actividad de estos virus en los porcinos, la información disponible acerca de esta enfermedad para el país es muy limitada, y es muy poco lo que se conoce sobre la diversidad genética y distribución geográfica de estos virus en Colombia. Investigaciones realizadas entre 1991 y 1994 demostraron que hubo seroreactividad del 6.5% para un IAV H1N1 y posteriormente, entre 1997 y 1999, en el departamento de Antioquia se reportó una seroreactividad del 41,3% para un IAV H3N2 y del 0,8% para la cepa IAV H1N1 (Mogollón et al., 2003). Otro estudio en 2001, reportó una prevalencia del 10% para la cepa IAV H3N2 y de 0,4% para la cepa IAV H1N1 en tres de las principales regiones productoras de cerdos del país (Pardo, 2001). Ramírez y colaboradores (Ramirez-Nieto et al., 2012), identificaron entre 2008 y 2010, IAV H1N1 pandémico (pdm) junto con otros subtipos clásicos porcinos en diferentes granjas del país. En 2013, en trabajos realizados por otros investigadores, se confirmó por secuenciación genómica de aislamientos virales, la presencia del virus pdm H1N1 en granjas del departamento del Meta (Karlsson et al., 2013). Estos hallazgos permiten sugerir que la enfermedad está muy presente en los sistemas de producción porcina, y que puede estar ampliamente distribuida en varias regiones del país. Adicionalmente, en Estados Unidos, ha sido demostrado que la prevalencia de granjas porcinas infectadas por IAV es del 57% (Chamba Pardo et al., 2017), situación que puede ser muy similar en el contexto colombiano pero a la actualidad se carece de evidencias que lo comprueben.

Por otra parte, la diversidad de los IAV que circulan endémicamente en porcinos en determinadas regiones geográficas del mundo ha sido ampliamente estudiada, sin embargo,

en Colombia la información que existe es muy limitada. La presencia de los IAV en cerdos colombianos ha estado documentada por años mediante técnicas de detección molecular o serológica, no obstante, es muy poco lo que se conoce sobre la diversidad genética y distribución de estos virus en el país.

Es importante recordar que existen medidas de bioseguridad como la vacunación, que han sido reconocidas como estrategias útiles para prevenir la propagación de la IP y/o mitigar los efectos de la enfermedad (White et al., 2017). En Colombia, es prioritario la búsqueda de estrategias novedosas de prevención de la enfermedad en la industria porcina, y que en conjunto ayuden a un mejor control y prevención de la influenza, sin embargo, para este fin se requiere de un conocimiento más profundo de la epidemiologia y características genómicas de los virus circulantes en el país. Esto parte desde los siguientes aspectos: (i) no existe una vacuna comercial o licenciada para el control de IP en cerdos en el país, (ii) la prevención y protección contra IP depende exclusivamente de las prácticas productivas implementadas y (ii) de las medidas generales de bioseguridad instauradas en las granjas para prevenir el riesgo de aparición de brotes epidémicos (Halloran et al., 1992).

Por otra parte, aunque se conoce que el IAV se encuentra circulando en granjas porcinas del país, el impacto a nivel de granja no ha sido totalmente evaluado, especialmente en las granjas infectadas de forma endémica. Por lo tanto, las pérdidas ocasionadas no son obvias y se hace muy difícil para los productores y los veterinarios realizar la evaluación de los beneficios de las medidas de control, como la vacunación. Por tanto, ayudar a eliminar, controlar y prevenir la incidencia de IP determina en parte la rentabilidad de las granjas.

Por tanto, en Colombia no se conoce a fondo sobre la prevalencia ni diversidad genética de los IAV que actualmente circulan en granjas porcinas tecnificadas, al igual que se desconoce su distribución a nivel regional. La caracterización genética de estos virus a nivel regional, permitirá entonces identificar su genoma, las relaciones filogenéticas, origen y diversidad presente en las principales áreas de producción de cerdos del país. Esta información es vital en los programas de vigilancia epidemiológica y en los programas sanitarios con el fin de entender la dinámica de estos virus en la industria porcina nacional, y que por tanto conlleven

al diseño o fortalecimiento de las estrategias de prevención, control y monitoreo del riesgo de transmisión entre los cerdos y de los cerdos hacia las personas. Por lo anteriormente resaltado, se hace entonces necesario realizar un estudio sobre la prevalencia, caracterización genética y distribución geográfica de los virus influenza tipo A en granjas porcinas tecnificadas con inventarios de más de 100 hembras de cría en el país.

1.3. Raciocinio científico

Los cerdos han sido propuestos como un hospedero intermediario en el ciclo de transmisión de los IAV, cumpliendo un rol primordial al facilitar la adaptación de cepas virales a los hospederos mamíferos. No obstante, a la actualidad se conocen más virus humanos establecidos en cerdos que virus porcinos establecidos en humanos. En este sentido, aun cuando el cerdo ocupa un lugar importante en el ciclo evolutivo de estos virus, el conocimiento de la epidemiologia de este virus y de la influenza porcina, en el caso particular de Colombia, es relativamente escaso en comparación a la comprensión de la influenza humana y a lo que ha sido investigado en otros países en torno a este patógeno. Es bien sabido que el conocimiento en la epidemiologia molecular y características antigénicas de estos virus permite establecer información invaluable sobre la diversidad, fuentes de infección y transmisión de la enfermedad en los cerdos que son base fundamental para establecer mecanismos y estrategias para su control y prevención.

Basado en estas consideraciones mencionadas anteriormente las siguientes hipótesis fueron abordadas en esta tesis:

a. Los virus de influenza porcina circulan activamente en las producciones tecnificadas del país causando infecciones epidémicas en la población.

b. El reordenamiento genómico es un fenómeno frecuente en los IAV porcinos circulantes en el país.

c. La detección del virus influenza está afectada por varios factores relacionados con la bioseguridad y prácticas de manejo de las granjas.

1.4. Objetivos

1.4.1. Estimar la prevalencia y distribución geográfica de IAV en granjas porcinas tecnificadas colombianas con más de 100 hembras de cría.

1.4.2. Realizar la caracterización genética de los IAV detectados en granjas porcinas tecnificadas colombianas.

1.4.3. Estimar las relaciones filogenéticas y el patrón evolutivo de los IAV obtenidos en el estudio a partir de la información genómica de mayor relevancia.

1.4.4. Identificar eventos de reordenamiento genético en los virus circulantes basado en el origen filogenético de los segmentos virales analizados.

1.4.5. Conocer la estructura y patrones característicos de la bioseguridad y medidas de manejo de los sistemas de producción porcina con más de 100 hembras de cría y estimar su relación con la detección de IAV.

1.4.6. Identificar factores epidemiológicos potencialmente asociados a la infección por IAV en las poblaciones porcinas estudiadas.

1.4.7. Generar un banco de cepas de IAV porcino que permita en un futuro el desarrollo de estudios inmunológicos, genómicos y de vacunas con virus locales.

1.4.8. Aportar al banco de datos mundial, las secuencias genómicas de IAV porcino de Colombia, que permita en un futuro el desarrollo de estudios genómicos más profundos.

1.4.9. Proveer información epidemiológica relevante que facilite la toma de decisiones para el manejo, control y prevención de la influenza porcina en Colombia.

CAPÍTULO II. MARCO TEÓRICO

En este capítulo, se abordan los conceptos y antecedentes sobre el virus influenza en porcinos y su importancia en sanidad animal y salud pública.

2.1. Historia e importancia de los Virus Influenza tipo A

La enfermedad llamada influenza es causada por un virus del género Alfainfluenzavirus que pertenece a la familia Orthomyxoviridae (Bouvier y Palese, 2008). A la actualidad existen cuatro tipos (A, B, C, y D) descritos de estos virus que representan cuatro de los siete géneros de la familia (ICTV, 2011), y se caracterizan por genomas de ARN de cadena negativa segmentados. La secuenciación genética de estos virus ha confirmado que comparten una ascendencia genética común; sin embargo, han divergido genéticamente (Bouvier y Palese, 2008). Los virus de influenza tipo A (IAV) están asociados con niveles mucho más altos de diversidad, infección y patogenicidad que los virus de influenza tipo B y C. Los virus tipo A serán el objeto de este trabajo.

El IAV ha sido causante de múltiples pandemias, epidemias y brotes estacionales a lo largo de la historia de la humanidad, manifestándose como una enfermedad de alta morbilidad y una mortalidad variable (Rivero y Carbonetti, 2016). Los mecanismos de evolución inherentes a la biología de estos virus, se traducen en una gran capacidad de adaptación, permitiendo la evasión de la respuesta inmune y el cruce de la barrera entre diferentes especies (De Graaf y Fouchier, 2014). En los últimos 100 años, se han registrado al menos cinco pandemias en humanos causadas por IAV, y las pandemias de 1918, 1957 y 1968 resultaron en una importante mortalidad humana (Viboud et al., 2016). Estudios filogenéticos sugieren la posible implicación de virus porcinos en las pandemias humanas como el virus H1N1 de 1918, H2N2 de 1957 y H3N2 de 1968, pero se carece de evidencias directas (Ma et al., 2009). Recientemente se han encontrado algunas evidencias del papel que los cerdos pueden tener en la facilitación de aparición de nuevas cepas con potencial zoonótico, como el caso de la pandemia H1N1 del siglo XXI, que ocurrió en 2009 (Mena et al., 2016), en la cual l nuevo virus H1N1 se derivó del reordenamiento de virus porcinos preexistentes con cepas humanas y aviares (Garten et al., 2009). No obstante, existen otras posiciones de expertos que sugieren también un rol más importante de los virus de origen

humano en la aparición de estas pandemias. A fines de 1918, en Estados Unidos se presentó una nueva enfermedad en cerdos que era muy parecida y concurrente con la presentación de la influenza humana. Tras varios años, se logró reproducir experimentalmente la enfermedad en cerdos, concluyendo que estos virus, provenientes de humanos y porcinos, compartían características similares, pero no eran idénticos (Weingartl et al., 2009). En consecuencia, con estos antecedentes se generó la hipótesis de que los cerdos habrían contraído la infección del hombre, pero otros investigadores sostuvieron una hipótesis contraria, donde la infección humana se originó desde los cerdos. Sin embargo, actualmente ha sido ampliamente demostrado que la transmisión viral puede ocurrir del hombre al cerdo o viceversa (Nelson y Vincent, 2015). A pesar de la incertidumbre que aún existe sobre los registros históricos de IAV en la era pre-virologica, todo el conocimiento que se genere actualmente sobre los virus pandémicos ayudaría a un mejor entendimiento de la compleja biología que rodea la formación de los virus pandémicos (Taubenberger y Morens, 2010).

Dado el papel potencial que tuvo el cerdo en pandemias pasadas, es importante comprender cómo circulan y evolucionan los IAV en estas poblaciones, a fin de centrarse en los estudios de vigilancia y prevenir la aparición de nuevas cepas con potencial pandémico o zoonótico. En la actualidad no está claro de qué región geográfica vendrá un nuevo virus pandémico ni de qué subtipo será ni de que especie provendrá. Incluso el conocimiento actual, es difícil que alguien pueda predecir con precisión una pandemia futura, o cuándo o dónde ocurrirá, qué subtipo será y qué impacto de morbilidad / mortalidad tendrá (Taubenberger y Morens, 2010). De hecho, la cepa pdm H1N1 2009 no se identificó como una amenaza hasta que causó la epidemia generalizada que no pudo ser contenida (Fraser et al., 2009).

Además de las consecuencias epidemiológicas y socio-económicas de las pandemias (Gasparini et al., 2012), los IAV están asociados con profundas pérdidas económicas para la industria animal, ya que por ejemplo, en la industria avícola un solo brote de influenza altamente patogénica puede provocar el sacrificio de varios millones de animales de producción con graves impactos socioeconómicos directos (Basuno et al., 2010). Recientemente, nuevas variantes virales y virus reordenandos con potencial zoonótico han sido reconocidos en cerdos (Everett et al., 2020). Por tanto, comprender la genética y biología

que anteceden a la aparición de estos virus causantes de epidemias particularmente graves en humanos, otros mamíferos y aves podría facilitar la identificación temprana de cepas con el potencial de causar pandemias e influir en las estrategias para su control.

2.2. Biología de los Virus Influenza tipo A

2.2.1. Taxonomía, estructura y clasificación.

Los Virus Influenza tipo A (IAV) son partículas pequeñas (80 a 120 nm de diámetro), envueltas por glicoproteínas (Bouvier y Palese, 2008). El genoma del IAV está organizado en ocho segmentos de ARN de polaridad negativa, numerados del 1 al 8 (Tabla 1 y Figura 1), de los cuales se transcriben en total 11 proteínas (Chen et al., 2001). El virión de influenza debe contener una copia de cada uno de los ocho segmentos de ARN para ser considerado como infeccioso (Jacobs et al., 2019). Los IAVs se caracterizan además por el subtipo de sus glicoproteínas de superficie, la hemaglutinina (HA) y la neuraminidasa (NA). Existen muchos subtipos genéticamente distintos que se han reportado (Samji, 2009). Actualmente se han identificado 18 subtipos HA y 11 NA (Mehle, 2014), sin embargo, solo tres subtipos de HA (H1, H2 y H3) y dos subtipos de NA (N1 y N2) han causado epidemias en humanos (Webster et al., 1992).

La hemaglutinina cumple la función de ligando del receptor viral, tiene forma de barra y estructura trimérica, sostenida por enlaces covalentes (figura 2). También define el tropismo de especie hospedera y la zona del sistema respiratorio que infecta, y está implicada en la eficacia de transmisión del virus. Estructuralmente tiene en el centro un tallo fibrilar que sirve como puente de unión para dos regiones polares: A) La región macroglobular externa es la porción más superficial donde se ubican los epítopos inmunogénicos capaces de generar la enorme diversidad antigénica tan característica del virus y que, al ponerse en contacto con el sistema inmune, induce la producción de anticuerpos neutralizantes. Otra función importante es servir como punto de enlace con los sialorreceptores de la membrana celular y de los eritrocitos; B) La región microglobular interna sirve principalmente como sitio de anclaje y propicia la inserción del material viral en la célula infectada (Bouvier y Palese, 2008). La neuraminidasa es una glicoproteína con forma semejante a un champiñón, cuya misión principal es la adhesión al ácido siálico y liberar el virión durante el ciclo vital del virus. Las

proteínas de la cápside M1 y M2 conforman la matriz viral; la primera envuelve al núcleo viral y tiene función de sostén, la segunda es un canal iónico que controla el pH y que permite que el genoma viral se libere durante el proceso de replicación. El péptido no estructural NS-1 actúa como un antagonista del interferón, por tanto, es un factor de virulencia que inhibe la respuesta inmune de hospedero, mientras que el péptido NS-2 trabaja como agente de exportación de las proteínas nucleares. Por último, la Nucleoproteína NP, se une al genoma viral, mientras que las proteínas PB1, PB2 y PA tienen actividad ARN polimerasa (Webster et al., 1992).

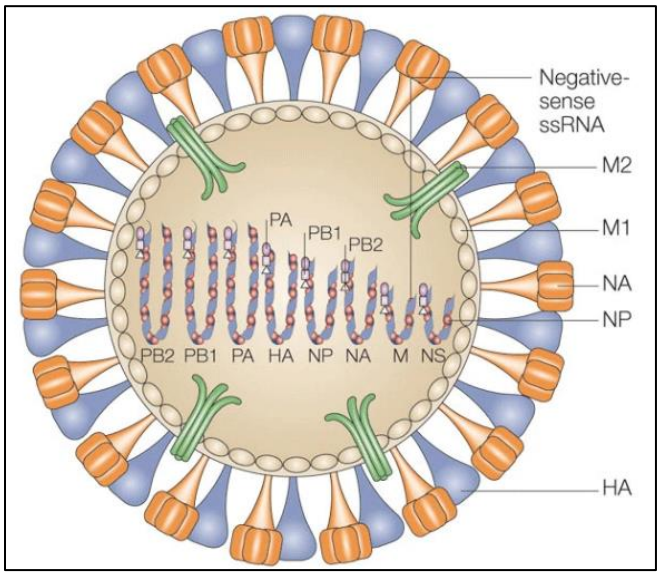

Figura 1. Esquema de la estructura y composición del Virus Influenza tipo A. Dos proteínas de superficie, HA y NA, y la proteína M2 están incrustadas en la envoltura viral, que se deriva de la membrana plasmática del huésped. El complejo de ribonucleoproteína (RNP) comprende un segmento de ARN viral asociado con la NP y tres polimerasas (PA, PB1 y PB2). La proteína de la matriz (M1) está asociada tanto a la RNP como a la envoltura viral. Tomado de Horimoto y Kawaoka. (Horimoto y Kawaoka, 2005)[1].

1 Derechos de autor Nature Reviews Microbiology ISSN 1740-1534.

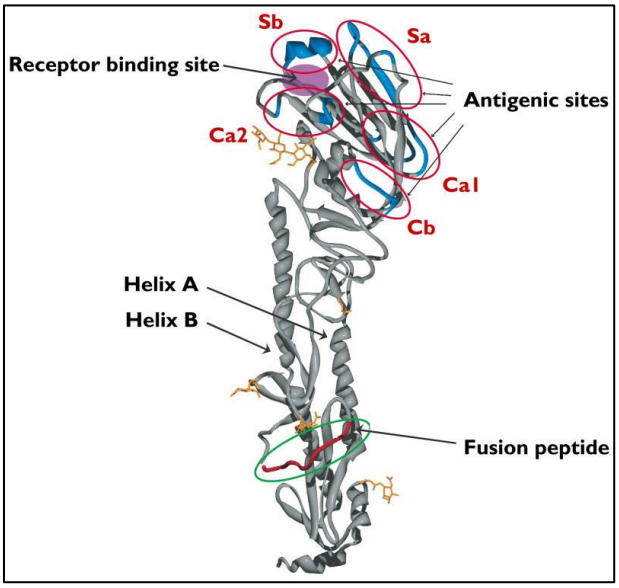

Figura 2. Estructura de la hemaglutinina del Virus Influenza tipo A (H1N1). La cabeza contiene el sitio de unión al receptor de ácido siálico, que está rodeado por cinco sitios antigénicos (Sa, Sb, Ca1, Ca2 y Cb). El tallo comprende las hélices A y B y el péptido de fusión. Tomado de Bouvier y col (Bouvier y Palese, 2008).

Tabla 1. Composición y características de las proteínas del Virus de la Influenza A (IAV)

Segmento (genes)	Tamaño (nucleótidos)	Proteínas codificadas	Número de aminoácidos	Función de la proteína
1	2.341	Polimerasa-2 (PB2)	759	Subunidad de polimerasa; reconocimiento de ARNm
2	2.341	Polimerasa-1 (PB1)	757	Subunidad de polimerasa; Elongamiento de ARN, actividad endonucleasa
		Polimerasa-1 F2 (PB1-F2)	87	Actividad proapoptótica
3	2.233	Polimerasa (PA)	716	Subunidad de polimerasa; actividad proteasa
4	1778	Hemaglutinina (HA)	566	Glicoproteína de superficie; principales actividades de antígeno, unión al receptor y fusión
5	1.565	Nucleoproteína (NP)	498	Proteína de unión a ARN; regulación de importación nuclear

6	1.413	Neuraminidasa (NA)	469	Glicoproteína de superficie; actividad sialidasa, liberación de virus
7	1.027*	Matriz (M1)	252	Proteína matricial; Interacción ribo nucleoproteína viral, regulación de exportación nuclear de ARN, gemación viral
		Matriz (M2)	97	Canal de iones; recubrimiento y ensamblaje de virus
8	809*	Proteína no estructural (NS1)	230	Proteína antagonista de interferón; regulación de la expresión génica del huésped
		Proteína no estructural (NS1) (NEP/NS2)	121	Exportación nuclear de ARN

*Tomado de Bouvier y Palese (Bouvier y Palese, 2008)

2.2.2. Transmisión y replicación

La transmisión de influenza A ocurre a través de microgotas de secreciones respiratorias y las condiciones climáticas relacionadas con la humedad relativa y la temperatura, repercuten en la eficiencia de esta transmisión (Neumann y Kawaoka, 2015). El contacto directo con superficies contaminadas con el virus son otra vía de infección. El virus penetra en el sistema respiratorio superior donde se inicia la replicación viral a nivel de las células epiteliales. Es importante señalar que el receptor reconocido por el virus, evento que realiza a través de la HA, es un residuo de ácido siálico que se encuentra en la membrana de las células epiteliales (Webster et al., 1992). Este residuo está unido a una galactosa por un enlace que puede ser de tipo alfa 2,3 o alfa 2,6. En los humanos el receptor predominante en vías aéreas superiores es alfa 2,6 y en aves es alfa 2,3 (Cheung y Poon, 2007). En el cerdo existe la presencia de ambos tipos de receptores en vías respiratorias superiores por tanto puede ser afectado por virus de origen humano, aviar y los propios del cerdo, constituyendo el reservorio ideal para que ocurran reestructuraciones genómicas del virus (Webster et al., 1992).

Una vez que ocurre la unión entre la HA y el ácido siálico, el virus penetra por endocitosis al citoplasma de la célula diana, el cambio de pH modifica la HA y libera al virus de la unión a la membrana. La proteína M2, cuya función es actuar como canal iónico, induce cambios de pH intraviral y permite, durante el ciclo replicativo, que el genoma viral se desprenda de la matriz y se libere al citoplasma celular y posteriormente al núcleo. El virus necesita una replicasa y la cooperación de NP con PB2 para sintetizar las hebras de ARN con polaridad negativa. Durante las primeras horas de la infección, en el citoplasma cargado con el ARNm se sintetizan los péptidos P, NS1, NS2 y M1, los que migran al núcleo y contribuyen a propiciar la síntesis del ARN genómico, junto con la replicasa. Posteriormente, el ARN genómico y la NP se desplazan hacia el citoplasma, mientras HA, NA y M2 permanecen todo el tiempo en el citoplasma. El péptido M2 funciona como señal del ensamblaje junto a la membrana celular, que ocurre cuatro a cinco horas después de la infección. La NA es la encargada de eliminar los residuos del ácido siálico, facilitando la liberación de los viriones, sin agregación. A veces, el cambio de un solo aminoácido o la presencia de inhibidores, pueden reducir el funcionamiento de la enzima o abolirlo. El ARNm sintetizado es cortado por la endonucleasa viral funcionando como molde para iniciar el proceso. El complejo PA, PB-1 y PB-2 se desplaza a lo largo de la cadena y PB-1 lleva el sitio activo del alargamiento, por ello, esta polimerasa condiciona la velocidad y calidad de la replicación viral siendo así, un factor principal de virulencia. La maquinaria celular sintetiza las nuevas proteínas víricas y se ensambla un nuevo virión que eclosiona de la célula (Bouvier y Palese, 2008; Cheung y Poon, 2007; Samji, 2009; Webster et al., 1992). Después de ocurrida la infección pueden transcurrir entre 18 y 72 h antes del comienzo de la sintomatología clínica, sin embargo, la excreción viral ocurre desde antes del comienzo de los síntomas (0-24 h) y continúa por 5-10 días (Vincent et al., 2014).

2.3. Evolución de los Virus Influenza tipo A

En la actualidad los IAV continúan representando una amenaza para la salud veterinaria y humana a pesar de la disponibilidad de vacunas, debido principalmente a la capacidad del virus para evadir la inmunidad existente a través de la evolución antigénica (Webster et al., 1992). Los genes de los virus Influenza mutan con alta frecuencia (Bush et al., 1999). La acumulación de mutaciones puntuales en los genes HA y NA puede conducir a un cambio

antigénico gradual de las glicoproteínas de superficie y a la aparición de cepas inmunológicamente distintas. Los cambios en la HA ocurren principalmente en la cabeza globular de esta glicoproteína que contiene el sitio de unión al receptor celular y cinco sitios antigénicos hipervariables (Wiley y Skehel, 1987). Estos cambios hacen que la variante del virus ya no sea reconocida por los anticuerpos, lo que permite que el virus se multiplique y produzca nuevos brotes de enfermedad (Schweiger et al., 2002). Adicionalmente, los cambios ocurridos en genes internos, principalmente en las polimerasas, pueden conferir una mayor virulencia, facilitar la adaptación a otras especies e incluso incrementar el potencial pandémico del virus (Shao et al., 2017). Además, estudios recientes han demostrado que la evolución de los IAV humanos circulantes se produce no solo por una deriva antigénica gradual, sino también por un reordenamiento intraclado que resultada en la importación de nuevas HA del mismo subtipo frente a las que existe un menor grado de inmunidad en la población, y que crea, por tanto, nuevas constelaciones de segmentos de genes virales (Rambaut et al., 2008).

2.3.1. Mecanismos de diversidad antigénica y genética

Los IAV pueden cambiar a variantes antigénicas distintas a través de dos mecanismos principales: Drift o deriva antigénica (mutaciones) y Shift o reordenamiento. La deriva antigénica es una acumulación lenta y gradual de errores cometidos por la ARN polimerasa que no posee un sistema de corrección de lectura y permite que ocurran un mayor número de sustituciones de nucleótidos durante el proceso de replicación viral. Algunas de estas mutaciones pueden ocurrir en sitios antigénicamente importantes de la HA o NA. Bajo presión de la inmunidad existente, estas variantes se seleccionan rápidamente, lo que eventualmente conduce a un virus antigénicamente distinto (Webster et al., 1992) (Figura 3). Cuando estas variantes son introducidas dentro de una población y no pueden ser reconocidas por los anticuerpos previamente generados, pueden ser capaces de iniciar un proceso de infección en animales susceptibles a esta nueva variante. Por otra parte, el reordenamiento que implica el intercambio de segmentos genéticos es un proceso rápido y más repentino impulsado por la adquisición de segmentos genéticos completamente nuevos durante la replicación. El reordenamiento es determinado por la naturaleza segmentada del genoma de los virus de influenza y puede ocurrir cuando dos o más IAV diferentes coinfectan y se

replican en una sola célula (Sandbulte et al., 2015). Durante la replicación, es posible que los segmentos genéticos de un virus de un subtipo en particular se intercambien con segmentos genéticos del otro virus de distinto subtipo, produciendo así un nuevo virus (Figura 4). Este mecanismo es lo que condujo a la creación del virus pdm A (H1N1) 2009. El virus de 2009 se creó a través de al menos tres eventos de reordenamiento genético independientes y temporalmente distintos que involucraron virus de origen porcino, humano y aviar (Smith et al., 2009).

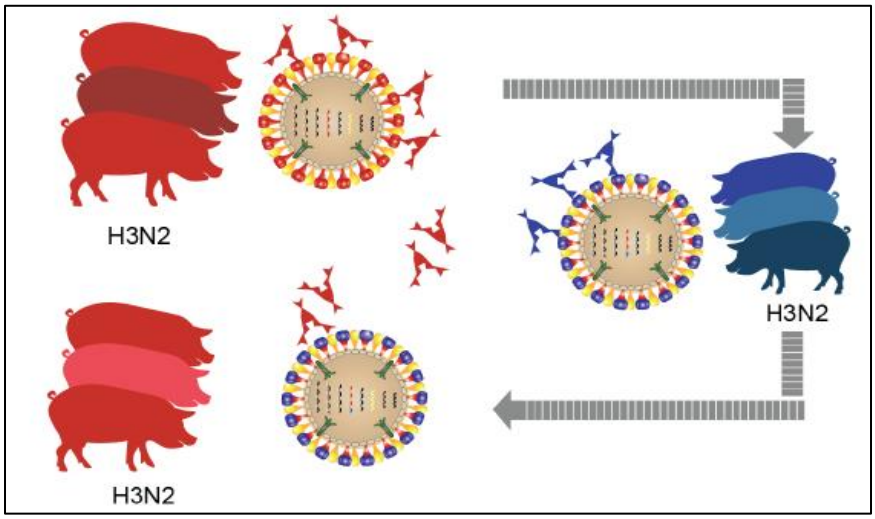

Figura 3. Representación del Drift o deriva antigénica. Ejemplo del mecanismo de variación antigénica que promueve la generación de cambios en un virus del subtipo H3N2 que inicialmente es reconocido por una población de cerdos (color rojo), pero al introducirse nuevas variaciones, estos nuevos virus son capaces de evadir la respuesta inmune e iniciar un proceso de infección (color azul). Adaptado de Sandbulte, et al. (Sandbulte et al., 2015)

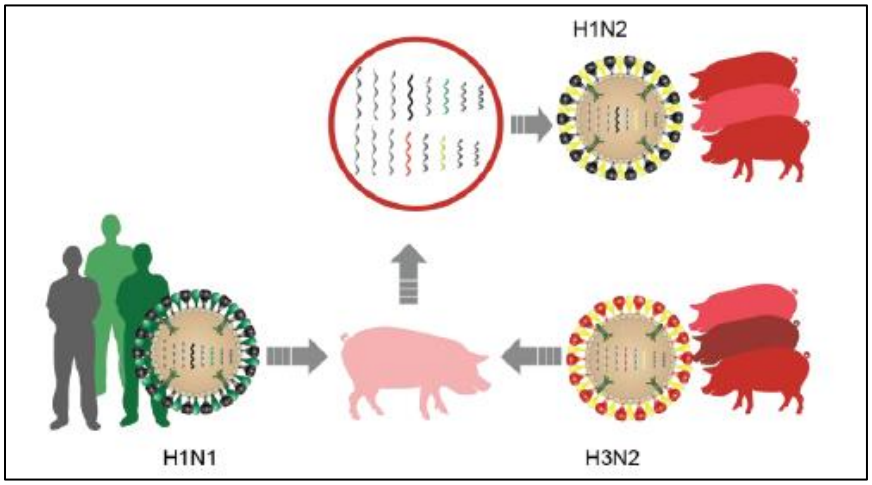

Figura 4. Representación del Shift o reordenamiento genético. Ejemplo del mecanismo de reordenamiento durante un proceso de co-infección de diferentes IAV, H1N1 de origen humano (color verde) y H3N2 de origen porcino (color rojo), resultando en la generación de una nueva cepa H1N2 que puede de ser transmitida de regreso a los cerdos. Adaptado de Sandbulte, et al. (Sandbulte et al., 2015)

2.4. Generalidades del IAV en cerdos

Los IAV están distribuidos mundialmente y son considerados patógenos prioritarios a nivel global por su potencial zoonótico y pandémico (Reperant et al., 2016) y también por que causan una de las enfermedades respiratorias más importantes en los cerdos. La Influenza Porcina (IP) es una enfermedad respiratoria aguda causada por el IAV, altamente contagiosa que presenta una alta morbilidad y un curso agudo, ocasionando fiebre y signos respiratorios. Usualmente los animales afectados presentan síntomas que incluyen tos, estornudos, secreción nasal, fiebre, inactividad, apatía, anorexia, pérdida de peso y retraso en el crecimiento, con duración de 5 a 10 días (Janke, 2014).

La IP se reportó por primera vez en cerdos en los EE. UU. en 1918 como una enfermedad respiratoria que coincidió con la pandemia de gripe española en humanos (Janke, 2014). Desde entonces, la influenza se ha convertido en una enfermedad importante para la industria porcina en todo el mundo. En 1930, se aisló por primera vez de cerdos, el virus IAV H1N1 emparentado con el virus de la gripe pandémica de 1918 (Taubenberger, 2006). Este linaje H1N1 se consideró estable en términos de genes y antigenicidad en los cerdos durante casi 80 años y se conoce hoy como IP clásica (Vincent et al., 2014). La IP es considerada una de

las enfermedades respiratorias más prevalentes en la producción porcina (Almeida et al., 2017). Estos virus son endémicos en cerdos en el mundo donde brotes epidémicos ocurren frecuentemente (Van Reeth, 2007). Adicionalmente, IAV es uno de los patógenos implicados en el desarrollo de la enfermedad del complejo respiratorio porcino (Thacker et al., 2001). Se ha reportado que la IP tiene gran impacto negativo sobre la productividad de las granjas afectadas (Vincent et al., 2009), debido principalmente al incremento en la conversión alimenticia y disminución en la ganancia de peso (Vincent et al., 2017), al incremento en mortalidad (Alvarez et al., 2015), y al incremento en los costos en la prevención y tratamiento de infecciones secundarias (Sandbulte et al., 2015; Van Reeth y Vincent, 2019). También se ha demostrado que los lechones nacidos de hembras infectadas con IP, tienen una menor ganancia de peso en su fase de engorde (Loeffen et al., 2003). Esta enfermedad se asocia con un impacto negativo significativo en la producción porcina, representando uno de los mayores desafíos en los sistemas de producción (Loeffen et al., 2009). En algunas condiciones la enfermedad puede ser endémica y a menudo la infección es subclínica y se asociada con otros agentes virales y bacterianos lo que resulta en una agudización de los signos clínicos (Janke, 2014).

Los subtipos del IAV que infectan con más frecuencia a los cerdos incluyen H1N1, H3N2 y H1N2, sin embargo, otros subtipos también han sido detectados, H1N7, H3N1, H4N6, H3N3, y H9N2 (Torremorell et al., 2012) y aunque solo los subtipos H1N1, H1N2 y H3N2 se consideran endémicos en los cerdos de todo el mundo, se puede encontrar una diversidad considerable no solo en los genes HA y NA, sino también en los demás genes. A la actualidad se ha evidenciado que los virus circulantes en las regiones de Europa tienen características antigénicas y genéticas distintas a los virus porcinos encontrados del continente americano (Vincent et al., 2009).

A diferencia del IAV humanos o de aves acuáticas silvestres, el transporte intercontinental controlado de cerdos ha provocado el establecimiento de linajes de virus genéticamente y antigénicamente distintos en el mundo (Van Reeth, 2007). En general, para el gen H1 porcino, existen tres linajes principales basados en la nomenclatura global: linaje porcino clásico (CS; 1A), linaje porcino de tipo aviar euroasiático (EA; 1B) y linaje humano estacional (HU; 1C). El linaje CS se divide en seis grupos (Anderson et al., 2016): α (1A.1),

β (1A.2), TR (1A.3.3.1), ϒ2 (1A.3.3.2), ϒ1 (1A.3.3.3) y pandémico (pdm; 1A.3.3.2). El clúster Alfa (1A.1) podría clasificarse además en tres subgrupos (1A.1.1; 1A.1.2; 1A.1.3). El linaje HU fue el resultado de dos introducciones separadas de virus H1 estacionales humanos en cerdos a principios de la década de 2000 y se dividen en virus del grupo 1B.1 (relacionados con un virus H1N2 que surgió en Gran Bretaña en 1994), y virus del grupo 1B. 2 (relacionados con los virus H1-δ1 (1B.2.2) y H1-δ2 (1B.2.1)) (Anderson et al., 2016). Para el gen H3 porcino, existen dos linajes principales basados en la nomenclatura global: linaje norteamericano (NAL) y linaje euroasiático (EUL). H3 NAL se puede clasificar además en cinco clados (I, II, III, IV y similar al humano) (Rajão et al., 2017). El Clúster H3 IV se puede dividir adicionalmente en seis clados (IVA a IVF) (Kitikoon et al., 2013).

La infección de IAV requiere de la unión de la proteína HA del virus al receptor celular (ácido siálico-AS) del huésped (Rajao et al., 2019). La distribución y abundancia de las especies de receptores de ácido siálico varía entre las especies animales, y se desconoce en gran medida el efecto biológico que tiene esta variación en el potencial de replicación de los IAV (Suzuki et al., 2000). La mayoría de los IAVs aviares se unen preferentemente a receptores α2,3-AS, mientras que los IAVs humanos y de otros mamíferos reconocen preferentemente los receptores α2,6-AS (Gambaryan et al., 2005). Los cerdos poseen ambos receptores celulares (α2,3-AS y α2,6-AS) en su tracto respiratorio superior y así reconocen cepas virales de influenza procedentes de humanos y aves, haciéndolos susceptibles a la infección por estos virus (Suzuki et al., 2000). Se ha descrito que esta condición biológica de los cerdos aumenta el riesgo para la aparición de nuevas variantes virales producto del reordenamiento genético (Ma y Richt, 2010).

Existe una gran diversidad de IAV dentro de los subtipos H1N1 / N2 y H3N2 que circulan en los cerdos a nivel mundial y hay diferentes linajes que predominan en regiones específicas del mundo (Rajao et al., 2019). Aunque la detección de virus humanos estacionales en cerdos es un evento periódico, el IAV humano completo rara vez se establece en los cerdos y por lo general en cerdos, estos virus se reordenan y emergen solo con algunos de los segmentos virales de origen humano, pero a menudo con marcadas diferencias genéticas con respecto a la cepa precursora (Nelson et al., 2014). Por tanto, existen otros factores de adaptación además de la especificidad del receptor para que los virus de origen humano se transmitan y

posteriormente se vuelvan endémicos en los cerdos (Rajao et al., 2019). Sin embargo, los virus humanos que han tenido una transmisión sostenida en cerdos ha conllevado al establecimiento de nuevos linajes de IAV en porcinos, contribuyendo a la diversidad genética y antigénica de IAV en los cerdos (Rajao et al., 2019). Varios estudios filogenéticos han demostrado que hay más virus influenza humanos adaptados a las poblaciones porcinas que virus porcinos adaptados a las poblaciones humanas (Ma et al., 2009) y tanto los IAV humanos como los porcinos se transmiten del hombre al cerdo y viceversa. No obstante, según el conocimiento actual la infección en los cerdos podría provocar que los virus IAV obtengan las adaptaciones necesarias para mantener la infección endémica, como un reservorio adicional para la infección en humanos, o proporcionar las condiciones para las mutaciones necesarias y permitir su transmisión a humanos. En consecuencia, el intercambio genético dado por el reordenamiento de segmentos virales de distintos IAV ha dado lugar a la aparición de pandemias como la del virus H1N1 (A/CA/04/2009/H1N1) y H3N2 (H3N2v), que causaron enfermedades tanto en humanos como en cerdos y que constituyen una amenaza para la salud pública (Vincent et al., 2014).

2.5. Epidemiología de la Influenza Porcina

La epidemiologia de la IP no está del todo definida. Existen múltiples factores que afectan la dinámica de infección y transmisión de los IAV en cerdos, entre otros, se ha descrito el flujo y movimiento de cerdos, así como la vacunación (Torremorell et al., 2012). Se sabe que estos virus se transmiten rápidamente entre hospederos por contacto directo, por aerosoles y por fómites (Crisci et al., 2013). En este contexto, la vacunación ha sido reconocida como la estrategia más común para prevenir la IP y/o mitigar los efectos de la enfermedad, sobre todo en las poblaciones consideradas a mayor riesgo (White et al., 2017). De acuerdo con la diversidad de cepas de IP, las vacunas para cada área geográfica se deberían producir localmente y estas pueden diferir en cepas, dosis de antígeno y formulación adyuvante (Van Reeth y Vincent, 2019). La vacunación con una vacuna de virus muerto reduce los signos clínicos y la carga viral en los pulmones en el caso de infección, pero no previene por completo la replicación y excreción viral (Van Reeth y Vincent, 2019). Se sabe que las vacunas son más efectivas si las cepas o variantes virales incluidas en ellas coinciden estrechamente con los virus que circulan en ese momento en los cerdos de esa región (Vincent

et al., 2017). En este sentido, la caracterización genética de los virus circulantes ayuda en la selección de cepas para incluir en las vacunas porcinas y que se asegure un emparejamiento para su efectividad. Sumado a la compleja epidemiologia de IAV en cerdos, también se requiere que se promuevan el diseño de estrategias novedosas de control de la enfermedad y el desarrollo de vacunas con acción universal y efectiva contra los diferentes subtipos de IAV (Sandbulte et al., 2015; Vincent et al., 2017).

2.5.1. Trayectoria evolutiva y epidemiologia de la Influenza Porcina

El estudio de la composición genética relacionado con la evolución de los IAV que afectan a los cerdos ha permitido agruparlos en linajes de manera que estos puedan ser comparados o relacionados con virus provenientes de diferentes orígenes geográficos, periodos y/o hospedadores. En este sentido, como resultado del proceso de adaptación evolutiva de IAV aparecen diversos linajes de diferente origen, entre otros aves, humanos, porcinos y equinos (Van Reeth, 2007). Los IAV se dividen en subtipos basados en las dos proteínas en la superficie del virus: hemaglutinina (HA) y neuraminidasa (NA). Hay 18 subtipos de HA diferentes y 11 subtipos de NA diferentes. Si bien existen potencialmente 198 combinaciones diferentes de subtipos de IAV, solo se han detectado 131 subtipos en la naturaleza (Ma et al., 2009). De acuerdo a la composición genética o al origen filogenético del IAV, los subtipos se pueden dividir adicionalmente en diferentes "clados" y "subclados" genéticos, cuyo agrupamiento a su vez conforma un respectivo linaje, que corresponde a una serie de mutaciones que se conectan filogenéticamente a un ancestro común (ver Figura 5). Los clados y subclados se pueden llamar alternativamente "grupos" y "subgrupos", respectivamente. Un grupo o clado de IAV es entonces una subdivisión adicional de los IAV basada en la similitud de las secuencias de los genes virales. Los clados y subclados se evidencian en árboles filogenéticos como grupos de virus que generalmente tienen características genéticas similares (es decir, cambios de nucleótidos o aminoácidos) y tienen un único ancestro común representado como un nodo en el árbol (ver Figura 5). Por tanto, esta división de IAV en linajes, clados y subclados permite a los expertos rastrear e identificar el origen filogenético de los diferentes virus en circulación. Es importante indicar que los clados y subclados que son genéticamente diferentes de otros no son necesariamente diferentes antigénicamente (es decir, los virus de un clado o subclado específico pueden no

tener cambios que afecten la inmunidad del huésped en comparación con otros clados o subclados).

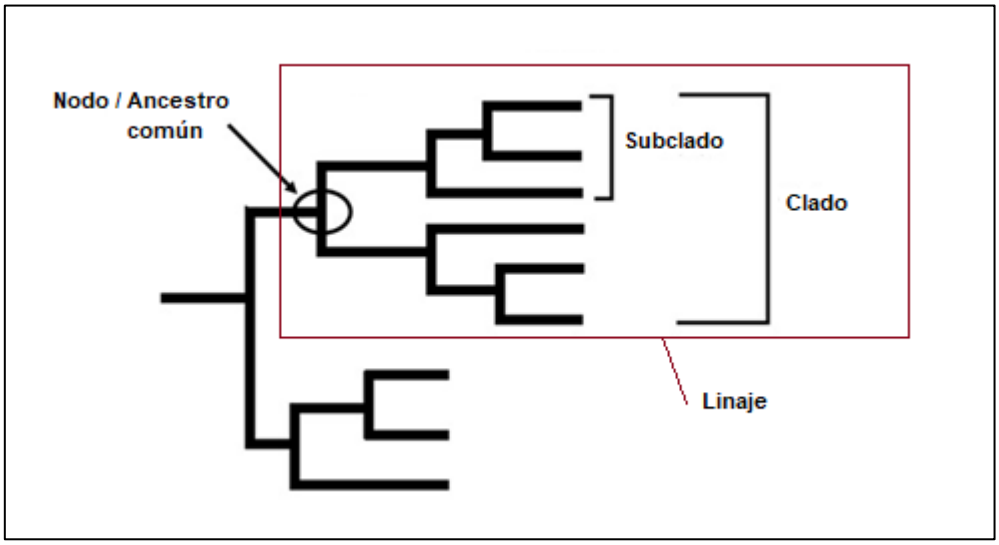

Figura 5. Imagen de un árbol filogenético. En un árbol filogenético, los virus relacionados se agrupan en ramas. Por ejemplo, los IAV cuyos genes HA comparten los mismos cambios genéticos y que también comparten un ancestro común (nodo) se agrupan en "clados" y "subclados" específicos.

A la actualidad, se han descrito al menos 69 linajes para los subtipos de HA, 46 linajes para NA y entre 7 y 11 linajes para los segmentos internos del virus (Zell et al., 2008). En cuanto a los linajes porcinos estos están determinados por la ruta evolutiva que ha ocurrido en cada uno de los continentes como se mencionó anteriormente, por ejemplo, para el gen HA existen entre otros los IAV derivados del linaje porcino clásico (H1α, H1β y H1γ) y los IAV del linaje estacional humano (H1δ1, H1δ2) (Vijaykrishna et al., 2011).

La circulación endémica de los linajes de IAV de los subtipos H1N1, H1N2 y H3N2 en cerdos varía según el continente y recientemente se ha descrito que incluye reordenamientos con el virus pandémico H1N1 (Vincent et al., 2014). Se ha reportado la introducción esporádica de virus humanos y aviares en cerdos, siendo los virus humanos H3N2 y H1N1 los que se detectan más comúnmente, sin embargo, la mayoría de estos virus humanos que infectan esporádicamente a los porcinos sólo se establecen en estas poblaciones luego de que eventos de reordenamiento genético han ocurrido (Lorusso et al., 2011). Los virus H3N1 han sido detectados en Asia, Europa, América del Norte y América Latina, y fueron virus

surgidos de cepas porcinas locales o de virus similares a los humanos (Choi et al., 2011; Lorusso et al., 2011). Ocasionalmente en cerdos de Asia se han aislado los subtipos H5N1 y H9N2 como recombinación de origen aviar, al igual que otros subtipos como H1N1, H3N2, H5N2, H6N6, H7N2, H4N8 y H11N6 detectados en cerdos en diferentes partes del mundo (Choi et al., 2011). Virus de otros orígenes también se han detectado esporádicamente en cerdos, por ejemplo, un subtipo H3N8 equino fue detectado en China (Tu et al., 2009) y un virus H1N7 de origen humano y equino fue detectado en Gran Bretaña (Brown et al., 1994). La epidemiología de la IP varía, por tanto, de un país a otro, debido entre otros, a las condiciones genéticas particulares del virus, los factores como el clima, la población y densidad de animales y las prácticas de manejo productivo y de bioseguridad. Las revisiones de literatura más recientes sobre la IP se han centrado principalmente en la evolución genética de las cepas que circulan en Europa (Brown, 2011), América del Norte (Lorusso et al., 2011), Asia (Choi et al., 2011). Algunos estudios se han centrado en la prevalencia, factores de riesgo y tasas de aislamiento, pero se han limitado a una región geográfica particular (Trevennec et al., 2011). Sin embargo, en comparación a otros lugares, pocos reportes existen para países de Latinoamérica.

2.5.2. Influenza porcina en el mundo

A principios de 2009, surgió en humanos un nuevo IAV H1N1 reordenado con segmentos genéticos de dos linajes de virus porcinos, iniciando la primera pandemia de influenza del siglo XXI (Nelson et al., 2015b). El virus tenía una composición genética compleja que no se había detectado previamente en cerdos (Garten et al., 2009). El análisis evolutivo de este nuevo virus norteamericano / euroasiático indicó que estos segmentos habían circulado sin ser detectados en cerdos durante al menos ocho años (Smith et al., 2009). El origen zoonótico del IAV pdm/H1N1 2009 sigue sin aclararse, debido a evidencias contradictorias. Estudios de la diversidad genética del virus pandémico H1N1 (pdmH1N1) proveen una fuerte evidencia de que el primer brote de pdmH1N1 ocurrió en humanos en México en 2009, lo que respalda la hipótesis de que el virus surgió allí por primera vez en humanos (Mena et al., 2016). Sin embargo, otros estudios han demostrado que el pdmH1N1 se derivó de varios virus que circulaban en los cerdos, y que la transmisión inicial a los humanos ocurrió varios meses antes del reconocimiento del brote en humanos (Smith et al., 2009). Los análisis

genómicos del virus pdmH1N1 no revelan directamente la fuente inmediata de origen del virus y se especula que es posible que las tres cepas parentales del virus pdm se hayan reunido en un lugar por medios naturales, donde se involucraron aves migratorias, virus porcinos y la actividad humana, junto con otras teorías que sugieren que para la aparición de este virus hubo incluso la posibilidad de que hayan estado involucrados errores de laboratorio (Gibbs et al., 2009).

En general, los virus de la IP en los cerdos se separan espacialmente en distintos linajes de América del Norte y Europa, aunque los virus de origen norteamericano y europeo circulan en Asia (Nelson et al., 2015b). Múltiples linajes virales circulan en los cerdos de América del Norte, incluidos (i) los virus porcinos "clásicos", que descienden de la pandemia H1N1 de 1918 (Memoli et al., 2009); (ii) virus porcinos de "triple reordenamiento", que surgieron a mediados de la década de 1990 con una combinación de segmentos humanos, porcinos y aviarios (Zhou et al., 1999); y (iii) virus “delta” (δ) que están estrechamente relacionados con los virus humanos H1 estacionales de principios de la década de 2000 (Vincent et al., 2009). Los principales linajes europeos incluyen virus H1N1 'tipo aviar' que pasaron de aves a cerdos en la década de 1970, virus H1N1 de origen humano de la década de 1980 y virus H3N2 de origen humano (Ottis et al., 1982). Se han identificado múltiples linajes porcinos de origen norteamericano y europeo en países asiáticos (Vijaykrishna et al., 2011).

En Latinoamérica, aunque en menor grado, también se han podido detectar eventos de reordenamiento genético entre linajes, como en Argentina, Chile, Brasil y Cuba donde se identificaron variantes virales (H1N1 y H1N2) en cerdos cuyo análisis genómico determinó que existían genes internos pertenecientes al subtipo H1N1 pandémico, mientras que otros genes como HA y NA tenían similitudes con los IAV estacionales humanos (Cappuccio et al., 2011; Nelson et al., 2015a; Pereda et al., 2011; Pérez et al., 2015; Schaefer et al., 2015; Tapia et al., 2020).

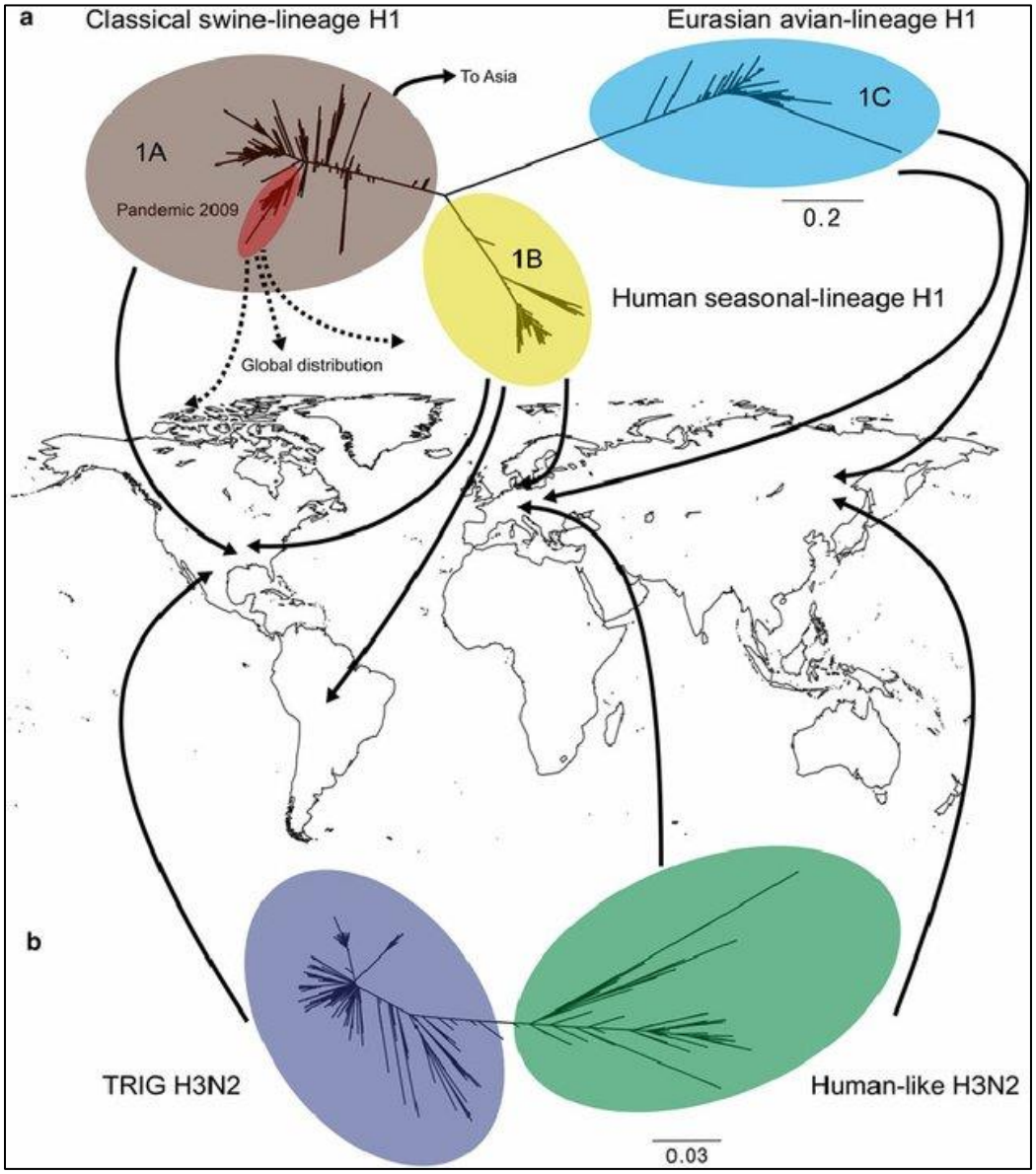

Figura 6. Principales linajes porcinos IAV H1 y H3 y su distribución geográfica. (a) Relaciones filogenéticas de virus H1; y (b) relaciones filogenéticas de virus H3. Linaje porcino clásico (marrón); Linaje del virus H3N2 reordenado triple (púrpura); Linaje del virus H1 estacional humano (amarillo). Linaje de virus derivados de H1N1 Eurasiático similar a las aves (azul); Linaje virus H3N2 similar al humano (verde); Linaje H1N2 similar al humano (amarillo). Linaje H1N1pdm09 (rojo). Tomado de Vincent, et. al. (Vincent et al., 2014).

En la figura 6 se ilustra la distribución de los linajes y subtipos más predominantes en cerdos en el mundo. Los datos filogenéticos sugieren que los subtipos IAV porcina de América del Norte se derivan de un linaje porcino clásico, un virus H3N2 reordenado triple o un virus H1 de linaje estacional humano (Figura 6), lo que da como resultado 10 linajes genéticos distintos. En Europa, hay tres linajes genéticos que circulan conjuntamente con virus derivados de un virus H1N1 Eurasiático similar a las aves, un H3N2 similar al humano v o un virus H1N2 similar al humano (Figura 6). En Asia, los linajes genéticos predominantes reflejan la dinámica observada en América del Norte y Europa con virus co-circulantes clasificados como linaje porcino clásico, H3N2 similar a los humanos o H1N1 similar a las aves de Eurasia. El H1N1pdm09 surgió del linaje porcino clásico H1 y experimentó una diseminación global a través de la transmisión de humanos a cerdos.

2.5.3. Linaje H1N1 porcino clásico

Durante la pandemia de 1918, la IP prevaleció durante mucho tiempo en cerdos en los EE.UU., en donde, después de su primera aparición, ocurrieron subsecuentemente brotes anuales en estas poblaciones y, en consecuencia, posterior al año 1930 después de que se aisló el virus en cerdos, a estos virus y aquellos relacionados estrechamente se les denominó como virus porcinos clásicos. Estos virus clásicos se observaron mucho tiempo después en otras partes del mundo, incluyendo Europa, África, Suramérica, Asia y Norteamérica (Kiss et al., 2010). Después de estos episodios, el virus aparentemente desapareció en muchas de estas locaciones, y no hubo evidencia de infecciones en Europa durante casi 20 años, hasta 1976, cuando el virus de la IP clásica se aisló de brotes ocurridos Italia y se relacionaron estrechamente con virus de la IP clásica de 1930 de los Estados Unidos (Nardelli et al., 2010), y es probable que el virus haya sido introducido durante la importación de cerdos desde los EE.UU. La enfermedad luego se propagó rápidamente a otros países y este virus se volvió endémico en los cerdos en toda Europa con una seroprevalencia de 25% (Brown et al., 1995b), sin embargo, a partir de 1979 estos virus fueron paulatinamente reemplazados por linajes similares a los aviares (Brown et al., 2000). Para el caso de los EE.UU. la prevalencia de la IP clásica H1N1 tuvo una marcada variación regional con un promedio del 51% (Chambers et al., 1991). En Asia, los virus H1N1 clásicos fueron aparentemente los más predominantes por un largo tiempo en cerdos (Guan et al., 1996).

2.5.4. Linaje de virus porcinos similares a los humanos

Algunos subtipos humanos predominantes también han infectado cerdos en condiciones naturales (Figura 7). El subtipo H3N2 humano fue aislado recurrentemente en cerdos en Asia y Europa (Nerome et al., 1995). Los H3N2 relacionados con la cepa humana de 1973, continuaron circulando en las poblaciones porcinas europeas mucho después de su desaparición en la población humana, causando infecciones frecuentemente caracterizadas por una alta seroprevalencia (Lange et al., 1984). Sin embargo, este nivel aparentemente alto de infecciones por H3N2 en Europa tuvo un marcado contraste con la baja prevalencia en cerdos en Norteamérica lo que sugirió que el virus no se establecía en las poblaciones porcinas americanas, sino que ocurría solo por la introducción infrecuente causada por humanos infectados (Easterday, 1980).

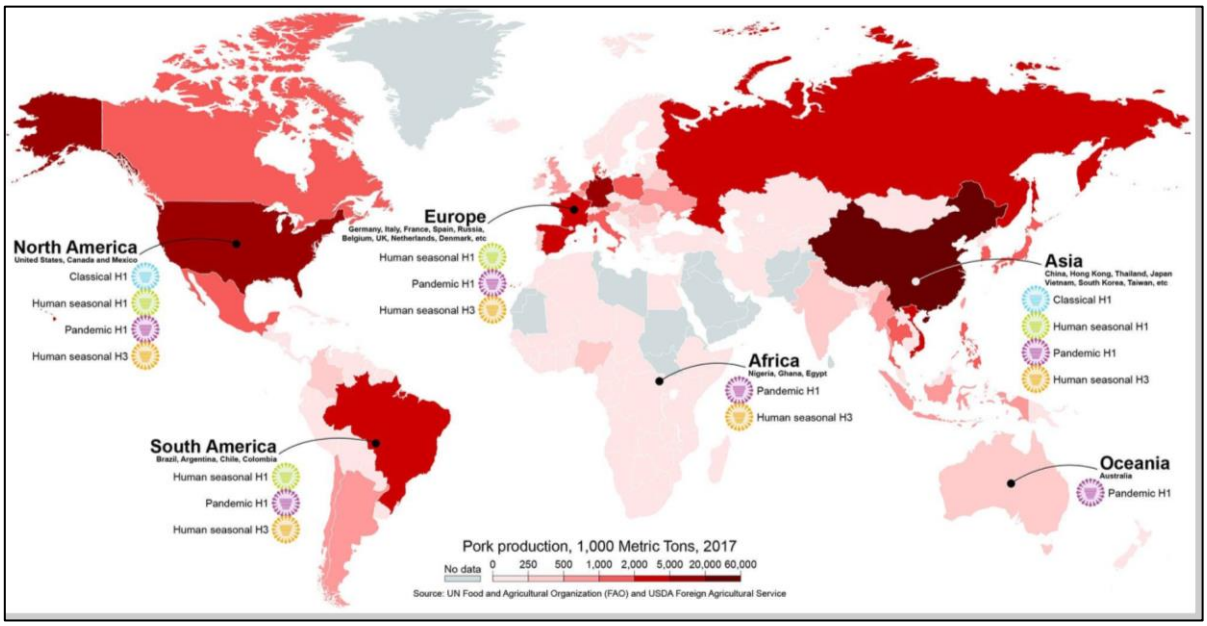

Figura 7. Diferentes subtipos / linajes de virus de influenza de origen humano que circulan en cerdos en diferentes continentes. El mapa está coloreado según la producción porcina en 1.000 toneladas métricas. Tomado de Rajao, et al. (Rajao et al., 2019)

2.5.5. Linaje de Virus porcinos similares a los aviares

Los virus H1N1 dominantes en los cerdos europeos desde 1979 han sido principalmente virus H1N1 “tipo aviar”, los cuales son distinguibles antigénica y genéticamente de los virus de la

IP clásica norteamericana H1N1, pero que están estrechamente relacionados (Pensaert et al., 1981), ya que todos los segmentos genéticos de los virus prototipo eran de origen aviar (Schultz et al., 1991), lo que indica que se había producido la transmisión de un virus aviar completo a los cerdos (Figura 6). Estos virus de tipo "aviar" parecen tener una ventaja selectiva sobre los virus H1N1 porcinos clásicos que están relacionados antigénicamente, ya que en Europa han reemplazado al virus de la IP clásica (Campitelli et al., 1997). Recientes estudios en Francia, los Países Bajos y Bélgica han demostrado que el subtipo más dominante ha sido H1N1 del linaje EU H1av (Chastagner et al., 2020; Chepkwony et al., 2021).

2.5.6. Linaje de Virus porcinos H1N2

Los virus de la influenza A H1N2, derivados de la IP clásica H1N1 y los virus H3N2 porcino "similares a los humanos" se han aislado en Europa y Asia (Gourreau et al., 1994; Sugimura et al., 1980). En Japón, estos virus parecen haberse establecido ampliamente en las poblaciones porcinas asociándose frecuentemente con epizootias respiratorias (Ouchi et al., 1996). Posteriormente, el subtipo H1N2 relacionado antigénicamente con los virus porcinos "similares a los humanos" ha surgido en los cerdos y se ha vuelto endémico en Europa a menudo asociados con enfermedades respiratorias (Brown et al., 1995a) y recientemente se han detectado en Estados Unidos, México y Chile (Komadina et al., 2014; Nelson et al., 2015).

2.6. Influenza porcina en Suramérica

Pocos estudios epidemiológicos de IP se han reportado para Latinoamérica, destacando la falta de vigilancia porcina en estos países, particularmente en Suramérica. Se cree que muchos linajes diferentes probablemente han estado circulando sin ser detectados durante muchos años debido a la falta de vigilancia y reporte (Mancera et al., 2020). Se han reportado linajes de IAV genéticamente y antigénicamente diferentes en algunos países. Brasil reporta una de las poblaciones de cerdos más grandes del mundo, sin embargo, hasta la aparición del IAV (H1N1) pandémico en 2009, no se consideró que la IP fuera endémica en los cerdos brasileros (Nelson et al., 2015a). Estudios filogenéticos de IAV circulantes en Brasil entre 2009 y 2012 han demostrado la circulación de los subtipos H1N1, H1N2, y H3N2, no obstante, también se detectaron virus reordenados de origen humano que no habían sido

caracterizados previamente y que estaban circulando en estas poblaciones porcinas por varias décadas (Rajão et al., 2013; Nelson et al., 2015a). En Chile y Argentina se han reportado múltiples linajes de IAV H1N1, H1N2 y H3N2 de origen humano, además de eventos de reordenamiento de virus que contienen genes internos del H1N1pdm09 (Pereda et al., 2010, 2011; Cappuccio et al., 2011). En Perú, el virus pandémico fue reportado en 2011 aislado de cerdos de traspatio (Tinoco et al., 2016). En Venezuela se ha reportado la presencia de anticuerpos contra IAV H1N1 y H3N2 en diferentes granjas de cerdos (Ramirez et al., 2005).

2.7. Influenza porcina en Colombia

En Colombia desde finales de los años 70 se ha evidenciado la circulación de IAV en cerdos, sin embargo, al igual que en otros países latinoamericanos, existen muy pocos reportes epidemiológicos de influenza porcina en el país. Evidencia serológica con detección de anticuerpos frente a los subtipos H1N1 y H3N2 (Hanssen et al., 1977) fue en un estudio llevado a cabo en 1977 (Mogollón et al., 2003). Posteriormente, luego de transcurrir unos 40 años se reporta por primera vez la detección molecular y el aislamiento de cepas relacionadas con el subtipo H1N1 pandémico en el país, y otras cepas asociadas al linaje clásico porcino aisladas en el año 2008 (Ramirez-Nieto et al., 2012). De forma similar en otro estudio en 2013 se reportó la detección y aislamiento del subtipo pandémico H1N1 en poblaciones de cerdos en la región de los Llanos entre 2010 y 2012 (Karlsson et al., 2013). Otro estudio realizado en cerdos en el año 2015 en plantas de beneficio de Colombia, demostró la circulación del IAV en cinco regiones principales de producción de cerdos del país con una detección molecular del virus que varió desde 5 a 18% de cerdos infectados con IAV (Flórez et al., 2018). Otro estudio caracterizó el papel específico del IAV en la presentación de complejo respiratorio porcino en Colombia, donde se examinaron 11 granjas porcinas ubicadas en las tres principales regiones productoras del país. Entre los hallazgos se destacó un incremento en la razón de odds para la seroprevalencia de *Actinobacillus pleuropneumoniae*, *Mycoplasma hyopneumoniae*, Circovirus porcino y virus del síndrome respiratorio y reproductivo porcino en las granjas que fueron positivas al IAV pandémico H1N1 (Jiménez et al., 2014). Finalmente, es importante mencionar que a la actualidad en el país no ha sido reportado el aislamiento o detección de IAV del subtipo H1N2 o H3N2 en cerdos.

2.8. Patogénesis de la Influenza Porcina.

La IP sigue el patrón típico de las infecciones virales respiratorias: el virus ingresa principalmente a través de aerosoles, y hay una progresión rápida de la infección en el epitelio de la cavidad nasal y las vías respiratorias (MacLachlan y Dubovi, 2011). Aunque la composición genética de los IAV porcinos varía, la enfermedad clínica y las lesiones inducidas por la mayoría de ellos son esencialmente similares. En los cerdos, los IAV infectan principalmente las células epiteliales que recubren la superficie del tracto respiratorio, desde la mucosa nasal hasta los alvéolos, pero también se han detectado virus en las células epiteliales glandulares asociadas con las vías respiratorias. Debido a que los IAV ingresan por las vías aéreas, estos infectan las células epiteliales que recubren la superficie del tracto respiratorio, causando la manifestación macroscópica de bronconeumonía craneoventral que afecta un porcentaje variable del pulmón. La lesión microscópica característica es la bronquitis necrosante y la bronquiolitis (Janke, 2014).

Los cerdos infectados con IAV desarrollan bronconeumonía que se caracteriza por lesiones pulmonares macroscópicas multifocales bien delimitadas de color rojo violáceo en las áreas craneoventrales de los lóbulos pulmonares conocidas como pulmón en tablero de ajedrez (Sreta et al., 2009). Las lesiones histopatológicas relacionadas IP consisten en daño de las células epiteliales, obstrucción de las vías respiratorias e infiltración de células mononucleares peribronquiales y perivasculares (Sreta et al., 2009; Lyoo et al., 2014). Algunos estudios experimentales han demostrado que infecciones con subtipo H3N2 pueden causar bronconeumonía intersticial (Lyoo et al., 2014). También se puede evidenciar colapso de espacios aéreos adyacentes, neumonía y enfisema (MacLachlan y Dubovi, 2011). Las lesiones microscópicas inducidas por SIV consisten en daño epitelial y en los bronquiolos con proliferación hiperplásica y bronquiolitis obliterante en fases avanzadas de la enfermedad. La infiltración linfocítica peribronquiolar y perivascular de leve a moderada ocurre en casi todos los niveles de las vías respiratorias (Sreta et al., 2009). Estudios experimentales han demostrado que los principales hallazgos histopatológicos luego de infección con el subtipo pandémico H1N1 en cerdos son la degeneración y necrosis epitelial, infiltración de linfocitos en el revestimiento epitelial y lámina propia de pequeños bronquios

y bronquiolos e infiltraciones de linfocitos peribronquiales y peribronquiolares (Valheim et al., 2011).

LA IP se caracteriza por causar anorexia, fiebre, disnea, tos y secreción nasal en los cerdos infectados. La infección generalmente da como resultado una inflamación aguda con una respuesta inmune que limita la propagación viral y promueve la eliminación completa del virus en el pulmón en 7-10 días aproximadamente (Brown et al., 2004). La inmunidad humoral a la infección está principalmente mediada por anticuerpos contra los antígenos de superficie viral hemaglutinina (HA) y neuraminidasa (NA). La HA es responsable de la unión del virus a los receptores que contienen ácido siálico en la superficie de la célula huésped y también de la fusión de la membrana viral y endosómica celular que permite la liberación del genoma viral en el citoplasma (Wesley y Lager, 2006). Los anticuerpos anti-HA evitan la conexión del virus con el receptor y, por lo tanto, bloquean la infección de las células (Pomorska-Mól et al., 2014). La NA cliva el ácido siálico y juega un papel importante en la entrada y liberación de virus (Cox et al., 2004). Los anticuerpos contra NA limitan entonces la diseminación del virus en animales infectados (Pomorska-Mól et al., 2014). Junto con la inmunidad humoral, también la respuesta inmune mediada por células tiene un papel importante en la defensa contra la enfermedad y juega un rol crucial en la eliminación del virus de los pulmones (Platt et al., 2011). Se han realizado muchos estudios en ratones donde se ha demostrado que la combinación de anticuerpos neutralizantes, con la respuesta inmunitaria de la mucosa y las células T es importante para la protección y recuperación de la enfermedad (Tamura et al., 2005). Además de la inmunidad humoral y celular específica, las citoquinas también juegan un papel importante en la inmunopatología de la IP y se ha documentado como la respuesta inflamatoria es responsable de la patogénesis (Van Reeth et al., 2001). El IAV se replica localmente sin generar viremia, por lo que las manifestaciones clínicas dependen fundamentalmente de la liberación sistémica de mediadores químicos con actividad proinflamatoria, como la interleuquina 6 (IL-6), el factor de necrosis tumoral alfa (TNF-α) y la interleuquina 1 (IL-1) (Liu et al., 2016). La respuesta a estos estímulos humorales causa una respuesta inflamatoria exuberante en el parénquima pulmonar que es en ultimas la responsable de la letalidad asociada a la enfermedad (de Jong et al., 2006). La actividad del óxido nítrico sintetasa también contribuye al daño durante la infección (Akaike

y Maeda, 2000). Investigaciones sobre la respuesta inmunitaria e inflamatoria en porcinos durante la fase aguda de la infección causada por IAV H1N1 han demostrado que el virus induce fuertes respuestas proinflamatorias e inmunosupresoras locales y que la concentración de algunas citoquinas y proteínas de fase de aguda esta positivamente correlacionada con la patología pulmonar, por lo cual se plantea que la producción de IL-1b, IL-8, TNF-a, proteína C reactiva y haptoglobina está involucrada en la inducción de lesiones pulmonares durante la infección por IAV (Khatri et al., 2010; Barbé et al., 2011; Pomorska-Mól et al., 2014). Uno de los mecanismos de respuesta cruciales frente a la infección en el hospedero es la producción de los interferones tipo I alfa y beta, no obstante, la proteína viral NS1 bloquea eficientemente este mecanismo permitiendo que el virus evada la respuesta inmune (Killip et al., 2015).

2.9. Diagnóstico de Influenza Porcina.

La IP se caracteriza por la aparición repentina de enfermedad respiratoria altamente contagiosa que puede confundirse con otras enfermedades infecciosas como las causadas por *Actinobacillus pleuropneumoniae* y *Mycoplasma hyopneumoniae* (MacLachlan y Dubovi, 2011). De hecho, las lesiones pulmonares macroscópicas y microscópicas en cerdos con IP pueden ser también comunes en otras infecciones como las causadas por *M. hyopneumoniae* (Thacker et al., 2001). Además del aislamiento viral, para el diagnóstico de IP se utiliza la prueba de RT-PCR gracias a su velocidad, sensibilidad, especificidad y capacidad de automatización (Kim y Poudel, 2013). El aislamiento viral puede ser a partir de huevos embrionados o mediante cultivo celular (Eisfeld et al., 2014). La identificación del subtipo del virus se realiza mediante pruebas de RT-PCR específicas para la detección de la HA y NA (Inoue et al., 2010) y su confirmación se hace por el análisis de secuencias genómicas (Wang et al., 2015). El virus también puede detectarse en muestras de tejido por inmunofluorescencia o por inmunohistoquímica (De Vleeschauwer et al., 2009). Se pueden usar pruebas serológicas (inhibición de la hemaglutinación y ELISA) para detectar anticuerpos contra el virus y diferenciar la infección natural en los animales vacunados (Kim y Poudel, 2013; MacLachlan y Dubovi, 2011).

2.10. Control y prevención de Influenza Porcina.

Actualmente las buenas prácticas de bioseguridad y la vacunación siguen siendo las estrategias principales para prevenir o reducir la transmisión del IAV entre cerdos y de cerdos hacia otras especies. Antes de introducir nuevos animales en una granja, los animales deben cumplir periodos de aislamiento y cuarentena (Torremorell et al., 2012) y se debe verificar por pruebas de laboratorio como la PCR que los animales son negativos a la infección por IAV (Salvesen y Whitelaw, 2021). La vacunación de porcinos con vacunas comerciales es una práctica común que conlleva a la disminución de las lesiones, los signos clínicos y la eliminación del virus (Sandbulte et al., 2015; Vincent et al., 2010). Las estrategias de vacunación actuales tienen varias limitaciones, ya que cuando se usan vacunas completas inactivadas, es difícil distinguir entre los animales vacunados y los infectados (Sridhar et al., 2015). Aunque las vacunas inactivadas contra IP han sido usadas en los Estados Unidos por más de 20 años (Sandbulte et al., 2015), existen concepciones de algunos veterinarios y productores porcinos acerca de que las vacunas contra IAV a veces tienen una baja eficacia en el campo (Lowe, 2008), esto debido a la falta de concordancia entre las cepas vacunales y las cepas circulantes del virus, pero bajo condiciones adecuadas, las vacunas pueden reducir la transmisión del virus (Romagosa et al., 2012). Algunos estudios han demostrado que las vacunas pueden ayudar a reducir la transmisión, pero muchas veces no impiden por completo la transmisión (Van Reeth et al., 2001). Aunque se dispone de información muy limitada sobre la transmisión del IAV en poblaciones de cerdos con diferente estatus inmune, algunos estudios por ejemplo, han evaluado las diferencias en la transmisión del IAV H1N1 en cerdos vacunados y basado en modelos experimentales observaron una reducción estadísticamente significativa en la transmisión viral medida por la tasa de reproducción (Ro) de la infección, en los grupos de animales vacunados en comparación con cerdos no vacunados (Romagosa et al., 2011), sin embargo, más investigaciones son necesarias para dilucidar con mayor profundidad este efecto debido a que la transmisión viral también puede verse afectada según la eficacia de la vacuna entre otros factores (Bikour et al., 1994; Van der Goot et al., 2005). Adicionalmente, para prevenir la transmisión de IAV entre rebaños y granjas porcinas, y reducir la probabilidad de que IP se vuelva endémica, se deben implementar entre otras medidas de bioseguridad, la cuarentena de los cerdos y sistemas sólidos de salud y seguridad para los trabajadores de las granjas (Salvesen y Whitelaw, 2021).

Normalmente en las regiones donde existen vacunas comerciales, las granjas practican la vacunación en cerdas reproductoras, con una mayor frecuencia entre cada ciclo de reproducción (Garin et al., 2015), a fin de reducir la presión de infección en la granja, de prevenir las consecuencias en el rendimiento reproductivo y para transferir anticuerpos maternales a la descendencia a través del calostro (Cador et al., 2016). Debido a que las personas pueden infectar a los cerdos y viceversa, las prácticas de bioseguridad también deben incluir medidas dirigidas a minimizar el riesgo de exposición de las personas a los cerdos y de los cerdos a las personas. Un plan integral de prevención incluye restringir la entrada de visitantes, y control de acceso de personas con síntomas similares a la gripe, de vehículos y de otras especies animales, implementar políticas de cambio de indumentaria y que el personal de la granja este vacunado contra la gripe estacional (Torremorell et al., 2012).

Algunos estudios han investigado los factores de riesgo asociados a la infección en granjas de cerdos. El tamaño de la granja y la densidad de los cerdos en el área alrededor de la granja se han identificado repetidamente como factores de riesgo (Maes et al., 2000; Suriya et al., 2008). También se reconoce que el principal factor de riesgo para la introducción inicial de IP es el movimiento de cerdos infectados a una granja, y se ha documentado que la introducción de virus en una granja se asocia más comúnmente con el ingreso de un animal infectado (Suriya et al., 2008). El movimiento de animales dentro y entre granjas también se ha identificado como un factor de riesgo en varios estudios (Suriya et al., 2008). El contacto cercano entre los cerdos y el número de cerdos por corral han sido también propuestos como factores de riesgo (Maes et al., 2000).

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CAPÍTULO III. USO DE FLUIDOS ORALES PARA EL MONITOREO EFICIENTE DE VIRUS INFLUENZA EN GRANJAS PORCINAS EN COLOMBIA.

En este capítulo se presenta el manuscrito científico como resultado de un estudio realizado para demostrar la efectividad de una técnica de monitoreo del IAV en granjas usando fluidos orales porcinos. El manuscrito se encuentra en revisión en la Revista Colombiana de Ciencias Pecuarias ISSN (online): 2256-2958. Este capítulo contribuye con el desarrollo de los objetivos específicos 1.4.1; 1.4.7 y 1.4.9 de este trabajo de grado.

Use of oral fluids for efficient monitoring of influenza viruses in swine herds in Colombia

Uso de fluidos orales para el monitoreo eficiente de virus Influenza en granjas porcinas en Colombia

Uso de fluidos orais para monitoramento eficiente do vírus Influenza em rebanhos suínos na Colômbia

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3.1. Abstract

Background: Influenza A virus (IAV) surveillance in swine is critical due to the direct impact of the disease in pork industry but also because the major role that pigs can have in the adaptation of new IAV viruses to the mammalian host, and therefore the threat it represents to the public health. Currently there are several diagnostic techniques available to

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detect IAV infection from nasal samples in swine, however, other samples such as oral fluids (OF) have been implemented as novel alternatives for pathogen detection. The OF allow an efficient and feasible detection of diseases at lower cost at a herd level, with lower risk of stress for animals. **Goal:** Describe a surveillance strategy of IAV at the herd level during respiratory disease outbreaks in swine farms in tropical settings using porcine oral fluids. **Methods:** Active surveillance strategy was conducted in five selected swine farms with past records of respiratory disease. Swine OF were collected for IAV testing. An OF sample was described as a pen-based specimen collected from a group of >20 pigs per pen and/or per barn (if they were housed individually but having close contact between them). IAV infection was investigated in OF by rRT-PCR testing, and confirmed by viral isolation in cell culture. **Results:** IAV detection was conducted in five purposively selected farms between 2014-2017. We investigated a total of 18 respiratory disease outbreak events. From the total 1444 OF samples tested, we found 107 (7.4%) positives to IAV by rRT-PCR. Additionally, 9 IAV isolates were obtained, and all were further identified as H1 subtype. **Conclusions:** Results of our study demonstrated how OF can be easily implemented as a novel, user-friendly, welfare-friendly, accurate and cost-effective sampling method for active surveillance and monitoring of IAV infections in swine farms in tropical settings.

Keywords: *Farms; infection; influenza A virus; molecular; RT-PCR; surveillance; swine.*

3.2. Resumen

Introducción: La vigilancia del Virus Influenza A (IAV) en los cerdos es fundamental debido al impacto directo de la enfermedad en la industria porcina, pero también por el papel principal que pueden tener los cerdos en la adaptación de los nuevos virus IAV al huésped mamífero y, por lo tanto, el riesgo que representa para la salud pública. Actualmente existen varias técnicas de diagnóstico disponibles para detectar la infección por IAV a partir de muestras nasales en cerdos, sin embargo, se han implementado otras muestras como los fluidos orales (OF) como nuevas alternativas para la detección de patógenos. El OF permite una detección eficiente y factible de enfermedades a menor costo a nivel de rebaño, con menor riesgo de estrés para los animales. **Objetivo:** Describir una estrategia de vigilancia de IAV a nivel de hato por medio de fluidos orales porcinos durante brotes de enfermedades

respiratorias en granjas porcinas en entornos tropicales. **Métodos:** Se llevó a cabo una estrategia de vigilancia activa en cinco granjas porcinas seleccionadas con antecedentes de enfermedades respiratorias. Se recolectaron OF porcinos para la prueba de IAV. Una muestra de OF se describió como una muestra grupal recolectada de un grupo de> 20 cerdos por corral y / o por establo (si estaban alojados individualmente, pero tenían un contacto cercano entre ellos). La infección por IAV se investigó probando OF mediante rRT-PCR y la confirmación mediante aislamiento viral en cultivo celular. **Resultados:** La detección de IAV se llevó a cabo en cinco granjas seleccionadas intencionalmente entre 2014-2017. Investigamos un total de 18 eventos de brotes de enfermedades respiratorias. Del total de 1444 OF muestras analizadas, encontramos 107 (7.4%) positivos a IAV mediante rRT-PCR. Además, solo se obtuvieron 9 aislamientos de IAV y todos se identificaron además como subtipo H1. **Conclusiones:** Los resultados de nuestro estudio demostraron cómo la OF puede implementarse fácilmente como un método de muestreo novedoso, fácil de usar, amigable con el bienestar animal, preciso y rentable para la vigilancia activa y el monitoreo de infecciones por IAV en granjas porcinas en entornos tropicales.

Palabras clave: Granjas; infección; molecular; porcino; RT-PCR; vigilancia; virus de la influenza A.

3.3. Resumo

Antecedentes: A vigilância do vírus Influenza A (IAV) em suínos é crítica devido ao impacto direto da doença na indústria de suínos, mas também porque o papel principal que os porcos podem ter na adaptação de novos vírus IAV ao hospedeiro mamífero e, portanto, o risco que representa à saúde pública. Atualmente, existem várias técnicas de diagnóstico disponíveis para detectar a infecção por IAV em amostras nasais de suínos, no entanto, outras amostras, como fluidos orais (OF), têm sido implementadas como novas alternativas para a detecção de patógenos. O OF permite uma detecção eficiente e viável de doenças com menor custo em nível de rebanho, com menor risco de estresse para os animais. **Objetivo:** Descrever uma estratégia de vigilância de IAV em nível de rebanho durante surtos de doenças respiratórias em granjas de suínos em ambientes tropicais por meio de fluidos orais suínos. **Métodos:** A estratégia de vigilância ativa foi conduzida em cinco granjas de suínos selecionadas com histórico de doenças respiratórias. Suínos OF foram coletados para teste

de IAV. Uma amostra OF foi descrita como um espécime baseado em curral coletado de um grupo de> 20 porcos por curral e / ou por celeiro (se eles foram alojados individualmente, mas tendo contato próximo entre eles). A infecção IAV foi investigada testando OF por rRT-PCR e confirmada por isolamento em cultura de células. **Resultados**: a detecção do IAV foi realizada em cinco fazendas selecionadas propositalmente entre 2014-2017. Nós investigamos um total de 18 eventos de surto de doença respiratória. Do total de 1444 amostras de OF testadas, encontramos 107 (7.4%) positivas para IAV por rRT-PCR. Além disso, apenas 9 isolados de IAV foram obtidos, e todos foram posteriormente identificados como subtipo H1. **Conclusões:** Os resultados de nosso estudo demonstraram como o OF pode ser facilmente implementado como um método de amostragem novo, amigável, amigável com o bem-estar, preciso e de baixo custo para vigilância ativa e monitoramento de infecções IAV em fazendas de suínos em ambientes tropicais. **Palavras-chave:** *Fazendas; infecção; molecular; RT-PCR; suínos; vigilância; vírus da influenza A.*

3.4. Introduction

Influenza A viruses (IAV) are important cause of acute respiratory disease in animals and humans (Janke, 2013). IAV have segmented RNA genome which facilitate the viral exchange of genetic material when an individual is coinfected with two different subtypes, resulting in new viral strains (E. G. Brown, 2000). Some IAV subtypes are restricted to mammalian hosts (Olsen *et al.,* 2000). Three viral subtypes are commonly detected in swine worldwide (Vincent *et al.,* 2017). Despite evidence of IAV reassortment in other species, swine are designated as key intermediate hosts for the emergence of new viruses (W. Ma et al., 2009). Several studies have demonstrated transmission of IAV from humans to pigs (Adeola *et al.,* 2010; Forgie *et al.,* 2011) and from pigs to humans (Yassine *et al.,* 2009). IAV transmission between these two species often result in emergence of new strains which spread between both populations (Xu *et al.,* 2011). Thus, active surveillance of IAV in these populations should be closely monitored for public health concerns (Kendall P. Myers et al., 2007).

Swine IAV (SIAV) infections have significant impact on the affected herd (Cornelison *et al.,* 2018). IAV affect pig performance, reducing feed conversion ratio, feed

intake and weight gain (Olsen *et al.,* 2002; Myers *et al.,* 2007). IAV disease could quickly disseminate within the herd, but also could be silent and pigs may not show clinical signs of infection (Olsen *et al.,* 2006). Thus, monitoring SIAV in a herd by clinical signs is rarely reliable and usually might result in misdiagnosis of the disease (Janke, 1995). Available sampling methods to detect SIAV infected individuals in a large swine population require high number of resources especially if virus prevalence is low (Muñoz-Zanzi et al., 2000). Pooled sampling has been proposed as a cost-efficient tool for detection of diseases in large populations (Rovira et al., 2008; Cortey et al., 2011). A "pooled sample" such as Oral Fluids (OF), is obtained from combining samples in equal portions (Cameron *et al.,* 2008), or from a group-based sample from different individuals in a pen (Fablet et al., 2017b). Effectiveness of pooled sampling is highly dependent on the dilution effect (Arnold *et al.,* 2009), however, several studies demonstrated a no dilution effect when detecting SIAV in OF using molecular detection methods (Panyasing *et al.,* 2016; Gerber *et al.,* 2017).

The SIAV surveillance have been extensively conducted worldwide but few studies have been reported in Colombia (Ramirez *et al.,* 2012). Some potential limitations include technical and economic challenges when testing a significative number of individual samples from a herd (Corzo *et al.,* 2013). Consequently, SIAV monitoring in pigs could be facilitated using methods that are easy, safe, and cost-effective. OF specimens are efficient and effective for viral surveillance in swine (Panyasing *et al.,* 2016; Fablet *et al.,* 2017). Therefore, we conducted a prospective observational study for SIAV surveillance at herd level using porcine OF, providing proof of an efficient, simple, cost-effective, animal welfare friendly and user-friendly sampling tool for IAV longitudinal monitoring in Colombian pig farms.

3.5. Materials and Methods

3.5.1. Ethic statement

Despite the minimal risk and not invasive sampling method used in this study, the protocol was submitted to the institutional animal care and research committee of the Universidad de Antioquia (approval act N°142). Additionally, the study protocol was reviewed and approved by the institutional committee of the Colombian national swine

producer´s association-PorkColombia (approval act N° 20111501). Additionally, swine producers provided written consent to participate in the study before sample collection. All samples were collected by trained veterinarians. Study was conducted in compliance with local regulations and international guidelines for ethical conduct in the use of animals in research were also followed **(**https://www.apa.org/science/leadership/care/guidelines**)**.

3.5.2. Study design

A list of farms located in the Province of Antioquia was obtained from the Colombian national swine producer´s association-PorkColombia. Candidate sites (owners of these farms) were then contacted by phone to ask willingness of participation and to obtain informed consent for this study. This prospective observational study was conducted in five selected swine farms that reported presence of influenza like illness in the past.

Sample size per farm was estimated for disease detection from pooled samples in a large population using the Epitools epidemiology online calculator (Sergeant, 2014), with the following parameters: Pool size of >20 animals per pool, test sensitivity at pool level of 80% (Romagosa et al., 2012), 95% confidence and an estimated disease prevalence of 10%. A sample of two OF per group was required to have a 90% probability to find at least 10% virus prevalence in the sampled subpopulation. For the purpose of this study an OF sample was described as a pen-based specimen collected from a group of >20 animals per pen and/or >20 animals per barn if they were housed individually but having close contact between them, i.e. breeding herd facility.

Sample collection in the farms was conducted upon request of the owners or farm technicians, once they suspected of respiratory disease outbreaks occurring in a group of pigs in the farm. At the time of the visit, the groups of pigs that were reported of suffering of respiratory disease were inspected and samples were collected at convenience. But other farm production units were inspected to record if there were other groups of animals showing clinical signs of influenza-like illness (sneezing, coughing, nasal discharge, difficulty to breath, lethargy, loss of appetite), however, samples were taken from different pigs including sows, nursery pigs, to breeding and finishing pigs regardless of whether they had symptoms

of respiratory illness or not. The OFs were collected in random groups (of any age) of pigs at each farm (selected at convenience), following the recommendations for this method (Henao-Diaz *et al.,* 2020; Rotolo *T* 2017). When the rope was saturated (chewing time of 30 to 60 min), fluid was extracted by manually squeezing while inside a clean plastic bag. Thereafter, 2 ml of sample was transferred into a tube containing 1 ml of virus transport media (VTM), and transported at 4°C to the laboratory within 8 hours. Samples were then processed or stored at -80°C until use. Additionally, individually housed pigs within same barn (e.g. Breeding females), were sampled hand-holding the rope, and individually rotated in 20 randomly selected pigs within the barn, assuring resampling of animals did not occur. VTM was composed of minimal essential medium with Earle's salts (MEM; Sigma-Aldrich, USA), 2x antibiotic–antimycotic solution [penicillin (300 IU/ml; Sigma-Aldrich, USA), streptomycin (300 lg/ml; Sigma-Aldrich, USA), gentamicin (150 lg/ml; Sigma-Aldrich, USA), amphotericin B (0.75 lg/ml; Gibco, USA)], in addition to gelatin (0.5%; Amresco, USA) and bovine serum albumin (0.5%; Sigma-Aldrich, USA).

3.5.3. rRT-PCR for IAV detection

RNA extraction from OF specimens was done using ZR viral kit (Zymo research, USA) following manufacturer´s instructions. rRT-PCR was performed in a 7500 fast thermocycler (Applied biosystems, USA) using TaqMan Fast Virus 1-Step Master Mix (Applied Biosystems) and specific primers/probe targeting a conserved region of IAV M gene (WHO, 2011). Positive (beta-propiolactone treated pandemic H1N1 virus) and negative controls were included in each run. Running conditions: 1 cycle at 50 °C for 5 min, 1 cycle at 95 °C for 20 s and 40 cycles of 95 °C for 15 s, 60 °C for 60 s. Analysis of amplification curves was done using thermal cycler system software. The baseline was set automatically and cycle threshold was visually set to halfway of the exponential phase of the positive control. Samples with a fluorescence cycle threshold value <39 were considered positive.

RT-PCR assay efficiency and analytical sensitivity (limit of detection) were determined for viral detection from OF samples. Virus spiked OF samples were used to investigate interfering factors in the assay efficiency. Briefly, five OF samples from healthy pigs were tested by adding 50 uL of beta-propiolactone treated pandemic H1N1 virus stock

(1×10^6 TCID 50/ml). Samples were tested in two independent replicates by performing 10-fold serial dilutions. Assay efficiency was calculated by Linear regression analysis of the serial 10-fold dilutions using formula (E) = [10 (1 / slope)] – 1 (Stordeur *et al.*, 2002). Limit of detection for the assay was defined as the highest dilution at which all replicates were positive.

3.5.4. Virus isolation

rRT-PCR IAV positive OF samples were selected for virus isolation. Briefly, confluent monolayers of MDCK cells were prepared in 24-well plates (Costar; Corning, NY, USA). Cell culture media was removed, and monolayers were washed three times with 1X PBS solution (Gibco, Grand Island, NY, USA). Prior to inoculation, sample was filtered using 0.45 um syringe filter (Millipore, Billerica, MA, USA. Viral inoculum was prepared using 300 ul of sample filtrate diluted in 700 ul of infection media (Decorte *et al.*, 2015). Each sample was inoculated into 2 wells (~0.5 ml/well) and then incubated at 37 °C with 5% CO_2 for up to 72 h. Cell cultures were daily observed for cytopathic effects (CPE). If CPE was present, cell culture supernatant was subjected for hemagglutination assay (HA). HA was performed following methods previously described (Killian, 2014). HA-positive cultures were further tested by rRT-PCR for IAV. Cells not showing CPE were exposed to a freeze–thaw cycle (-80 and 37°C) and then tested by HA. Cultures negative for HA were subjected to two additional passages by re-inoculating into fresh confluent MDCK cells. At 72 h after third passage if CPE and HA were negative, samples were considered negative for IAV isolation. Isolates obtained were partially sequenced by sanger sequencing service at a external facility (Macrogen, Korea) and identified using BLAST tool for the Hemaglutinin viral gene (Van den Hoecke *et al.*, 2015).

3.5.5. Statistical analysis

Data were tabulated and classified as positive or negative based on a cycle threshold (Ct) cutoff point of 39. A swine group was classified as IAV positive if at least one pen-based OF sample tested positive by rRT-PCR. Descriptive statistics were obtained for each variable according to their data type. Comparison of infection status between groups (age and farms)

was tested by Chi squared test. All analyses were performed using R studio software v3.5.0 (RStudio Team, 2016).

3.6. Results

Swine farms monitored

A list of 21 candidate farms was obtained from the associates linked the national pork producer's association. From these 10 swine producers accepted to participate in this study and five of these were selected for IAV monitoring. These farms were located in the State of Antioquia (Municipalities of Yolombo, Támesis, Betulia, Caldas and Barbosa) of Colombia (Figure 1). Molecular detection of IAV was carried out in porcine OF collected from January 2014 to December 2017. The main characteristics of these farms included: farrow to finishing facilities, common biosecurity practices (disinfection of facilities, quarantine periods for entering personnel and animals, shower and changing clothes at entry, boots disinfection at barn or pen entry) were applied. The size of the breeding herd per farm varied from 100 to 500 female pigs. Replacement gilts were obtained from the same farm but two farms had external reposition. Some of these farms had documented serology testing for SIAV, but none had records for virus detection by molecular methods. None of the farms vaccinated against SIAV. Environmental conditions, altitude and climate varied from farm to farm, however these factors were not considered for the analysis of this work.

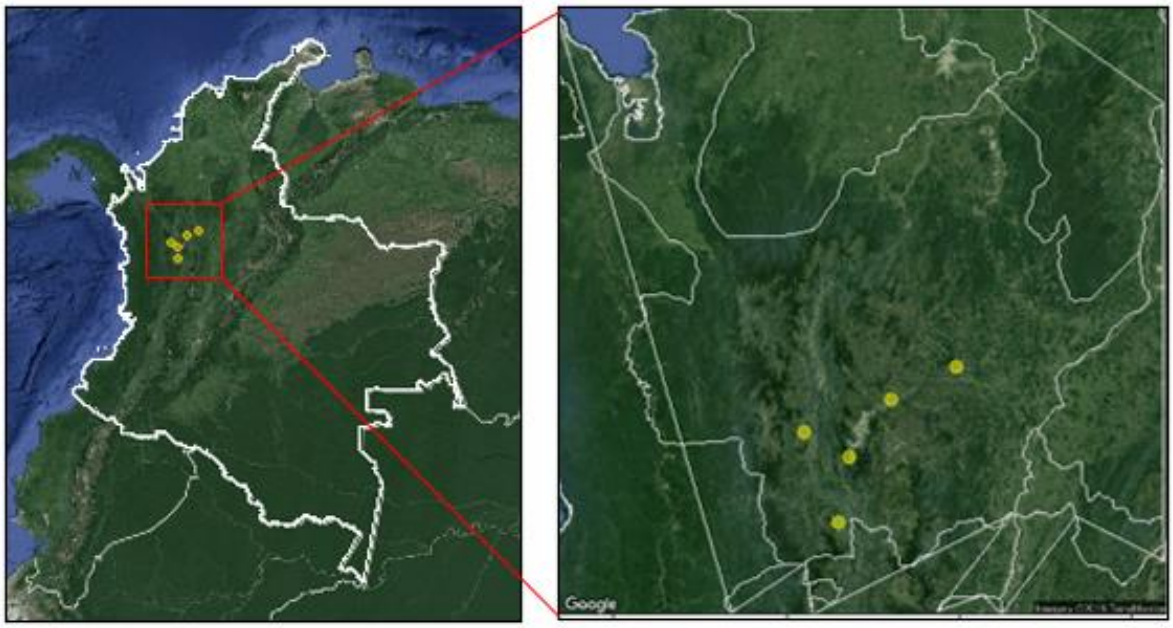

Figure 1. Location of the study sites (pig farms) in Colombia and in the State of Antioquia.

IAV detection and viral isolation

In this study five swine farms were monitored for IAV detection. In overall, from the total (n=1444) OF samples tested, 105 (7.3%) samples were positive to IAV by rRT-PCR. Total number of samples collected at each farm and testing results are summarized in table 1. Furthermore, farm I was monitored two times (August 2015 and March 2016) detecting in total 11 samples positive (12.9%; n=85) to IAV by rRT-PCR. Farm II was monitored three times (October 2015, March and April 2016) detecting in total 14 samples positive (11.3%; n=124) to IAV by rRT-PCR. Farm III was monitored 10 times (October and December 2015, February to September 2016, and February 2017) detecting in total 36 samples positive (5.3%; n=674) to IAV by rRT-PCR. Farm IV was monitored three times (January and August 2014, and February 2015) detecting in total 4 samples positive (7.1%; n=56) to IAV by rRT-PCR. Farm V was monitored eleven times (from February 2016 to February 2017) detecting in total 42 samples positive (8.3%; n=505) to IAV by rRT-PCR.

Table 1. Number of samples collected per year at each farm and rRT-PCR testing results for IAV detection.

Year and farms	IAV rRT-PCR results			
	Negative	Positive	Total	% Positive
2014	**32**	**4**	**36**	**11,11**
Farm IV	32	4	**36**	11,11
2015	**152**	**25**	**177**	**14,12**
Farm I	39	7	**46**	15,22
Farm II	17	9	**26**	34,62
Farm III	76	9	**85**	10,59
Farm IV	20	0	**20**	0,00
2016	**998**	**71**	**1069**	**6,64**
Farm I	37	2	**39**	5,13
Farm II	93	5	**98**	5,10
Farm III	500	27	**527**	5,12
Farm V	368	37	**405**	9,14
2017	**157**	**5**	**162**	**3,09**
Farm III	62	0	**62**	0,00
Farm V	95	5	**100**	5,00
Total	**1339**	**105**	**1444**	**7,27**

We confirmed IAV infection in symptomatic as well as in asymptomatic groups of pigs. IAV positive samples were found in wide range of pigs including sows, breeding herds, nursery and finishing pigs at each farm (Table 2). Also, significative differences ($P < 0.05$) in IAV detection were found between age groups (Breeding, Finishing, Nursery, Sow) and date of sample collection (month) but not between farms (Farm I to IV). Nine viral isolates were successfully obtained from IAV rRT-PCR positive OF samples after cell culture isolation on MDCK cells (table 3). Thus, confirming IAV active infection and circulation in in 4 out of the 5 farms the farms monitored. All IAV isolates were identified as H1N1 pandemic subtype.

Table 2. Number of samples collected per age group at each farm and rRT-PCR testing results for IAV detection.

Farms and age groups	IAV rRT-PCR results		
	Negative	Positive	Total
Farm I	**76**	**9**	**85**
Breeding	8	0	8
Finishing	48	6	54

Nursery	20	3	23
Farm II	**110**	**14**	**124**
Breeding	78	3	81
Finishing	15	0	15
Nursery	17	11	28
Farm III	**638**	**36**	**674**
Breeding	179	2	181
Finishing	246	7	253
Nursery	186	27	213
Sow	27	0	27
Farm IV	**52**	**4**	**56**
Breeding	14	1	15
Finishing	8	0	8
Nursery	15	1	16
Sow	15	2	17
Farm V	**463**	**42**	**505**
Breeding	80	2	82
Finishing	32	0	32
Nursery	182	16	198
Sow	169	24	193
Total	**1339**	**105**	**1444**

Table 3. Number of inoculated samples and virus isolates obtained after IAV cell culture.

	Results for MCDK cell IAV isolation		
Farms	**Negative**	**Positive**	**Total**
Farm I	5	1	6
Farm II	10	3	13
Farm III	22	1	23
Farm IV*	36	0	0
Farm V	32	4	36
Total	**69**	**9**	**78**

*Ct values of positive samples were above 35.

No inhibition factors for the rRT-PCR test were present in the specimen (porcine oral fluid) after investigation using spiked OF samples. Good performance of the testing method was found with a limit of detection of $10^{5.5}$ TCID50/ml observed and a test efficiency (R^2) greater than 99%.

3.7. Discussion

This was a prospective study that provided important information about using OF samples for the detection of IAV in swine herds as an efficient method for disease surveillance and monitoring. This is the first study describing the use of pooled samples as a screening method to monitor IAV in swine farms over time in Colombia. This study showed how to overcome the common diagnostic challenges under field conditions in swine farms, where the testing of a number of individual pigs is limited, the number of pigs per pen differs, and where the true prevalence of IAV within a pen is unknown. Therefore, for our study conditions, we conclude that the monitoring and detection of SIAV from OF pen-based samples in swine herds was successful.

Routinely surveillance of swine diseases has become more important in recent years as part of the health programs and has increased attention after the impact seen from pandemic diseases, however, collecting appropriate numbers of individual samples can be stressful for the pigs, costly and labour-intensive (Gerber *et al.,* 2017). Additionally, individual surveillance and molecular testing of large number of individual samples become a challenge for swine producers. Sampling methods such as OF, overcome this limitation, which allows pools sample testing, greatly reducing costs and giving valuable information at a herd level.

Pen-based OF samples can be also collected in the field by trained personnel demonstrating the easiness of this sampling method. Thus, OF requires minimal training and allow testing of large sample numbers providing a non-invasive and useful approach for active surveillance in swine populations in a cost-effectively manner (Panyasing *et al.*, 2016). The results of our study demonstrated how IAV can be efficiently monitored in swine herds using OF as a screening method. The application of this approach in swine productions in tropical settings can facilitate the detection and monitoring of important pathogen of interest for animal and public health.

Virus isolation from samples such as OF become challenging sometimes. Several factors contribute to it. Among others, virus inactivation may occur by naturally occurring enzymes or other components present in the OF. Salivary proteins and other components in saliva have been found to contribute to significant inhibit influenza viruses in humans (White *et al.,* 2009). Such components have not been examined in porcine saliva but experiments have shown inhibitory activity of OF against H1N1 virus (Hartshorn *et al.*, 2006). However, IAV have been isolated in experimentally spiked swine OF (Decorte *et al.,* 2015) which suggests that the components in these specimens do not completely inhibit infectivity and viral growth, and confirmed in our study with the success on the virus isolation. On the other hand, sensitivity of molecular detection of IAV have been described as higher for OF samples than others sample types (Henao-Diaz *et al.,* 2020).

Interestingly, in our study most of the groups of pigs found infected with IAV, did not show respiratory disease signs of infection. Therefore, if an infected pig do not become symptomatic it makes more difficult the selection and collection of appropriate individual samples for disease testing (Grøntvedt *et al.,* 2011; Buehler *et al.,* 2014), but highlight at the same time how critical is to implement strategies to detect disease at a herd level. The results of our study showed that OF can be used for detection of IAV in swine herds as a routine method whether clinical demonstration of respiratory disease occur or not. In addition, these specimens can be at the same time a good source for testing of other pathogens and other laboratory methods such as viral isolation and subsequent subtyping and sequencing of the virus. The applicability of sampling based on OF for surveillance of infections in pig populations have becoming more frequent and well accepted as a valuable tool for detection of many other important swine pathogens (Trang *et al.,* 2014; Hernández-García *et al.,* 2017; Atkinson *et al.,* 2019; Barrera-Zarate *et al.,* 2019; Henao-Diaz *et al.,* 2020).

The findings of our study have provided valuable information regarding the use of OF for molecular detection of IAV in swine farms in one of the main pork producing regions of Colombia. OF were used to demonstrate the application of a pen-based sampling method as a routine, efficient and cost-effective tool for active surveillance of respiratory viral diseases. Our results also suggested that IAV might be representing a common cause of

respiratory disease in swine farms. Therefore, routine use of pen-based sampling using OF facilitate detection of pathogens at a herd level and contribute as an efficient strategy to monitor viral swine diseases of great importance.

3.8. Declarations

Conflict of interest

The authors declare they have no conflicts of interest in reference to this work.

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Author contributions

Karl Ciuoderis: Writing - original draft, data curation, formal analysis, writing - review and editing. Laura Perez: writing - review & editing, laboratory analysis. Andres Cardona: writing - review & editing, laboratory analysis. Jorge Osorio: writing - review and editing. Juan Hernandez: writing - review & editing.

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CAPÍTULO IV. MANUSCRITO: BIOSEGURIDAD Y DETECCIÓN DEL VIRUS INFLUENZA A EN GRANJAS PORCINAS: ESTUDIO EN COLOMBIA.

Este capítulo corresponde al manuscrito científico como resultado de la investigación entre aspectos de bioseguridad, prácticas de manejo y la prevalencia del Virus Influenza A en granjas porcinas del país. El manuscrito se encuentra en revisión en el Journal Preventive Veterinary Medicine ISSN (online): 0167-5877. Este capítulo contribuye con el desarrollo de los objetivos específicos 1.4.1; 1.4.5; 1.4.6; y 1.4.9 de este trabajo de grado.

Farm Biosecurity and Influenza A virus detection in Swine Farms: A Comprehensive Study in Colombia.

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4.1. Abstract

Several swine viral pathogens cause a significant impact in the pork industry. Biosecurity protocols (BP), and good management practices (MP) are key for protection against diseases in swine herds, as they are intended to reduce the risk of introduction and transmission of infectious diseases into the industry (Amass and Clark, 1999). In this study survey data were collected from 176 pig farms with inventories over 100 sows randomly selected in Colombia. We combined several analysis methods on the survey data obtained to explore relationships

in complex datasets to understand the farm structure, identify clustering patterns of swine farms in Colombia and estimate its association with SI. Two principal dimensions (eigen values >0.2) that contributed to 27.6% of the dataset variation were identified using Multiple Correspondence Analysis (MCA). Farms with highest contribution to dimension 1 were larger farrow-to-finish farms, using self-replacement of gilts that implementing most of the measures evaluated. In contrast, farms with highest contribution to dimension 2 were medium to large farrow-to-finish farms, but that implemented biosecurity in a lower degree. Additionally, two farm clusters were identified by Hierarchical Cluster Analysis (HCA), and the odds of influenza A virus (IAV) detection was statistically different between clusters (OR 7.29, CI: 1.7,66, p=<0.01). Moreover, logistic regression methods were used to estimate the association between variables and IAV detection. Three important variables were associated with higher odds of IAV detection: 1) "location in an area with a high density of pigs", 2) "farm size", and 3) "after cleaning and disinfecting, the facilities are allowed to dry before use". Our HCA results revealed two different clustering patterns of the swine farms, thereby providing a systematic approach to identify relationships between biosecurity, husbandry practices and the SI farm status. More importantly, we provided an approach for the analysis of complex survey data as a baseline to identify gaps on biosecurity and key elements for designing successful strategies to prevent and control swine respiratory diseases such as SI in the swine industry.

Key words: Swine Biosecurity, Husbandry Practices, Swine Farms, Univariable Analysis, Multiple Correspondence Analysis, Hierarchical Cluster Analysis, Generalized Linear Model, Epidemiology, Swine Influenza.

4.2. Introduction

Infectious diseases have a tremendous impact on swine health and production worldwide (VanderWaal and Deen, 2018). Hence avoiding the transmission of infectious agents between herds is important for swine health (Alarcón et al., 2021). Biosecurity is a set of protocols and practices that once implemented can reduce the risk of introduction and transmission of diseases into swine farms (Rojas et al., 2014). Intensive pork production, especially in areas of high densities of farms, rely on strict BP to minimize the impact of infectious diseases on swine production (Alarcón et al., 2021). Therefore, assessing herd disease risks factors can lead to design better BP (Bottoms et al., 2015). Finding novel

strategies to better understand the complex relationships of biosecurity and epidemiology of infectious diseases in animal productions is also highly recommended (Pokludová, 2020).

In the swine industry, epidemiological surveys are used to assess the association between biosecurity, husbandry practices and diseases (Boklund et al., 2004). Analysis of survey data using multivariable methods help to identify gaps on biosecurity in the pig farms (Kouam and Moussala, 2018). These statistical methods allow the extraction of fundamental information regarding the structure of the production systems to understand relationships in complex datasets (Dohoo et al., 1997). However, full understanding of the variability and complexity of biosecurity is not a straightforward process, and it is usually not easy to achieve. Therefore, applying different and complementary methods like multiple correspondence analysis (MCA) and hierarchical cluster analysis (HCA) may facilitate the understanding of BP and MP (as a whole and not as single interventions) and their relationships with disease outbreaks in livestock (Costa et al., 2013).

Influenza A viruses are endemic in swine populations causing Swine Influenza (SI) (Brown, 2000). Swine Influenza is the most prevalent respiratory disease in pig productions worldwide (Almeida et al., 2017). Increase on feed conversion and reduce average daily gain of pigs are the main negative effects of SI (Er et al., 2016). Additionally, IAVs are associated with higher mortality rates of piglets (Alvarez et al., 2015) and increased cost of production (Sandbulte et al., 2015). The epidemiology of IAVs in pig farms is not fully understood. Multiple factors affecting IAV infection in pig farms have been described (López-Robles et al., 2012). Furthermore, the dynamic genome of IAV and its molecular epidemiology in sow herds (Diaz et al., 2017b) and in pigs after weaning (Diaz et al., 2017a) increases the complexity of SI in pigs, highlighting the importance of good biosecurity to reduce the risk of disease transmission between farms.

In Colombia, few information is available regarding SI and very limited studies about IAV epidemiology in Colombian pig farms. IAV was first reported in Colombia in the 70's (Hanssen et al., 1977); while pandemic H1N1 virus (Karlsson et al., 2013) and classical H1 virus (Ramirez-Nieto et al., 2012) were detected during the last decade. Additionally, there is no commercial or autogenous vaccines against IAV available in the country. Understanding the BP and MP and their association with IAV in pig farms is important to find better strategies to reduce the risk of disease transmission. Therefore, the main goal of

our study was to investigate the biosecurity, husbandry practices of Colombian swine farms to identify patterns, relationships, and possible associations with SI. We conducted a survey on 176 swine farms and applied an approach that combined several multivariable analysis methods to explore relationships in complex data to understand the farm structure, identify clustering patterns of swine farms in Colombia and estimate its association with SI. Our results provide for the first time an analysis of complex survey data of pig farms in Colombia, identifying key elements of farm biosecurity and factors associated with IAV detection in these farms in the country.

4.3. Materials and methods

4.3.1. Colombian Swine industry description

According to the available data for the national swine census published by the Instituto Colombiano Agropecuario "ICA" (Colombian animal health authority), about 234 thousand pig productions were registered for year 2017, of which 81% were backyard productions systems and 19% were 'technified' systems or farms producing pigs indoors, and from these 'technified' pig farms 91.6% were farms with inventories of 100 sows or less. A total of 5,331,493 pigs (sows, hogs, piglets and growing pigs) were reported for the country in this year, where 65% of them were held in 'technified' pig farms. Pigs from these 'technified' farms were distributed as follows: 34,2% were pigs until 60 days of age, 28,4% were pigs from 61 to 120 days of age, 26,2% were pigs from 121 to 180 days of age, 9.5% were sows and 1,7% were hogs (ICA, 2017). On the other hand, for year 2019, ICA reported about 240 thousand swine productions, from which one third were backyard systems and about two third were 'technified' farms. Of these 'technified' farms 95.5% were farms with were farms with inventories of 100 sows or less. Also, about 6.7 millions of pigs were reported for the country, of which 94.9% were pigs from 'technified' farms (ICA, 2020). Overall, about more than a half of the pigs (2.9 million) are held in the most pig dense states; Antioquia (1.7 million), Cundinamarca (0.5 million) and Valle del Cauca (0.7 million) (ICA, 2020).

4.3.2. Farm selection

Sampling framework was established from national census of 1397 pig farms registered in the National Association of Pork Producers PorkColombia-Fondo Nacional de la

Porcicultura and the "Farm" was the unit of interest. The inclusion criteria were: (i) geographic location within the area of study, (ii) willingness to participate, (iii) minimum inventory per farm of 100 sows, and (iv) ease of access to the facilities. A list of 370 farms fulfilled inclusion criteria from which a sample size was calculated using the following estimation parameters: 95% confidence level, 5% relative standard error, and 50% proportion for factors (Levy y Lemeshow, 2008). Sample size was proportionally stratified by dividing the number of registered farms into mutually exclusive and non-overlapping groups of sample units (departments of the country).

4.3.3. Farm data and survey information

An epidemiological questionnaire (Supplementary material 1) was designed based on previously published data (Almeida et al., 2017; Baudon et al., 2017; Chamba Pardo et al., 2017), and adjusted after consulting swine veterinarians overseeing the production systems in Colombia. Additionally, considerations were included on the questionnaire after reviews by the National Association of Swine producers board and by international swine influenza experts. Survey is available in Spanish upon request to the corresponding author.

The final survey had 80 questions about BP, farm characteristics and MP. Most questions had multiple choice, but some (n=16) had the option "other" in which the producer could describe a response. To avoid bias during interviews, all questions regarding operating procedures were clarified with study personnel prior to data collection. Additionally, prior to study data collection, a survey pilot test was conducted in three different farms to verify that the content and interpretations of the questionnaire resulted in reliable and valid measurements, however, these pilot data were not included in the data analysis of this study. All survey data were processed anonymously to protect the confidentiality of the participants. The survey data obtained were coded and transcribed by double entry into a database using Epi Info™ software (Dean et al., 2011) and Microsoft Excel (2016).

Selected farms were first classified according to the size of breeding herd into: a) small (100 to 300 sows), b) medium (301 to 1000 sows), and c) large (>1000 sows). Then, farms were classified based on production type into: a) breeding and nursery farms (production of piglets for sale up to a weight of 22-30 kg), b) farrow-to-finish farms (pigs are raised from birth to market weight), and c) genetic core (production only of gilts or boars). Only four genetic

core farms were classified in this category, however, we considered very important for the study as one of the main sources of gilts in the regions, therefore for the study analysis they were included as farrow-to-finish to avoid their exclusion. Farm location was divided in three regions based on geographical place: a) Region one, an area with the 9.51% swine national population, comprising the Colombian states of Córdoba, Sucre, Bolívar, Magdalena, Cesar, Atlántico, La Guajira, and Norte de Santander; b) Region two, an area with the 74.4% of the national swine population, comprising the Provinces of Antioquia, Caldas, Risaralda, Quindío, Valle del Cauca, Cauca, Tolima, and Cundinamarca; and c) Region three , an area with the 16.0% of the national swine population, comprising the Provinces of Santander, Boyacá, Arauca, Casanare, Meta, Caquetá, Putumayo, Huila, Chocó, and Nariño.

4.3.4. IAV sampling, testing and farm characterization

To detect animals infected with IAV, nasal swabs (NS) and oral fluids (OF) were collected from piglets (between 3 and 12 weeks of age), because they are considered a swine subpopulation with higher odds of IAV infection (Cappuccio et al., 2017). The number of NS samples collected at each farm was calculated to estimate the prevalence of infection at the subpopulation level using an imperfect test (Humphry et al., 2004). A total of 40 NS were randomly collected at each farm to detect at least one positive sample assuming a within-farm prevalence of 13% (Karlsson et al., 2013) or higher, with a 95% confidence and a maximum error of 15%, and with a rRT-PCR test that offered a specificity and sensitivity of 95% and 95% respectively (Shu et al., 2011). In addition, two OF samples were also collected as a pooled sample from each sampled pen with 95% probability of detecting IAV prevalence of 0.13 (Christensen and Gardner, 2000) and with 80% of sensitivity and 95% specificity for the rRT-PCR test (Romagosa et al., 2012). Sample collection was performed following recommendations previously described (Detmer et al., 2011; Romagosa et al., 2012). OF were collected at each farm from groups of pigs in four different pens of at least 30 pigs per pen. Farms had at least 6 pens per barn where the sampled pigs were housed. Ropes were hanged in opposite points of the pen and OF were collected simultaneously after the ropes were saturated (chewing time of 30 to 60 min). All samples were transported within 12 to 36 hours at 6-10°C (using gel packs) to the lab for testing or stored (-80°C) until processing. Temperature of the sample's transporting container was verified upon reception at the

laboratory. For purposes of this study, we use the term “herd” to mean any group or aggregate of pigs within a farm. Therefore, the individual sites of a multi-site production or the whole aggregate of sites can be defined as a herd.

Viral RNA extraction from NS and OF samples was performed using ZR viral kit (Zymo® research, USA) following manufacturer instructions. Universal IAV molecular detection of matrix gen was carried out with real time reverse transcriptase polymerase chain reaction (rRT-PCR) according to previous methods (Shu et al., 2011). Briefly, Fast One Step rRT-PCR kit (Applied Biosystem®, EE.UU.) was used with 0.6 uM of each primer (InfA Forward 5´-GAC CRA TCC TGT CAC CTC TGA C; InfA Reverse 5´-AGG GCA TTY TGG ACA AAK CGT CTA) and 0.2 uM of probe (InfA Probe 5´-GC AGT CCT CGC TCA CTG GGC ACG). Then, 3 uL of extracted RNA were added to 20 uL final reaction volume. Cycling conditions were 50°C x 5 min, 95°C x 20 seg, followed of 40 cycles of 95°C x 15 seg and 60°C x 1 min. An ABI 7500 Fast (Applied Biosystem®, EE.UU.) thermocycler was used. To increase testing capacity NS (same group of pigs and type of sample) from the same farm were tested in pools of 10 per pool (Van et al., 2012) and if a pool was rRT-PCR positive, then samples composing the pool were tested individually.

4.3.5. Data analysis

A farm was classified as IAV positive if at least one sample (either NS or OF) resulted positive by rRT-PCR. We use descriptive statistics to summarize data. Categorical data were cross-tabulated and quantitative variables were categorized. Differences on IAV detection on NS and OF was evaluated using Chi-square test. Tabular methods were used to distribute the number of observations per category. Initial analysis utilized simple non-parametric univariable methods using Chi-square test was applied to identify those variables with some evidence of association with IAV detection (using a P value of less than 0.2 to suggest some association). All variables identified in the previous stage were added to a Bayesian logistic regression model. Several multivariable statistical methods were used to process and analyze the data and to estimate associations with IAV detection at the farm level. Prior to analysis, the number of variables with missing data was assessed using an algorithm to identify missing values and outliers (Wilkinson, 2018), once identified, data were inspected to fix any

possible data entry or measurement errors (Kwak and Kim, 2017). Missing values in the dataset were handled by imputation analysis using a previously reported method using the 'MIMCA' procedure in the 'missMDA' package for R (Audigier et al., 2017). Initial data processing was conducted using Microsoft® Excel, and all further data processing and analysis was conducted using R and RStudio v3.5.0 (RStudio Team, 2020) using the packages readxl, viridis, ggplot2, MASS, FactoMineR, missMDA, Amelia, factoextra, ggpubr, corrplot, VarSelLCM, epitools, chest, nlme, caret, DAAG, rcompanion, HDoutliers, chest, lmtest, questionr, magrittr, arm and interplot. Bonferroni-corrected t-test was conducted to identify outliers using 'outlierTest' function in the 'car' package for R.

4.3.6. Multiple correspondence analysis (MCA)

MCA was used for data exploration and further statistical analysis to estimate relationships between the categorical data (Husson et al., 2010). The purpose of the MCA is to identify the most important variables, graphically representing the results and suggesting relationships as principal dimensions during exploratory data analysis (Lyra et al., 2010). A stepwise pre-selection approach for variable selection was followed before final analysis (Bergman et al., 2019). Data set was divided into different subgroups containing similar variables (farm characteristics, management practices and biosecurity variables) and these sets were preprocessed separately using MCA. Square cosine criterion "cos2" (> 0.2) was used to select the most representative variables based on their quality during each preprocessing step (Bergman et al., 2019). Chosen variables were retained and a second MCA was constructed until the final data set was obtained. Inertia (eigenvalue >0.2) and Cronbach's alpha score (Costa et al., 2013) were used to define the number of dimensions to retain in the final MCA. Results of the first two dimensions were plotted to illustrate relationships between variables as MCA principal dimensions. The variables selected and retained in the final MCA were the most informative according to their contribution to the characterization of the farms. MCA was performed using the 'MCA' procedure in the 'FactoMineR' package for R.

4.3.7. Hierarchical clustering analysis (HCA)

In addition to exploring the data set to identify graphical patterns, MCA was also used as a method for classification purposes. MCA results were used subsequently to perform a HCA (Handl et al., 2005). The clustering analysis with object scores method was used to identify

groups sharing similar characteristics within each of the identified dimensions from the MCA, which also aimed to discover an a priori unknown partition among the pig farms. In this sense, we applied a statistical method to classify the farms into clusters based on the level of similarity within and between members of different clusters, thus the relevant clustering trend of the data was obtained by HCA (Husson et al., 2010; McShane et al., 2002). To evaluate the quality of clustering results, a validation method was performed for determining the number of clusters of the model using a partition-based algorithm by the BIC criterion (Guo et al., 2017). HCA was performed using the 'HCPC' procedure in the 'FactoMineR' package for R. Hypergeometric test was used to compare the distribution of the variables and to identify which subcategories were overrepresented or underrepresented in the characterization of each cluster (Husson et al., 2010).

4.3.8. Multivariate regression analysis

After data exploration and univariable analysis of all predictors of IAV detection, we applied a logistic regression analysis using a logistic regression model (GLM) to analyze the potential factors associated with IAV detection in the swine farms. Because the unit of analysis was the farm, it was assumed that all samples from pigs in that farm were nested within it. The IAV infection status of the farm was defined as the dependent variable. In order to build the GLM, an identification of a priori variables was made using statistical and non-statistical criteria (Hosmer et al., 2013). All variables that were plausible for the presence of the virus or that had clinical, epidemiological or biological relevance were considered, with special attention to those IAV risk factors reported previously on the literature. Building strategy for GLM was conducted in three steps. In the first step, a selection of the candidate variables was made, considering the importance of the variables according to multiple criteria (scientific or biological) under the concept of purposeful selection to maximize the control of confounders. Regarding the statistical criteria, a bivariate analysis by the $\chi 2$ test of independence was performed to identify an association ($p < 0.25$) between the frequency of IAV positive and negative farms in the presence of the variable of interest. Additionally, variables that most contributed to the inertia in the MCA solution were also considered. In the second step, GLM was built with all retained variables from first step and then selection of variables to be retained followed a stepwise approach (Zhang, 2016). Model selection was

based upon a manual and automatic stepwise removal process according to their Wald test P values, and a likelihood ratio test was used to identify possible contribution to the model (with a P value of 0.05 or less suggesting some contribution). Variables not contributing to the model ($p>0.05$) were excluded. Confounding was assessed by monitoring coefficients ($\Delta\beta$) of other variables before and after variable removal, with a change of 20% or more suggestive of possible confounding (Ciaburro, 2018). If a change of >20% on $\Delta\beta$ was observed after any variable exclusion, the variable was a potential confounding variable and then returned to the model (Thorpe, 2017). Finally, plausible interactions among the main variables were evaluated by automatic stepwise process using the 'stepAIC procedure in the 'MASS package for R, adding interaction terms to the model and using the likelihood ratio test (p-value ≤ 0.1) as suggestion of a significant effect (Mastin et al., 2011). After the evaluation of interactions, the best fit model was selected by AIC. Post-hoc test of best fit model was performed using Tukey's Honestly Significant Difference (Tukey's HSD) post-hoc test. If post-hoc test was not significant, the interaction was removed from the final model (Helios, 2015).

Model diagnostics were conducted (Vakhitova and Alston-Knox, 2018). Variables were then removed sequentially from the final model, using a likelihood ratio test of 0.05 or less to suggest model contribution. This cycle of inclusion/exclusion of variables continued until the best-fitted model was obtained. The fit of the final model was assessed using Pearson's $\chi 2$ test ($p <0.05$), the proportion of deviance (%) and the Pseudo-R2 coefficient of determination (Mangiafico, 2015). Additionally, estimation of phi coefficient (phi ≤ 1) was applied to identify overdispersion in the main GLM (Vakhitova and Alston-Knox, 2018). Variance inflation factor (VIF) was also calculated to assess multicollinearity of variables. Finally, adjusted odds ratios (ORs) were used to estimate the association of IAV infection and selected variables or potential risk factors (Dohoo et al., 2009).

4.3.9. Ethics Statement

This study was conducted in accordance with international ethical guidelines and standards for the use of animals in research, under the approved protocol by institutional review board of the National Swine producers Association of Colombia (approval act 20111501).

Additionally, informed consent was obtained from all swine farm owners participating in this study. All data were used guaranteeing protection and confidentiality of information.

4.4. Results

Farm data collection. One hundred and seventy-six farms out of 187 (94.11%) farms selected from the census agreed to participate in this study. Data and sample collection started in October 2016 and ended in July 2017. Distribution of farms sampled by region, state, and results of IAV detection are summarized in Table 1. The total sow inventory in the farms sampled (n= 75,665) represented 34.8% of the total national inventory of sows (n=216,837) registered in 2017. Overall, we observed a wide variability of the BP implementation in the farms sampled. For example, applying disinfection to the facilities after washing was one of the most implemented practice, observed in most farms (95.9%), while down time for new gilt introduction was one of the least implemented practice, observed only in 13.4% of the farms. The survey variables and frequencies found in the farms evaluated are summarized in Table 2.

Table 1. Location and influenza A virus (IAV) status of 176 swine farms that participated in a biosecurity and husbandry practices survey in Colombia in 2016-17. Farms were tested for IAV using RT-PCR on nasal swabs (NS) and oral fluids (OF).

Zone	State	Negative farms	Positive farms	Total farms	Total NS collected	Total OF collected	IAV Positive NS	IAV Positive OF
1	Atlántico	4	2	6	200	40	4	4
	Bolivar	2	0	2	80	16	0	0
	Cordoba	1	0	1	40	8	0	0
	Magdalena	3	0	3	120	24	0	0
	Norte de Santander	1	0	1	40	8	0	0
2	Antioquia	45	20	65	2779	560	84	58
	Caldas	8	2	10	360	72	7	2
	Cundinamarca	22	5	27	1088	216	25	17
	Quindío	2	4	6	239	47	20	11
	Risaralda	3	1	4	200	40	9	4
	Tolima	3	1	4	160	32	0	3
	Valle del Cauca	15	18	33	1358	249	94	42
3	Boyacá	4	1	5	240	48	0	1
	Caquetá	1	0	1	40	8	0	0

	Cauca	0	3	3	120	24	34	9
	Huila	0	1	1	40	8	1	3
	Meta	2	1	3	120	24	10	3
	Nariño	1	0	1	40	7	0	0
Total number of farms		**117**	**59**	**176**	**7264**	**1431**	**288**	**211**

Table 2: Frequencies of biosecurity measures and husbandry practices of 176 swine farms surveyed in Colombia in 2016-17. Variables and categories included in the study analysis, and their observed frequencies in the farms surveyed. Variables in bold italic were associated with swine influenza farm status upon univariable analysis ($p<0.25$)

I. Farm characteristics			
Code	Variable	Categories	Frequency
V1	***Farm location based on regional pig densities***	≥10 pigs/km^2	114 (64.8%)
		<10 pigs /km^2	62 (35.2%)
V2	Near (≤5 km) to other farms	Yes	87 (49.7%)
		No	88 (50.3%)
V3	Farm type	Breeding and nursery	44 (25.0%)
		Farrow-to-finish	128 (72.7%)
		Genetic core	4 (2.3%)
V4	***Number of production sites***	One site	80 (46.8%)
		Two sites	40 (23.4%)
		More than two sites	51 (29.8%)
V5	***Farm size***	Small (100 to 300 breeding females)	106 (61.6%)
		Medium (301 to 1000 breeding females)	52 (30.2%)
		Large ((>1000 breeding females)	14 (8.1%)
V6	***Total animal inventory***	Less than 2500 pigs	106 (60.2%)
		More than 2500 pigs	70 (39.8%)
II. Biosecurity - Infrastructure			

V7	Floor	Concrete or washable material	169 (97.7%)
		Other	4 (2.3%)
V8	Quarantine area independent of production	Yes	109 (63.0%)
		No	33 (19.1%)
		Do not have	31 (17.9%)
V9	***Perimeter barrier***	Yes	159 (90.3%)
		No	17 (9.7%)
V10	Bird nets in swine facilities	Yes	59 (36.0%)
		No	105 (64.0%)
III. Biosecurity - Cleaning and disinfection			
V11	Access system for personnel entry	Clothing changing only	5 (2.9%)
		Shower, clothing and shoes change	116 (66.7%)
		Clothing and shoes change	43 (24.7%)
		None	10 (5.7%)
V12	Use of access system is mandatory	Yes	137 (81.1%)
		No	32 (18.9%)
V13	***Disinfection system for vehicles at entry***	Spray arch	48 (29.1%)
		Wheel bath	7 (4.2%)
		Backpack pump sprayer	95 (57.6%)
		None	15 (9.1%)
V14	Cleaning protocol between vehicle usage	Washing	129 (76.8%)
		Washing and disinfection	32 (19.0%)
		None	7 (4.2%)
V15	***Record vehicle disinfection***	Yes	129 (73.3%)
		No	47 (26.7%)
V16	***Record disinfection of facilities***	Yes	115 (65.3%)
		No	61 (34.7%)
V17	***Apply disinfection to the facilities after washing***	Yes	164 (95.9%)

		No	7 (4.1%)
V18	Down time of the facility after cleaning and disinfecting	Two days or less	19 (11.5%)
		Three days	43 (26.0%)
		Over four days	89 (53.9%)
		None	14 (8.5%)
V19	***Quality of water for animal consumption is verified***	Yes	124 (78.0%)
		No	35 (22.0%)
V20	***After cleaning and disinfecting, the facilities are allowed to dry before use***	Yes	134 (92.4%)
		No	11 (7.6%)
IV. Biosecurity - Animal movements			
V21	Mixture of pigs from different origins	Yes	21 (12.8%)
		No	143 (87.2%)
V22	Other animals within the pig farm	Poultry and cattle	17 (9.7%)
		Poultry	2 (1.1%)
		Cattle	91 (51.7%)
		Horses	8 (4.5%)
		Sheep / Goats	1 (0.6%)
		None	57 (32.4%)
V23	***Other animals have access to the swine facilities***	Yes	44 (27.0%)
		No	119 (73.0%)
V24	Source of gilts in the last semester	External (from other farms)	79 (46.7%)
		Internal (from same farm)	80 (47.3%)
		None	10 (5.9%)
V25	Origin of gilts in the last semester	Only one source	99 (83.9%)
		Multiple sources	19 (16.1%)
V26	Farm of gilt source	Genetic core	106 (77.4%)
		Commercial farm	16 (11.7%)
		Imported	3 (2.2%)
		Other (animal fair - market)	12 (8.8%)

V27	***Quarantine time for new gilts***	20 days	17 (13.4%)
		20 to 30 days	33 (26.0%)
		More than 30 days	77 (60.6%)
V28	Wash and disinfects quarantine area between batches	Yes	52 (29.7%)
		No	123 (70.3%)
V29	***Wash and disinfects gestation barn between groups***	Yes	37 (21.1%)
		No	138 (78.9%)
V30	Wash and disinfects nursery rooms between groups	Yes	83 (47.4%)
		No	92 (52.6%)
V31	Wash and disinfects areas between groups of growing pigs	Yes	100 (57.1%)
		No	75 (42.9%)
V32	Wash and disinfects area between groups of finishing pigs	Yes	72 (41.1%)
		No	103 (58.9%)
V. Biosecurity - Transport and personnel			
V33	***Independent vehicle to transport animals and feed***	Yes	101 (82.8%)
		No	21 (17.2%)
V34	***Record entry of vehicles***	Yes	132 (78.1%)
		No	37 (21.9%)
V35	***Specialized vehicles to transport animals***	Yes	130 (76.9%)
		No	39 (23.1%)
V36	***Specialized vehicles to transport feed***	Yes	121 (73.3%)
		No	44 (26.7%)
V37	Type of vehicle to transport animals	Metal bodywork vehicle	75 (43.6%)
		Wooden bodywork vehicle	92 (53.5%)
		Other	5 (2.9%)

V38	Control access of visitors to the farm	Yes	162 (95.9%)
		No	7 (4.1%)
V39	***Exclusive personnel to each farm section***	Yes	146 (84.4%)
		No	27 (15.6%)
V40	Down time for visitors before entering to the facilities	24 hours	26 (15.2%)
		48 hours	86 (50.3%)
		72 hours	46 (26.9%)
		None	13 (7.6%)
V41	***Records entry of personnel***	Yes	129 (78.7%)
		No	35 (21.3%)
V42	***Exclusive provision for staff and visitors***	Yes	155 (92.8%)
		No	12 (7.2%)
V43	Vehicles for exclusive use on the farm	Yes	94 (66.2%)
		No	48 (33.8%)
VI. Biosecurity - Health			
V44	***Flies control program***	Yes	162 (94.7%)
		No	9 (5.3%)
V45	Rodent control program	Yes	164 (95.3%)
		No	8 (4.7%)
V46	***Medication and deworming protocols***	Yes	133 (82.6%)
		No	28 (17.4%)
V47	***Record biosafety programs***	Yes	144 (81.8%)
		No	32 (18.2%)
V48	***Record health care programs***	Yes	127 (72.2%)
		No	49 (27.8%)
VII. Management practices			
V49	Ventilation system	Natural	151 (85.8%)
		Mechanic	3 (1.7%)

		Other	22 (12.5%)
V50	Frequency of technical assistance	Permanent	34 (20.6%)
		Weekly	54 (32.7%)
		Biweekly	25 (15.2%)
		Monthly	52 (31.5%)
V51	Type of professional providing technical assistance	Veterinarian	145 (84.8%)
		Animal science	11 (6.4%)
		Agronomist	6 (3.5%)
		Other	3 (1.8%)
		None	6 (3.5%)
V52	***Frequency of training of technicians and operators***	Annual	11 (6.8%)
		Biannual	41 (25.3%)
		Quarterly	101 (62.3%)
		None	9 (5.6%)
V53	Tail docking in piglets	Yes	169 (90.4%)
		No	18 (9.6%)
V54	***Breeding system***	Natural breeding	7 (4.4%)
		Artificial insemination	151 (95.6%)
V55	***Some method of castration (chemical or physical)***	Yes	41 (24.4%)
		No	127 (75.6%)
V56	Type of food supplied	Feed	170 (98.3%)
		Farm-own made mix	3 (1.7%)
V57	***Feeding system***	Manual	124 (73.8%)
		Automatic	37 (22.0%)
		Other	7 (4.2%)
VIII. Other supplementary variables			
V58	Geographical location or region	Zone One (Córdoba, Sucre, Bolívar, Magdalena, Cesar, Atlántico, La Guajira, Norte de Santander)	13 (7.38%)
		Zone Two (Antioquia, Caldas, Risaralda, Quindío, Valle del Cauca, Tolima, Cundinamarca)	149 (84.6%)

		Zone Three (Santander, Boyacá, Arauca, Casanare, Meta, Caquetá, Putumayo, Huila, Cauca, Nariño)	14 (7.95%)
V59	Percentage of response to the questionnaire	Answered more than 90% of the questions (High)	148 (84.1%)
		Answered between 70 and 90% of the questions (Medium)	26 (14.8%)
		Answered less than 70% of the questions (Low)	2 (1.1%)
IAV	Influenza virus infection status of the farm	Positive	59 (33.5%)
		Negative	117 (66.5%)

Note 1: For some variables, there may be additional response options that were not selected. These categories are not presented in the table.

The demographic and general characteristics of farms participating in the study are presented in Table 2 (section I and VIII). Approximately three third (84.6%) of the farms were in the same geographical location (zone two) composed by seven states, including the most important pork producing areas in the country. About two third (64.8%) of the farms were located in a region with pig densities ≥10 pigs/km^2. Most participating farms were small and farrow-to-finish (61.6% and 72.7% respectively). There was no significant difference in the proportion of herd sizes reported for each farm type ($P > 0.05$). Almost half of the producers interviewed (49.7%) reported residing in close proximity (<5 km) to other pig farms.

Biosecurity practices of participating farms are shown in Table 2. During the univariate analysis, farm size was significantly associated with the following variables: farm type (V3), total animal inventory (V6), entry protocol (V11), disinfection system for vehicles at entry (V13), access of other animals to the swine facilities (V23), independent vehicle to transport animals and feed (V33), specialized vehicles to transport feed (V36), exclusive personnel to each farm section (V39), down time for visitors before entering to the facilities (V40), frequency of technical assistance (V50) and influenza virus infectious status (IAV). Additionally, type of farm was significantly associated with the following variables: farm location based on regional pig densities (V1), farm type (V3), total animal inventory (V6),

bird nets in swine facilities (V10), Farm of gilt source (V26), wash and disinfects nursery rooms between groups (V30), wash and disinfects area between groups of finishing pigs (V32) and feeding system (V57).

Biosecurity and management practices related to animal movements are summarized in Table 2 (section IV). Approximately 18% of farmers reported not having any quarantine practices in place for incoming pigs, compared to 63% of farms having quarantine area independent of the production facility. Above a third (46.7%) of all producers did not maintain a closed breeding herd, regularly introducing new gilts to the main herd. The majority of producers (>80%) introduced new gilts from one source of origin. Regarding sources of newly introduced gilts in the previous 6 months, genetic core farms seemed to be an important source for all producers. The proportion of mixed farming practices (keeping domestic animals in addition to pigs) was impressively common (67.6%) among all farmers interviewed (Table 2, section IV). However, large farms were less likely to keep other domestic animals than medium and small ($P < 0.05$).

Cleaning and disinfection practices and measures related with transport and personnel are summarized in Table 2 (section III). Majority (76.9%) of farmers reported using specialized vehicles to transport animals and half of the farmers used wooden bodywork vehicle to transport of the pigs which were in a majority (66.2%) of exclusive use on the farm. Washing rather than washing and disinfecting was the most common (76.8%) practice applied for vehicle hygiene after usage and using a backpack pump sprayer was the most common (57.6%) disinfection system for vehicles at entry. In the majority (95.9%) of farms a disinfection method was applied after washing of the facilities.

Health practices and management practices applied are summarized in Table 2 (section VI and VII). Most of the farmers (>70%) reported implementation of pest control, deworming, biosafety and health care programs. The majority (>80%) of farms implement natural ventilation system, manual feeding, artificial insemination, provide commercial formulated food or feed to animals, apply tail docking in piglets and depend on a veterinarian professional for technical assistance.

Finally, missing data were evaluated using a diagnostic tool from the Amelia package for R software (Honaker et al., 2011). We observed that 5% of total data collected were missing and only three of the consulted variables (V25 “Origin of gilts in the last semester”; V26 “Farm of gilt source”; V27 “Quarantine time for new gilts”) were found with more than 20% of missing values. The highest percentage of missing data found was 33%. To handle missing values a previously reported multiple imputation method for categorical variables was implemented (Audigier et al., 2017) before further data analysis. Additionally, after dataset inspection, no outliers’ values were identified.

IAV testing and characterization. We tested a total of 8,375 samples (6,999 NS and 1,378 OF) from 176 farms and detected IAV by rRT-PCR in 33.55% (n=59) of the farms. Positive farms were widely distributed within the main pork producing regions in the country (Figure 1). Furthermore, the percent of positive samples at the farm level ranged from 2.08% to 39.58%. We also found significantly (p-value <0,05) less positive NS (3.93%, 275 out of 6,999) than OF (11.03%, 156 out of 1,378), indicating that OF were more sensitive for IAV detection at the population level than NS.

Multiple correspondence analysis. MCA was used to estimate relationships between 57 qualitative variables that included farm characteristics, herd MP, and BP. MCA solution expressed the amount of variation (inertia) for these relationships between several categorical variables. The cos^2-criterion ($cos^2 > 0.2$) was used to select representative variables in each of the pre-processing steps on the MCA, where 34 out of the 57 variables were preselected according to their contribution ($cos^2>0.2$) to the inertia (Figure 2), but a total of 23 variables in addition to one supplementary variable (region of location) were included in the final MCA (Table 3). Inertia (eigenvalue >0.2) was used to define the number of dimensions to retain in the final MCA. Relationships between the most important variables to the inertia were mainly expressed in 10 dimensions (Figure 4), which accounted for 63% of the variance of the data set but main data analysis was performed taking into account the first two dimensions, which accounted for more than 27% of the variance of the data set (27.6% cumulative variance). The first dimension explained 20.1% of the inertia and the second the 7.5%. Variables and

their categories that most contributed to the inertia in the MCA are showed in Figure 3. The five variables that most contributed to the creation of the first five dimensions were: for dimension 1 "(V41) records entry of personnel= no", for dimension 2 "(V29) wash and disinfects gestation barn between groups= yes", for dimension 3 "(V14) cleaning protocol between vehicle usage= wash and disinfect", for dimension 4 "(V35) Specialized vehicles to transport animals=No", for dimension 5 "(V40) down time for visitors before entering to the facilities =72h". Final MCA was performed on dataset before and after data imputation, obtaining similar results (26.7% cumulative variance for the first two dimensions on the MCA performed on the dataset with missing values), however dataset with imputation was selected to reduce bias and also because work better for the MCA method of the r software package used.

Table 3. Variables (names and meanings) included in Multiple Correspondence Analysis computed from biosecurity and husbandry practices survey data collected on 176 swine farms in Colombia in 2016-17.

Variable name	Variable code	Variable meaning
Total animal inventory	V6	Total number of pigs (piglets, breeding sows, hogs and growing-finishing) housed in the farm
Access system for personnel entry	V11	Describe the protocol followed by visitors and workers "physical barrier" before entering to the farm
Use of access system is mandatory	V12	Describe whether or not the application of the entry protocol was enforced to visitors and workers before entering to the farm
Cleaning protocol between vehicle usage	V14	Describe the method of cleaning applied to vehicle after usage
Apply disinfection to the facilities after washing	V17	Describe whether or not disinfection was applied to the facilities after washing
Down time of the facility after cleaning and disinfecting	V18	Describe the number of days waited before using again the facility after cleaning and disinfecting
After cleaning and disinfecting, the facilities are allowed to dry before use	V20	Describe whether or not before using again the facility after cleaning and disinfecting
Other animals have access to the swine facilities	V23	Describe which animals have access to the swine facilities
Wash and disinfects quarantine area between batches	V28	Describe whether or not quarantine area is washed and disinfected between batches
Wash and disinfects gestation barn between groups	V29	Describe whether or not gestation area is washed and disinfected between batches

Wash and disinfects nursery rooms between groups	V30	Describe whether or not nursery area is washed and disinfected between batches
Record entry of vehicles	V34	Describe whether or not a registry of vehicles that have entered to the farm is kept
Specialized vehicles to transport animals	V35	Describe whether or not specific vehicles are used to transport animals
Specialized vehicles to transport feed	V36	Describe whether or not specific vehicles are used to transport feed
Exclusive personnel to each farm section	V39	Describe whether or not the farm workers have specific assignation to a farm section
Down time for visitors before entering to the facilities	V40	Describe the time (in days) enforced for visitors recently coming from another farm
Records entry of personnel	V41	Describe whether or not a registry of people that have entered to the farm is kept
Exclusive provision for staff and visitors	V42	Describe whether or not people have to share protective equipment (boots, work clothes, etc) with others while working or visiting in the farm
Flies control program	V44	Describe whether or not the farm has an active control program for flies
Medication and deworming protocols	V46	Describe whether or not the farm has routine medication and deworming protocols
Breeding system	V54	Describe the type of breeding system used
Influenza virus infection status of the farm	IAV	Influenza virus infection status of the farm based on RT-PCR testing results
Supplementary - State	Region	Describe the zone where the farm was located based on the political-administrate division of the country

Hierarchical clustering analysis. Results from the final MCA solution (23 variables plus one supplementary variable) were subsequently used to perform an agglomerative hierarchical clustering analysis (HCA). Regarding HCA, the partitioning of the tree clustering resulted in a selection of two clusters (Figure 5). Description of the most influencing variables in clusters selection of the HCA are summarized in table 4. Model selection was performed according to the BIC criterion and variable selection using VarSelLCM package showed that 18 (78.26%) of the 23 variables included (plus one supplementary variable) were relevant for clustering. The most discriminative variable on the cluster selection was “records entry of personnel (V41)” followed by “record entry of vehicles (V34)”. Hypergeometric test was significant with $p < 0.05$ and no over or under-representation of the data for each category was observed. In total, 85.2% (n=150) of the farms belonged to cluster 1 and 14.8% (n=26) of the farms belonged to cluster 2 (Table 5).

Clustering patterns of data were represented on a factor map (Figure 6). As described in table 5. Cluster 1 was characterized by farrow to finish farms located in region 2 with more than two production sites while in cluster 2 farms were most frequently small breeding herds located in region 1 with only one production site. Additionally, most farms in cluster 1 (85 out of 150) and cluster 2 (23 out of 26) were small size farms with 100 to 300 sows per farm. In contrast, 34.6% (52 out of 150) were medium size farms in cluster 1, while only 7.7% (2 out 26) were medium size farms ibn cluster 2. Furthermore, the odds of IAV detection were 7.35 times higher in cluster 2 compared to cluster 1 (95% CI: 1.7, 66; $p<0.01$).

Table 4. Description of the most influencing variables in the selection of clusters of a Hierarchical Cluster Analysis of biosecurity measures and husbandry practices of 176 swine farms surveyed in Colombia in 2016-17. Results of R software analysis using VarSelLCM package.

Variables	**Disciminative power**	**Disciminative power (%)**	**Disciminative power (% cum)**
V41 Records entry of personnel	38.45	14.92	14.92
V34 Record entry of vehicles	34.33	13.32	28.24
V46 Medication and deworming protocols	20.26	7.86	36.10
V12 Use of access system is mandatory	18.24	7.08	43.18
V35 Specialized vehicles to transport animals	15.05	5.84	49.02
V11 Access system for personnel entry	14.88	5.77	54.79
V39 Exclusive personnel to each farm section	14.27	5.54	60.33
V23 Other animals have access to the swine facilities	14.13	5.48	65.81
V6 Total animal inventory	12.25	4.75	70.56
V42 Exclusive provision for staff and visitors	11.51	4.47	75.03
V44 Flies control program	11.51	4.47	79.49
V36 Specialized vehicles to transport feed	10.55	4.09	83.59
V58. Region of location	6.67	2.59	86.18
V54 Breeding system	6.29	2.44	88.62

Variables	Disciminative power	Disciminative power (%)	Disciminative power (% cum)
iav	6.23	2.42	91.04
V14 Cleaning protocol between vehicle usage	6.18	2.40	93.43
V20 After cleaning and disinfecting the facilities are allowed to dry before use	5.45	2.12	95.55
V40 Down time for visitors before entering to the facilities	5.03	1.95	97.50

Table 5. Common farm characteristics among the farm clusters obtained after Hierarchical Cluster Analysis of biosecurity measures and husbandry practices of 176 swine farms surveyed in Colombia in 2016-17.

Variable	Category	Cluster		Total (n=176)
		1 (n=150)	2 (n=26)	
		n (%)	n (%)	n (%)
V3: Farm type	Breeding and nursery	37 (84.1)	7 (15.9)	44 (25)
	Farrow-to-finish	109 (85.2)	19 (14.8)	128 (72.7)
	Genetic core	4 (100)	0	4 (2.3)
V5: Farm size	Small (100 to 300 breeding females)	85 (78.7)	23 (21.3)	108 (61.4)
	Medium (301 to 1000 breeding females)	52 (96.3)	2 (3.7)	54 (30.7)
	Large ((>1000 breeding females)	13 (92.9)	1 (7.1)	14 (8.0)
V4: Number of production sites	One site	66 (79.5)	17 (20.5)	83 (47.2)
	Two sites	35 (85.4)	6 (14.6)	41 (23.3)
	More than two sites	49 (94.2)	3 (5.8)	52 (29.5)
V58: Geographical location	Zone 1	4 (30.8)	9 (69.2)	13 (7.4)
	Zone 2	136 (91.3)	13 (8.7)	149 (84.7)
	Zone 3	10 (71.4)	4 (28.6)	14 (8.0)
IAV: Influenza virus infection status of the farm	Positive	93 (79.5)	24 (20.5)	117 (66.5)
	Negative	57 (96.6)	2 (3.4)	59 (33.5)

Farm factors association with IAV infection. Univariable analysis identified a total of 28 variables (Table 2) potentially associated with farm swine influenza status. The second stage, which involved multivariable analysis of these variables and plausibility and biological relevance, identified a total of 20 variables associated with farm swine influenza status (the $\chi 2$ test; $p < 0.25$). After final processing steps and building of the final GLM, stepwise approach resulted in the selection of five potential predictors: "farm size (V5)", "facilities are allowed to dry after cleaning and disinfecting (V20)", "location in an area with a high density of pigs in the region (V1)", "Flies control program (V44)", "Frequency of training of technicians and operators (V52)" associated with the detection of IAV in the evaluated farms. After assessing interactions using stepwise approach resulted in the selection of three potential predictors including an interaction. Additionally, cofounding effects of the excluded predictors (V44 and V52) were assessed by examining the difference in effect estimates between models with and without this specific factor, thus confirming no confounding effect was presented. Therefore, main GLM (iav ~ V5 + V20 + V1 + V5:V1) included only three predictors ("farm size (V5)", "facilities are allowed to dry after cleaning and disinfecting (V20)", "location in an area with a high density of pigs in the region (V1)", in addition to the interaction between V5 and V1), which were associated with IAV detection (p-value ≤ 0.05). After post-hoc test comparisons, significative contribution was found for all predictors. Adjusted OR for each of the predictors ranged from 0.27 to 34.9 (Table 6). Final GLM only explained 25.17% of variability observed in the frequency of testing IAV positive attributed to the predictors. From this percentage, 19.52% was caused by partial effects of the predictors and remaining percentage was related to contribution of complex interrelationships among predictors (concomitance). Overdispersion was not identified for the model and outliers data were not identified.

Table 6. Summary of results of multivariable regression analysis (Generalized Linear Model) of four predictors of Influenza A Virus (IAV) positive detection in 176 swine farms surveyed in Colombia in 2016-17. Farms were tested for IAV using RT-PCR on nasal swabs and oral fluids. Table show results after controlling potential confounding factors and interactions.

Variables	Level	NSC	OR	CI 95%	Collinearity

		β	SE		Low lim	Upp lim	T	VIF
Intercept		-5.62	1.62	0.003	0.00	0.08	-	-
Farm size (V5)	1001-7500	3.55	1.11	34.9	3.92	311.8	0.39	2.54
	301-1000	2.18	0.67	8.88	2.38	33.06	0.28	3.50
Facilities are allowed to dry after cleaning and disinfecting (V20)	Yes	3.21	1.55	24.89	1.18	524.3	0.97	1.02
Location in an area with a high density of pigs in the region (V1)	>10 an/km^2	1.61	0.59	5.05	1.56	16.3	0.44	2.25
Farm size (V5) : Location in an area with a high density of pigs in the region (V1)	1001-7500: >10an/km2	-1.20	1.25	0.29	0.02	3.4	0.40	2.48
	01-1000: >10an/km2	-1.30	0.76	0.27	0.06	1.2	0.27	3.58
AIC= 189.92; BIC= 219 Nagelkerke's R^2: 0.33 p value= 4.14e-09 Over dispersion coefficient (phi): 1.04 Deviance = 0.269; Concomitance = 5.65%; Main effects sum= 19.52%								
NSC: Non-Standardized Coefficient; SE: Standard Error; CI: Confidence interval; T: Tolerance; VIF: variance inflation factor; OR: Odds ratio; AIC: Akaike criterion; BIC: Bayesian information criterion; R^2: adjusted correlation coefficient. Italic bold: variables with statistical significance								

4.5. Discussion

The present study intended to assess the biosecurity, MP and farmer´s behaviors of pig farms of Colombia, a country experiencing a very rapid growth in the pig population. The study focused firstly on farms with inventories over 100 sows. In this epidemiological study we used a combination of different methods for analysis of complex survey data to identify and characterize the structure and clustering patterns of Colombian swine farms. Also, we estimated how these patterns were associated with IAV detection at the population level. This study was unique with regards to the methodology used and depth of information gathered, providing a baseline reference tool for the industry in Colombia. To our knowledge, this is the first study analyzing complex survey data from swine farms in Colombia. This methodological approach allowed to identify two clustering patterns, structure and biosecurity of 176 swine farms, providing key baseline data to further investigate the specific

BP and MP of these farms and help recognize gaps on biosecurity and failure on the current strategies for disease control and prevention in the pork industry. Our results also demonstrate the need of more awareness campaigns to reduce risky practices in the swine farms in the country.

The use of a multivariable method such as MCA is, highly relevant for studies where a complex dataset of qualitative variables is collected (Ayele et al., 2014). During MCA, categorical data are transformed into cross tables and the results interpreted in a graphical manner (Costa et al., 2013). Thus, the first dimension explains as much variance as possible of the dataset, the second dimension is orthogonal to the first and displays as much of the remaining variance as possible, and so on. Based on these criteria, in our model, a two-dimension MCA solution was considered the most adequate. The first two dimensions represented 27.6% of total inertia; thus, the relationships between the data are reasonably (albeit not perfectly) suited for the MCA. Other studies have reported comparable findings using this multivariable analysis (Ribbens et al., 2008; Bergman et al., 2019). This tool helped us to identify which BP and MP variables were the most important to obtain a structural characterization and understand the diversity among Colombian pig farms to provide a baseline assessment of their practices and facilities.

To explore the existence of farms with different patterns of BP, MP and farmer´s behavior, multivariate methods (MCA and HCA) were applied. When combining such methods, a complex dataset is reduced in profiles that maximize inter-cluster variation and intra-cluster correlation (Holt et al., 2016) allowing us to explore, analyze and identify clustering patterns of swine farms and providing key insights on relationships between data (Costa et al., 2013). In our study, this combined approach allowed us to recognize two clustering patterns of farms, based on their level of similarity and thereby illustrate the structure variability of the swine farms in the country (Greenacre, 2016; Husson et al., 2010). Additionally, one of these clusters was associated with higher odds of IAV detection, thus rising new research questions for future studies that help identify specific gaps on biosecurity that may represent each of the farm clusters. Multiple studies have explored risk factors of IAV infections in pig farms (Culhane and Detmer, 2014), however, few studies have focused on analysis of clustering

patterns of BP and MP (as a large dataset of complex interactions) and associations with IAV detection at a farm level. Results from multiple factor and hierarchical clustering analyses on data regarding MP and BP in swine farms in Corsica, revealed interesting patterns in the introduction and spread of infectious disease in four clusters of swine farms with different levels of risk (Relun et al., 2015). In this sense, data provided from these multivariable analyses could be useful to more realistically understand which factors are affecting the transmission of infectious diseases among pig farms and highlight the need of campaigns among the swine farmers to reduce risky practices.

Both farm clusters had in common several measures such as the use of barriers before the entry of people, and access control for vehicles, however about a third of the farms in cluster 2 did not have barriers preventing the entry of people, and most of these farms were small farms. These results agree with those by other studies (Bottoms et al., 2013; Simon-Grifé et al., 2013), which reported that larger farms located in high pig density regions implement higher BP. In our study, most of the larger farms are likely to have a higher technical standard in biosecurity, but risky practices such as mixing of livestock species is still observed in some of these farms. Studies have documented that applying measures to reduce the risk of disease introduction by visits and vehicles is one of the most important BP to the farmers and veterinarians (Simon-Grifé et al., 2013), but at the same time these practices were not applied on a considerable number of farms in our study. Although two clusters of farms were identified, the percentage of variance explained by the analysis was relatively low (27.6%). This finding suggests that the combination of BP and MP implemented by a given farm in conjunction with the farmer´s behaviors have a certain degree of randomness and, consequently, the clusters still contain internal variability. Similar findings are reported in other studies conducted in pig farms in Argentina (Alarcón et al., 2019). Therefore, to our understanding this is an indication of the complexity surrounding the evaluation of biosecurity, but also acknowledging that compliance of the measures evaluated was not assessed in our study, and that the heterogeneity is probably connected to the different degree of expertise and experience of veterinarians and farmers, their personalities, their interpretation of biosecurity principles and their access to sources of technical information (Racicot et al., 2012; Simon-Grifé et al., 2013). Recent studies have shown that compliance

of BP varies depending on farmer´s behaviors, work experience and education (Racicot et al., 2012). In this context, it has been noticed that increase in the biosecurity standards by pig farmers could be also motivated by the presence of an outbreak of a new disease in the region (Nöremark et al., 2009).

While there is a perception of good biosecurity implemented by swine farmers, our results showed that may be a failure in understanding of the biosecurity principles and compliance of protocols. High percentage of farms reported implementation of most measures assessed in the survey, however, among others risky practices, raising other livestock within the same farm was still identified. Attitudes towards biosecurity can be influenced by additional factors such as the lack of credibility, trust and confusion as to the specific recommendations that farmers should follow (Simon-Grifé et al., 2013). Potential for cross-species transmission of animal diseases that affect multiple species will be facilitated in areas where concentrations of different animal species co-exist (Schembri et al., 2015). Of concern in this study, was the number of farms engaging in mixed livestock production systems. This is a risky practice for disease transmission since vertebrates may serve as potential reservoirs of pathogens in a farm. For example, having other domestic animals on the same farm has been associated with a greater prevalence of salmonellosis in swine farms (Rajić et al., 2007). Our results suggest that small-size pig farmers (<300 sows) are more likely to rise other livestock species.

Regarding the protocol for access of people and vehicles to the farm and their associated biosecurity measures, it is important to note the number of farms (<20%) that do not carry out the registration of vehicles and people that enter the farm. These results highlight the need to improve the use of these records which facilitate to identify and control potential sources of disease transmission in the event of outbreaks (Simon-Grifé et al., 2013). In relation to farm visits, the surveyed farms had similar or better BP to those reported in other studies carried out in other countries (Lambert et al., 2012; Simon-Grifé et al., 2013). However, entrance protocols representing a physical barrier to people in the prevention of disease introduction was not carried out at 10 (5.7%) of the pig farms. Although small number of farms were not applying this physical barrier, it is well known that BP at entrance are preventive measures to reduce the risk of disease spread in pig farming systems (Bellini et

al., 2016). On more than half of the farms had down time of 48 hours or less for visitors before entering to the facilities. In addition, few (<9%) farms did not implement a disinfection procedure for vehicles at entry nor preventive measures for the access of personnel to the farm. Recent studies showed that the absence of BP for access of vehicles and people are risk factors for disease transmission (Alarcón et al., 2021). Furthermore, workers and visitors entering the swine farm have been identified as a major pathway of disease transmission during outbreaks of highly contagious diseases in studies conducted (Lambert et al., 2012; Pineda et al., 2020). Transportation and other mechanical vectors such as fomites (boots and containers), and the movement of personnel has been identified as risk factors to herd health and disease spread (Dee et al., 2002). In this sense, few farms (4.2%) farms reported not implementing a cleaning protocol between transport vehicle usages, and 15 (9.1%) farms did not apply disinfection protocols for vehicles at entry of the farm which represent a risky practice. Also, veterinarians were identified as the main source of technical assistance and information for pig farmers with majority of farmers receiving assistance at least 3 times per year (Gunn et al., 2008). But in the present study, results showed that an improvement of BP at the access to the farm is still needed, which clearly point to the need for veterinarians and other assistance professionals to continue raising farmers' awareness of biosecurity issues.

Moreover, 87 (49.7%) farmers in this study were located within a 5 km radius of other "commercial" pig farms and 114 (64.8%) farms in this study were located in an area of high density of pigs/km2. Failure in compliance of farm BP may increase the risk of disease introduction or spread from or to other farms and may represent a real threat, particularly in high pig density and mixed farming areas (Ribbens et al., 2008). Our results also showed the lack of application of some BP in smaller herds located which were mainly located in low pig density regions, findings that have been observed in other studies (Alarcón et al., 2019; Ribbens et al., 2008). Although few farms (8.8%) acquired their gilts from animal fairs/markets or livestock saleyards and 16 (11.7%) acquired their gilts from other commercial farms, studies on farms purchasing animals from livestock saleyards have identified several factors potentially increasing the probability of disease transmission and spread (Shirley and Rushton, 2005). Infected pigs moving from one farm to another (re-

stockers) pose a greater risk for disease spread (Andres and Davies, 2015). Similarity, 19 (16.1%) farms reported that received their gilts from multiple sources in the last semester. Animals from different sources and health status that are mixed in the same area, provide an excellent opportunity for disease transmission (Schembri et al., 2015). In regards to some husbandry practices, a small proportion of farms (<5%) did not implemented pest control programs and used farm-own made feed mix as a food source. The type of feed, added to other factors such as poor hygiene, and the presence of rodents are generally considered to be important aspects associated with a high prevalence of salmonellosis in pig farms (Rajić et al., 2007).

Despite the sample size and selection criteria of farmers included in the analyses in this study, we believed the trends in the findings of this study may generally represent farmers in Colombia. However, very limited assumptions can be made regarding the attitudes and behaviors for biosecurity practices compliance of farmers. Nevertheless, at the conclusion of this study, a detailed report was provided to the national swine producer association. Therefore, we believe the provision of this report, alongside other advisory recommendations served as an incentive to review and update a number of gaps in biosecurity.

Using multivariable regression methods (GLM) we also found that three variables were associated with higher odds of IAV detection: “location in an area with a high density of pigs in the region (V1)”, “farm size (V5)”, and “facilities are allowed to dry after cleaning and disinfecting (V20)”. Several studies have demonstrated that farm size, distance between farms in areas with high densities of pigs, and density of pigs in a specific area are risk factors associated to several viral diseases (Robertson, 2020). In our study, we found that approximately half of the farms were located to less than 5 km from other farms and were located in a pork production area with a local animal density greater than 10 pigs/km2. In addition, we observed that some swine farms raised other livestock species making more complex the biosecurity scenario. Previous studies in the United Kingdom, Netherlands and Korea reported great level of difficulty in preventing the spread of viral diseases in dense livestock areas, mainly where a mixture of pigs with other animal species was present (Hayama et al., 2016). Likewise, a modeling study in China demonstrated how areas of high

density of swine productions are highly associated with an increased risk of outbreaks of IAV (Fuller et al., 2013). Published data have showed that prevalence of IAV is highly associated with high densities of pigs, defined in terms of number of farms and number of pigs (Baudon et al., 2017). In our study, we found that the frequency of positive farms was significatively higher (p-value = 0.03) in geographical areas with higher densities of pigs. In this sense, pig density becomes a key element in biosecurity of the farms specially for new facilities. We also found that a positive IAV status increased significantly in larger farms (those with inventories of 300 or more sows), but information about association of farm size to IAV infection in piglets in production systems of Colombia is very limited. Several studies have identified that farm size is a risk factor for IAV infection in pigs in other locations of the world (Poljak et al., 2008). A metanalysis conducted in 2017, showed that high densities and number of pigs per farm are associated with higher IAV prevalence, suggesting that larger pig farms have higher odds of IAV detection or persistent infections (Baudon et al., 2017). Other studies revealed that large number of pigs as one influential risk factor for IAV seropositivity in sows and fattening pigs (Maes et al., 2000), and this factor may also strongly impact on the incidence of subclinical IAV infection (Takemae et al., 2016).

We found an IAV herd-level prevalence ranging from 2.1 to 39.6% (Median 14.6%) which is lower but similar to findings made for swine farms located in important pork producing regions in the world (Chamba Pardo et al., 2017). It also has been reported that IAV herd-level prevalence may vary over time showing seasonal patterns (Chamba Pardo et al., 2017). We also demonstrated that IAV is actively circulating in piglets and probably established across swine herds in the country. For IAV detection at the farm level we selected piglets between 3 and 12 weeks of age because they act as reservoir for enzootic infections in swine populations (White et al., 2017). Moreover, piglets play a pivotal role in maintaining IAV endemicity in pig populations (Diaz et al., 2015; Pileri et al., 2017). Swine IAVs can endemically persist in farrow-to-finish farms, causing repeated disease outbreaks in pigs around 8 weeks of age (Cador et al., 2017). Additionally, different factors contribute to IAV persistence within swine herds including population dynamics of farrow-to-finish farms, immune status of animals and the co-circulation of distinct subtypes (Cador et al., 2017). Furthermore, the odds of IAV detection in farms were 7.35 times higher in cluster 2 compared

to cluster 1, and cluster 2 was mainly represented by farms with only one production site, therefore these characteristics may also be contributing factors to the endemically persistency of the virus. Therefore, active surveillance should be routinely recommended to better understand the transmission within and between the swine production systems in Colombia.

The general need for routine monitoring of diseases in swine has pointed to the use of new sampling/testing methods which be adapted to the pig population structure and production practices of contemporary swine farms. In this sense, sample pooling strategies have been used for herd monitoring of influenza virus and other infectious diseases in swine an other animals (Muñoz-Zanzi et al., 2000; Ssematimba et al., 2018; Van et al., 2012). This design based on sampling individual groups of animals provides flexibility in monitoring farms ranging widely in size and complexity. Furthermore, provides a powerful approach for detecting infection and similar methods have been widely applied for detecting Porcine Reproductive and Respiratory Syndrome Virus infection in one barn using as few as 2 OF samples. Under these same assumptions, if 2 oral fluid samples are collected from each of 3 barns on one farm, the probability of detection increases to 81% (Rotolo et al., 2017). In our study, 8 OF samples were collected from 4 different groups of pigs within the same farm, and using the formula given by other authors (Rotolo et al., 2017) (assuming a within-herd IAV prevalence above 13%), the probability of IAV detection is greater than 40%. Because of the known high transmissibility of influenza viruses, it is expected that a large proportion of animals would face the exposure to the virus following IAV entry to a herd. A study conducted in vaccinated and non-vaccinated pigs showed that probability of detecting IAV in OF was 99% when within pen prevalence was higher than 18% (Romagosa et al., 2012). In this sense, other studies reported that OF collected from pigs resulted in higher odd ratios of detecting a positive IAV sample by RT-PCR compared to individual pooled samples (nasal swabs), however, individual samples were most likely to yield a successful virus isolation (Garrido-Mantilla et al., 2019). Similarity, pooling specimens to increase efficiency of testing and cost effectiveness is another advantage obtained from the sample pooling strategies (Furstenau et al., 2020). Studies performed using pools of 10 nasopharyngeal swine specimens containing either single or multiple positive specimens or specimens positive for other respiratory viruses showed no negative effect on IAV detection by RT-PCR (Van et al.,

2012). In our study, testing of 5 pooled individual samples helped to increase our testing capacity and resource savings. A study reported that testing pools of 5 individual-samples did not affect the detection of IAV on oral swabs but increased testing capacity without sacrificing sensitivity (Ssematimba et al., 2018). The sensitivity of pooled samples may be lower than the sensitivity of individual samples and this practice may actually reduce the chances of detecting the virus since it may result into lowering the virus titer of the pooled sample, however, studies have shown that pen-based collection of OF is a sensitive method to detect influenza even when prevalence of the disease is low (Romagosa et al., 2012). Thus, monitoring of IAV in swine farms using sample pooling strategies such as OF to detect virus infection at a herd level is a recommendable approach to follow under limited resource settings such as the swine productions in Colombia.

We demonstrated that IAV is widely circulating in Colombian swine populations at the main producing regions for pork industry. Therefore, the virus is not restricted to a particular geographic location, even if BP are in applied in the farms. In addition to the scarce information available regarding active surveillance and prevalence of IAV in the pork industry in Colombia, which could be interpreted as a little interest on detection of SI in the local industry, thus we hypothesize that conjunction of the lack of active detection of IAV in swine farms, no vaccine availability, and either deficient or not strict compliance of BP could be adding more difficulty on controlling IAV spread within and between pig farms in the country. Therefore, highlight the need for awareness campaigns to reduce risky practices and implementation of a vaccination program against SI (guarantying that vaccine strains match circulating viruses in swine) would be highly advised. Vaccination integrated with other BP is the most effective strategy to reduce IAV transmission (Romagosa et al., 2011) and to control spread of SI (Sandbulte et al., 2015), even when the disease is persistently endemic in piglets (White et al., 2017).

It is well known that BP rely on the type of production system, type of farm and specific risks of disease transmission (Silva et al., 2019). For example, sow farms that receive and replace their gilts from external sources may have a higher risk of disease introduction compared to farms that produce their own gilts. In this sense, eradication and disease control programs at

the farm level could be achieved through different management procedures including herd closure (Corzo et al., 2010). For example, recent studies have shown that IAV can settled within farrow-to-finish farms and endemically persist causing recurrent infections in successive batches of pigs and leading to repeated swine flu outbreaks in the farm (Cador et al., 2017).

Controversially, in our study farms in which the facility to dry after cleaning and disinfection were associated with a higher odd of IAV detection. Given known facts, cleaning and disinfecting are critical parts of all biosecurity programs (Ford, 1995) since the goal of this process is to decrease pathogens load significantly to a point where disease transmission does not occur. However, many other factors may be affecting the persistency of the virus after cleaning and disinfection. For example, air and surfaces in swine barns during outbreaks of IAV can contain different viral loads representing different exposure levels for animals. A study showed that during SI outbreaks, detection of IAV from air was sustained up to 11 days from reported onset representing an exposure hazard to both swine and people (Neira et al., 2016). Other experimental studies have demonstrated that IAV transmission is strongly modulated by temperature and humidity (Lowen et al., 2007). Therefore, it is possible that our finding is confounded by environmental factors such as persistence of airborne viral particles, local temperature, and humidity or that the period time to let dry the surfaces or the cleaning and disinfection process is not properly executed. Another aspect to highlight is the compliance of the measure surveyed. Even that majority of the farmers indicated that after washing and disinfecting they allowed to dry the facilities before use, based on the controversial finding, we may think that compliance to that measure was not completely applied. Social desirability is also a paradigm for measurement bias in surveys. Studies have showed that people can provide answer to surveys based on a normative behavior to appear correct to interviewers (Brenner and DeLamater, 2016). These and some other possible sources of bias could have influenced the results of our study, therefore, our findings should be taken with caution until they can be validated with further studies, in which environmental factors, compliance and other possible affecting variables are assessed.

Additionally, in our analysis using an independent vehicle to transport animals and feed was also found to increase the odds of IAV detection. Studies have revealed that feed transportation system and other mechanical vectors can be source of transmission of diseases in swine farms (Dewey et al., 2014), however, this finding could be biased because we did not investigate the origin and prior movements of the vehicles, and if both vehicles used were properly cleaned and disinfected between uses. Thus, this finding also requires further investigation to clarify, including the type of animals transported in each vehicle evaluated.

Seasonality of human influenza is well studied. It is generally accepted that seasonal influenza in people peaks in the winter months (Lipsitch and Viboud, 2009) and similar findings have been reported for swine (Chamba Pardo et al., 2017) but causes of such seasonality are still not completely understood (Lofgren et al., 2007) and little information is currently available to describe the pattern of IAV in swine over different seasons (Poljak et al., 2014). IAV prevalence in our study may be subjected of variation due to seasonality because of the long timeframe (from October 2016 to July 2017) to complete the sample collection, however, seasons do not occur in Colombia as a tropical area in which variations in climate factors between months are not strongly marked in comparison to temperate regions.

We acknowledge some limitations of our study. Selecting farms according to some pre-specified rules may have introduced selection bias in the study. Also, the inclusion of farms was based on the voluntary participation of farmers; and thus, selection bias was probably introduced. Lack of clarity and validity of the answers during analysis of survey data may include bias (Stärk et al., 1998), but rigorous design and validation of the questionnaire can help to control biases (Enlund et al., 2019). In our observational study, prior data collection, several training sessions and preliminary tests with farm owners (not included in the study analysis) were performed to reduce bias. The questionnaire was conducted by 6 veterinarians. Efforts were made to ensure the clarity of the questions during training sessions, however, the interviewers may have unintentionally influenced the responses, so interviewer bias cannot be completely excluded. Most of the questionnaires were closed, excepting those questions that could allow interviewees to provide more details answers or clarifications.

Low response rate is another reported disadvantage of surveys (Ribbens et al., 2008), then questions with a minimum response rate of 70% are also required to reduce biases during the analyzes (Thrusfield, 2005), however our study significantly exceeded that minimum response rate. Although the overall response rate was high, one cannot neglect the fact that some questions had a response rate under 95% and this could lead to answers non representative of the whole study area population, so bias could also be influencing our results. Additionally, compliance to the measures of biosecurity surveyed in the swine farms was a factor not assessed in our study and may represent a limitation. We acknowledge that farm biosecurity has evolved over time as swine diseases have been better understood, but effectiveness of farm biosecurity depends largely on the compliance by the personnel involved in the production system (Racicot et al., 2012). Thus, compliance of BP is always a challenging issue when analyzing biosecurity in animal productions. Poor biosecurity compliance has been reported in animal productions (Makita et al., 2020; Racicot et al., 2011) and it is frequently related to lack of knowledge or comprehension of the biosecurity principles, but also to human dimensions such as personality and attitudes (Merrill et al., 2019). Also, the survey data was collected in a cross-sectional study from interviews which may have led to bias towards answers stating measures believed to be applied on farm rather than confirming measures really applied. Perception of a given biosecurity measure by a pig farmer can be also strongly influenced by the BP actually applied on the farm (Casal et al., 2007). Finally, as this was a cross-sectional observational study, causal relationships should not be inferred from the results presented.

4.8. Conclusions

Livestock raising is an important sector of the Colombian economy and swine production occupy an important place among livestock industries. Despite the great efforts made to increase biosecurity in the pork industry, fails are still noticed as outbreaks of swine diseases are reported in this sector. Because of the above there is an increased need to provide tools for swine veterinary services to identify gaps on biosecurity in these animal productions and data about potential farm factors associated with swine diseases detection. Such approaches should to be based on a thorough understanding of farmer's behaviors, biosecurity and management practices involved the pig production, which are poorly unknown and highly

diverse within the country. To gather such information in a quantifiable manner, a survey approach was developed to better understand the Colombian swine farms, covering biosecurity and husbandry practices, as well as other aspects such as detection of swine influenza virus.

Multivariable techniques such as MCA and HCA were useful tools to analyze the complex and large survey data to assess biosecurity in pig farms. These methods allowed to identify profiles and characterize the pig farms based on the farmer's behaviors, biosecurity and management practices involved the pig production. Also, these farm profiles were further used to estimate the odds of pathogen detection identifying key elements to design appropriate strategies for swine health monitoring and disease control. The present study of the pig production in Colombia increases our knowledge on the different pig farm characteristics, traditional management/husbandry practices and biosecurity protocols applied, which also describe the structure of the main swine production systems in the country. The analysis of the survey data allowed to identify biosecurity gaps and risky behaviors or risky farm profiles, which could be critical control points across the production chain where to implement mitigation measures. Also, our study revealed and highlighted patterns of farms possible associated with higher odds of IAV detection in pigs. Thus, our survey approach for data collection and analysis can be a promising tool to assess biosecurity in swine farms and to identify factors associated with detection of swine pathogens. The main recommendations from this work are to improve communication strategies to and between swine farmers to enhance biosecurity in the country.

4.9. Declarations

Credit authorship contribution statement

Karl Ciuoderis: Writing - original draft, data curation, investigation, methodology, formal analysis, project administration, writing - review and editing. **Andres Diaz**: investigation, writing - review & editing, validation, investigation. **Jorge Osorio**: investigation, writing - review and editing. **Carlos Muskus**: investigation, writing - review and editing. **Mario Peña**: Supervision, writing - review & editing. **Juan Hernandez**: writing - review & editing.

Declaration of competing interest

None

Supplementary materials

Supplementary material associated with this manuscript can be found in appendices

S1 Database. Database used for the MCA of survey data. (XLSX)

S2 Database. Database used for the HCA of survey data. (XLSX)

S3 Database. Database used for the GLM of survey data. (XLSX)

S1 Appendix. Questionnaire form. (PDF)

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CAPÍTULO V. DIVERSIDAD FILOGENÉTICA DE AISLADOS DEL VIRUS DE INFLUENZA PORCINA EN COLOMBIA DURANTE 2011-2017.

Este capítulo corresponde al manuscrito científico como resultado del análisis genómico y filogenético de diferentes virus porcinos obtenidos durante la realización de este trabajo de doctorado en Colombia. El manuscrito aún no se encuentra en revisión de una revista para publicación. Este capítulo contribuye con el desarrollo de los objetivos específicos 1.4.2; 1.4.3; 1.4.4; 1.4.7; 1.4.8 y 1.4.9 de este trabajo de grado.

Genetic Diversity and Phylogenetic Analysis of Swine Influenza Viruses Isolated in Colombian pig farms from 2011-2017

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5.1. Abstract

Swine Influenza is a disease which cause damage to respiratory tract of pigs. The evolutionary origins of the Influenza A virus (IAV) in swine in many countries, included Colombia, remain unclear, highlighting the lack of swine surveillance in Latin American countries. Although pork industry is one of the largest animal productions in Colombia, influenza is not thought to be endemic in swine and its thread to public health is underestimated. Therefore, the aim of our study was to perform a genetic characterization and identify the genetic diversity of the swine IAV in Colombian pigs. Through phylogenetic analysis of 159 genome sequences of IAV, the H1N1pdm, rH1N2 and rH3N2 subtypes were identified circulating in the Colombian swine population during 2011–2017 in farms from 11 states of the country. We identified previously uncharacterized influenza viruses of human-like origin that have circulated undetected in swine in this country. Particularly for the rH3N2 and rH1N2 subtype, this is the first time that its isolation and sequencing has been

reported in Colombian pigs. The analysis of these sequences demonstrates that in the swine population of Colombia, circulate viruses that have suffered rearrangements of their proteins with different subtypes, conserving a backbone from H1N1pdm lineage. Thus, viral diversity has increased by reassortment between co-circulating viruses, including pandemic subtype. The fact that most swine viruses from Colombia had a backbone from the pandemic subtype highlights the need for additional surveillance in swine, and the circulation of multiple divergent hemagglutinin lineages challenges the selection of future effective cross-protective vaccines.

Keywords: Influenza A Virus, Swine, Pigs, Colombia, Evolution, Diversity, Reassortment, Humans, Influenza, Zoonoses, Phylogeny

5.2. Highlights

- Higher prevalence of H1pdm over H3 viruses was found for Swine viruses obtained.
- Phylogenetic analysis of pdmH1 HAs revealed reverse zoonotic events.
- Phylogenetic analysis of H1N2 and H3N2 revealed reassorting of endemic viruses with the pandemic subtype.
- Genetic analyses of circulating strains suggest the future selection of matching vaccines.

5.3. Introduction

Influenza A viruses (IAVs) are single stranded RNA Orthomyxoviruses with a segmented genome (Kerr, 2009) which are known as cause of the one of the most important zoonotic diseases in pigs (Reperant et al., 2016). IAVs are distributed worldwide and it is one of the most prevalent respiratory disease in swine (Simon-Grifé et al., 2012), resulting in a great economic burden on the pork industry worldwide (Holtkamp et al., 2008). Hemagglutinin (HA) and neuraminidase (NA) are the two surface proteins recognized by the immune system after infection and vaccination, (Janke, 2014) which also serve to determine viral subtypes (Choi et al., 2002). Genetic and antigenic mutations at these two surface proteins result in virus change and evolution (Shao et al., 2017). Genetic changes of IAVs and their capability

to reassort have raised public health concerns of new strains emerging with pandemic risk for animals and humans (Nelson & Vincent, 2015).

Currently, IAVs of the H1N1, H1N2, and H3N2 subtypes are the most frequently detected in pigs worldwide (Anderson et al., 2021), but other subtypes have been also reported (Lewis et al., 2016). Unlike IAV from humans or wild aquatic birds, the limited intercontinental transport of pigs has caused establishment of genetically and antigenically distinct virus lineages in the world (Van Reeth, 2007). In overall, for swine IAV H1 gene, there are three major lineages based on global swine H1 nomenclature: Classical swine (CS; 1A), Eurasian avian-like swine (EA; 1B) and Human seasonal (HU; 1C). The CS is further divided into six clusters (Anderson et al., 2016): α (1A.1), β (1A.2), TR (1A.3.3.1), ϒ2 (1A.3.3.2), ϒ1 (1A.3.3.3), and pandemic (pdm; 1A.3.3.2). Alfa (1A.1) clusters could be further classified into three subgroups (1A.1.1; 1A.1.2; 1A.1.3). The HU lineage was the result of two separate introductions of human seasonal H1 viruses into swine in the early 2000s and they are further divided into the 1B.1 viruses, related to a reassorting H1N2 (rH1N2) virus that emerged in Great Britain in 1994 , and 1B.2 viruses, related to the H1-δ1 (1B.2.2) and H1-δ2 (1B.2.1) viruses (Anderson et al., 2016). For swine IAV H3 gene, there are two major lineages based on global swine H3 nomenclature: North American lineage (NAL) and Eurasian lineage (EUL). H3 NAL can be further classified into five clades (I, II, III, IV and human-like clade) (Rajão et al., 2017). H3 Cluster IV can be further divided into six clades (clades IVA to IVF) (Kitikoon et al., 2013). However, the genetic diversity of SIAVs in the current pork industry is not fully understood (Diaz et al., 2017a).

Phylogenetic diversity and distribution of IAVs have been analyzed worldwide (Zhuang et al., 2019), and much of this diversity is the result of bidirectional transmission between swine and humans (Powell et al., 2021), followed by evolution (mainly driven by the mutation and re-assortment of viral genomes) within swine host populations (Shao et al., 2017), and diffusion of the virus through live animal transport and trade (Anderson et al., 2021). Genetic diversity of SIV has been observed in intensive swine production regions of the world (Walia et al., 2019). Co-circulation of different IAVs lineages and subtypes could result in virus reassortment increasing genetic diversity (Rajão et al., 2017).

Pandemic H1N1 virus became a predominant genotype in swine in several regions (Nasamran et al., 2020). Persistence pdm H1N1 in pigs may facilitate emergence of novel virus strains with higher pathogenicity to humans and pigs (Cador et al., 2017). Adaptations or mutations occurred after transmission of human-origin viruses to pigs have been reported as a key contribution for the fitness of the viruses (Nasamran et al., 2020). Therefore, routine genetic characterization and monitoring of SIV should be conducted (Walia et al., 2019) to understand how IAV with pandemic potential evolve (Nelson et al., 2015a). However, genetic diversity of swine influenza virus (SIV) is not well documented and SIAVs have not been monitored closely enough. For example, scarce information is available for Colombia. First report of SIV occurred in the 70's (Hanssen et al., 1977). SIV pdmH1N1 was reported in swine farms (Jiménez et al., 2014), but phylogenetic diversity of SIV circulating in swine populations is still unclear in this country. Evidence of potential introduction of human IAV strains into Colombian swine herds have been reported previously (Karlsson et al., 2013). Thus, phylogenetic analysis of circulating SIVs is also important to identify potential adaptations or mutations that could correlate with zoonotic potential or higher pathogenicity of the viruses.

Pork production in Colombia is relying on more than 200,000 pig farm sites. Most of the farms are located in few pigs' dense provinces. One third of the pigs are raised in backyard systems and about two third in farms producing pigs indoors. Weaned piglets (age of 21 to 28 days) are commonly transported to other farms where they reach the market weight. Pigs are capable of generating novel reassorting viruses with the potential to be highly infectious and transmissible in humans (Ma et al., 2009), and when infected pigs from different sources are moved and mixed, the opportunity to viral reassort is greatly increased. Thus, insights into patterns of swine IAV genetic and antigenic diversity are critical to identify emerging viral threats which also provide criteria for updating influenza diagnostics and disease prevention strategies (Anderson et al., 2013).

Given the need to better understand the genetic diversity of SIV molecular epidemiology of SIV in Colombia, here we report and characterize a total of 159 SIV isolates obtained from

10951 samples collected between 2010 and 2017 from 207 pig farms widely distributed in the main pork producing regions of the country, and reveals genotypic diversity of SIV with strong evidence of predominance of pandemic strain in Colombian swine, as well as detection of a novel human-like origin reassorting H3N2 subtype and reassorting H1N2 subtype for the first time in the country.

5.4. Materials and methods

5.4.1. Sample collection

Between January 2011 and June 2017, we collected 10951 samples obtained from 207 different swine farms widely distributed in several regions of Colombia, representing the main pork production areas of the country. Nasal swabs (NS) and oral fluids (OF) were collected from selected pigs at different ages at each farm as part of an active surveillance for detection of SIV. The sample collection was performed following protocols and methods previously described (Romagosa et al., 2012). For laboratory analysis, all specimens were transported to the laboratory within 48 h under cold chain (6-10°C) until processing, or stored at -80°C until use. Filtered samples were inoculated onto MDCK cell cultures in the presence of antibiotics and anti-mycotic to prevent contamination as described previously (Eisfeld et al., 2014). Cell supernatants were collected from infected cells 48h pi and used in hemagglutination test (HAT) to determine presence of virus. Sample with a HAT titer of ≥4 units per 50 µL was considered positive for isolation. The HAT-positive cell supernatants were aliquoted and stored at 80°C until use. For virus confirmation, HAT-positive cell supernatants were also tested by rRT-PCR as described below.

5.4.2. IAV molecular screening

RNA was extracted from NS and OF samples using ZR viral kit (Zymo® research, USA) following manufacturer instructions. Initial IAV screening was conducted by real-time reverse transcription PCR (rRT-PCR) selective for the matrix protein (MP) gene. Sequences for the primers and probe were based on the assay described (Shu et al., 2011). Briefly, Fast One Step rRT-PCR kit (Applied Biosystem®, EE.UU.) was used with 0.6 uM of each primer and 0.2 uM of probe. Then, 3 uL of extracted RNA were added to 20 uL final reaction

volume. Cycling conditions were 50°C x 5 min, 95°C x 20 seg, followed of 40 cycles of 95°C x 15 seg and 60°C x 1 min. An ABI 7500 Fast (Applied Biosystem®, EE.UU.) thermocycler was used. To increase testing capacity, NA samples (same group of pigs) from the same farm were tested in pools of 10 per pool (Fablet et al., 2017a) and if a pool was rRT-PCR positive, original samples were tested individually. rRT-PCR result was interpreted by cycle threshold (Ct) values, where Ct value between 0 and 39 were considered as positive and Ct of 0 and ≥40 as negative. rRT-PCR positive samples were further investigated by Next Generation Sequencing to determine the HA and NA subtype.

5.4.3. Genomic Characterization of SIVs

Full-length characterization of SIV genes was conducted on all virus isolates. Some viruses were subjected of whole genome sequencing while other were partially sequenced (HA, NA and or M gene). RNA was extracted from 140 µl of specimens using QIAamp Viral RNA Mini Kit (Qiagen) according to the manufacturer's instruction. cDNA was made from each RNA sample using SuperScript III One-Step RT-PCR System with Platinum Taq High Fidelity DNA Polymerase (Invitrogen, USA), with Uni12/Uni13 primers (Zhou et al., 2009) or with Opti1-F1/F2 primers (Mena et al., 2016), according to the manufacturer's instructions. Each reaction was analyzed by agarose gel electrophoresis and SYBR green staining (10 µl/sample) and visualized by UV transillumination.

5.4.4. Partial HA, M and NA genes Sequencing

HA, M and NA gene bands were individually cut out of the agarose gel, and sent to a sequencing facility (Macrogen, South Korea) to be sequenced by chain termination technology. Sequences results retrieved were viewed on the BioEdit software and edited using the Lasergene package (Hall, 1999).

5.4.5. Virus Whole genome Sequencing (WGS)

SIV isolates were selected for WGS using Next Generation Sequencing (NGS) and nanopore sequencing. The selection criteria of SIA for genomic characterization were based on time

of sample collection, subtype, place of collection, and Ct value (virus titer). Selection criteria considered isolation year, place of origin and IAV subtype.

5.4.6. Sequencing of PCR Products Using the Illumina Sequencer

The MiSeq Platform (Illumina, USA) was used for NGS as described previously (Van den Hoecke et al., 2015). WGS was performed from amplification of eight segments of IAV using FWuni12 and RVuni13 primers (Inoue et al., 2010). For sample preparation, cDNA synthesis was performed using Superscript III RT (Life Technologies, USA) combined with 0.1 mM of each primer. The thermocycler conditions for IAV genome amplification were a RT step at 50°C for 20 min, 10 min of RT inactivation at 95°C and 40 cycles of 95°C for 15 s and 60°C for 45 s. For library preparation, purified PCR products were quantified using the Qubit 4 Fluorometer (Thermo scientific, USA) following the manufacturer's protocol. AMPure XP beads were used for DNA purification. For the NGS library preparation, the Nextera DNA Library Preparation kit (Illumina, USA) was used following the manufacturer's protocol. Samples were pooled together and loaded into the cartridge of the Illumina MiSeq Reagent Kit V2. Sequencing was performed as multiplex double read libraries for 400 cycles. Quality control metrics of from raw sequences were evaluated using FastQC (Leggett et al., 2013) and then the sequencing adaptor and unpaired ends were removed by using the Trimmomatic package (Bolger et al., 2014). A score of 30 was used as the filter threshold for high quality of reads. Genome assembly was performed by de-novo assembly method using SPAdes (Bankevich et al., 2012). From the resulting contigs, Influenza gene segments were manually identified and annotated using Blastn. Assembled sequences were compared with reference sequences database by BLAST and then subject to hemagglutinin (HA) classification. The best BLAST hit was used to preliminary classify the HA based on the highest nucleotide identity (>90%) and trimmed sequences were used for read mapping to this reference using Bowtie2 (Langdon, 2015) and SAMTools (Li et al., 2009).

5.4.7. Sequencing of PCR Products Using the MinION Sequencer

MinIon plataform was used for nanopore sequencing as described previously (King et al., 2020). To enable use of multiplex MinION sequencing (Oxford Nanopore Technologies-ONT) specific barcode tag was added at the start of each reverse primer for eight viral RNA

segments and followed a protocol as described previously (Imai et al., 2018). PCR products were analyzed using the Agilent 2100 Bioanalyzer with an Agilent DNA 7500 Kit. Each PCR product was consequently re-purified with Agencourt AMPure XP according to the manufacturer's instructions. PCR amplicons were also measured by Qubit 3.0 Fluorometer (Thermo Fisher Scientific) and then processed for library preparation using the SQK-LSK308 1D2 Sequencing Kit (ONT) according to the manufacturer's instructions. After the MinION Platform QC run, the DNA library derived from a pool of 12 samples was loaded into a single MinION Flow Cell (FLOMIN107 R9.5 Version) and the run protocol was initiated using MinKNOW software (v 1.4.1, ONT). Real time basecalling was performed with the integrated Guppy v3.0.4 software (ONT) to produce fast5 and fastQ files. Low-quality reads and < 500- and > 3000-length reads were removed using SeqKit (v 0.8.1). Demultiplexing and adapter trimming was performed using Porechop (v 0.2.2), and all data were collected and mapped with Minimap2 (v 0.7.15) using the reference sequences for influenza A (PB2 [CY099076.1], PB1 [CY099309.1], PA [CY045233.1], NP [CY009919.1], M [DQ150436.1], NS [CY050162.1], H1 [FJ789832.1], H3 [KC992248.1], N1 [GU236519.1], and N2 [KC866483.1]). Single nucleotide variant calling from mapped data was obtained by Samtools and BCFtools (v 1.5.0). Mapped data were visualized by IGV software (v 2.3.8) and analyzed by Samtools and Qualimap (v 2.2.1).

5.4.8. Phylogenetic analysis

For phylogenetic analysis, the nucleotide sequences of each SIV gene from this study were compared to sequences representative from different areas of the world available at the Influenza Research Database (**https://www.fludb.org**), Global Initiative on Sharing All Influenza Data's (GISAID) Epiflu database and the NCBI database (**https://www.ncbi.nlm.nih.gov/genbank/**). Representative sequences were randomly selected distribution in hosts, isolation years, place of origin and genetic diversity, and a representative sequence should share the same or similar host, isolation time, and those sequences that shared the same or similar host, isolation time, and isolation place with most of a group of viruses located together in a phylogenetic tree were selected as a representative sequence. Phylogenetic trees were constructed by comparing the sequences from this study to 126 HA1, 103 HA3, and 111 NA reference viruses respectively. For hemagglutinin and

neuraminidase virus clade and lineage classification, several reference sequences were used (Table 1). Sequences reported from Pork main producing regions in the world were included.

The Sequences were aligned using Clustal W method in MEGA software (Kumar et al., 2018) and then trimmed by the closest-neighbor trimming method (Yonezawa et al., 2013) to thin out densely sampled sequences without the sampling bias. The best-fit substitution model was estimated by the Bayesian Information Criterion using the MEGA and J-modeltest (Kumar et al., 2018; Posada, 2008). Phylogenic trees were constructed using nucleotide sequences of each of the gene of the SIVs (both reference viruses and from this study) by using the Bayesian Markov Chain Monte Carlo (MCMC) algorithm in MrBayes (Ronquist et al., 2012) for exploratory analysis and deeper analysis were conducted on phylogenic trees constructed using Beast program (Drummond et al., 2012). For the analysis in MrBayes, MCMC sampling was conducted in two simultaneous runs for 2,000,000 generations and an average standard deviation of split frequencies < 0.10. For the analysis in Beast, MCMC sampling was conducted in two simultaneous runs for 50,000,000 generations and an average standard deviation of split frequencies < 0.10. Posterior probabilities were calculated and checked for convergence with Tracer v1.7 software (Rambaut et al., 2018). The resulting tree was visualized and edited using FigTree software (Rambaut, 2009). After comparing the nucleotide similarity of the sequences obtained from this study, if one shared a >95% nucleotide similarity to other, then that sequence was considered as identical; thus, it was not displayed in the final phylogenetic tree for that respective gene. To assign genotype of the SIVs, lineages or clusters of each gene of the virus were assigned based on the comparison to reference viruses. After lineages or clusters of gene were assigned the combination of the eight lineages or clusters was assigned as genotype or genetic constellation of SIVs.

5.4.9. Ethics statement

This study was conducted under approval of Institutional Review Board of the National Swine Producers Association-PorkColombia (reference number 20111501). All animal procedures were performed in accordance with international ethical guidelines and standards and Colombian regulations for the use of animals in research.

Table 1. Reference sequences used for hemagglutinin and neuraminidase clade and lineage classification

Gene Type	Lineage		Accession no
HA1	Classical	Alpha (1A.1)	CY099119
		Beta (1A.2)	CY157999
		Gamma (1A.3)	CY158217
		Pandemic (1A.3.3.2)	NC026433
	Human-like	rH1N2 (1B.1)	KC866483.1
		Delta 1 (1B.2.2)	KC355809
		Delta 2 (1B.2.1)	JN652518
HA3	Cluster	I	AF251427
		II	AF268128
		III	AF268124
		IV	CY099027
	Novel human-origin		KP137795
NA	N1		GU236519.1
	N2	Swine H3N2 IV	KC866483.1
			CY099249
		Human H3N2	CY001105
		Human H1N2	DQ280226
		Swine TRIG H3N2	CY045585

5.5. Results

Phylogenetic diversity of the isolated SIVs

After RT-PCR testing, 672 out of the 10951 samples were positive to IAV, from which 483 samples had a Ct value below 35, as selection criteria for cell culture isolation. Out of the 483 clinical specimens inoculated in this study, 170 viruses were successfully isolated. Due to resources limitation, sequencing of the samples was conducted in two ways. Partial virus sequencing of HA and NA genes of the majority of the isolates was conducted in order to identify virus subtype. Then selection of sequences was conducted for whole genome sequencing (WGS). A list of all the specimens sequenced in this study can be found in supplementary table 1 (Table S1).

Out of the 170 clinical specimens isolated in this study, whole genome was sequenced from 162 while partial genome was sequenced from 8. To identify the subtype of the SIV isolates obtained in this study, nucleotide sequences of HA and NA genes were compared with

nucleotide sequences in the GenBank NCBI database using BLAST program. BLAST results revealed that most of the viral isolates were H1 subtype (95,3%, 162 out of 170), followed by H3 subtype (1,2%, 2 out of 170) and HA subtype could not be determined in 6 of the sequences due to bad quality. Additionally, BLAST results revealed that main NA subtype of the viral isolates was N1 (85,9%, 146 out of 170), followed by N2 (7,1%, 12 out of 170). Subtype for the NA gene could not be determined in 12 of the sequences due to bad quality. Sequencing results for both NA and HA genes from the same virus was only obtained in 89,4 % of the IAV isolates (152 out of 170). After analysis of the sequencing results, 82,4% (140 out of 170) of the specimens were identified as H1N1 subtype, 5,9% (10 out of 170) as H1N2 subtype, 1,2% (2 out of 170) as H3N2 subtype, but in the remaining specimens the subtype was not identified, finding 3,5% (6 out of 170) as HxN1, 7,1% (12 out of 170) as H1Nx. Tables 2 and 3 show additional summary details on the virus subtypes identified based on year of collection and place of origin.

The HA gene of the clinical specimens sequenced in this study displayed a similarity of 83.5%-99.9% while that for the NA gene; 84.5%-99.8%, when compared amongst themselves. We identified 21 isolates with >95% identity in both HA and NA genes, therefore these viruses were excluded in the selection for WGS. From WGS, we identified six isolates with bad quality results for PB2, PA and HA genes; thus, partial genome analysis of these viruses was conducted. Sequences from 31 (29,8%) isolates were obtained using Illumina sequencing technology while 72 (70,2%) sequences were obtained using Oxford Nanopore sequencing technology. Genome depth of coverage ranged in average from 88% to 99% for the different genes segments amongst the samples sequenced with a deep of sequencing from 88x to 3000x.

Phylogenetic tree of HA1 gene was constructed by comparing the isolates from this study to SIV-H1 refence viruses. As shown in Figure 1, the gene segment 4 of the specimens sequenced in this study clustered within the pdm clade (1A.3.3.2) of the CS lineage (Table 2). Generally, the specimens clustered according to a timeframe of one or two years of their year of isolation. It seems that CS clade pdm of endemic Colombian viruses in pigs are genetically similar to swine H1 viruses, circulating in North America. Phylogenetic tree of

HA3 gene was constructed by comparing the isolates from this study to SIV-H3 refence viruses. As shown in Figure 2, the gene segment 4 of the specimens sequenced in this study with North American lineage of the clade cluster II (Table 2). In contrast to that in Figure 1, specimen M12966 was isolated in year 2017, but clustered within the human lineage, together with some strains isolated in 2009 and 2012. No classical alpha, beta or gamma clades of HA1 nor cluster I, III, or IV of HA3 were identified in this study.

Additionally, phylogenetic tree of NA gene was constructed by comparing the isolates from this study to SIV-NA refence viruses. As shown in Figures 3, some specimens sequenced in this study clustered with NA1 pandemic lineage, while as shown in Figure 4, other specimens were clustered with NA2 subtype (Table 2). Therefore, some NAs were included in the clade of H1N1, which could be classified into the pandemic lineage while the others could be classified into the N2 lineage of human H3N2 and H1N2 viruses. Specimen M10721 clustered separate but together with some strains from similar years. These results suggest that the NA2 genes of the M10721 virus is phylogenetically distant from those of the other contemporary Colombian isolates and therefore have a different ancestry.

Phylogenetic trees of the internal genes (M, NP, PA, NS, PB1, PB2) of Colombian SIV were constructed by comparing SIV isolates from this study to 120 refence viruses, including CS, EA, TR, HU lineages and pdm IAV. Phylogenetic analysis of each internal gene showed that all internal genes of the SIV-H1N1, SIV-H1N2 and SIV-H3N2 from this study were clustered with pdm clade indicating that most if the SIV sequenced in this study acquired the backbone (internal genes) from pdmH1N1-2009 virus (Supplement figures 1-8).

Regarding the evolutionary structure of the inferred topologies from the HA gene, the analysis of the sequences indicated that the HA proteins of the H1 viruses from this study were found in a clade clustered with the Colombian H1N1 pdm influenza viruses isolated in swine between 2009 and 2015 (Figure 1). Interestingly the H3N2 isolate (M12966) from this study was found in a clade of H3 Cluster II influenza viruses closely related with a novel human origin virus isolated in 2012 in the United States (Figure 2). On the other hand, the evolutionary structure of the inferred topologies from the NA gene, the analysis of the

sequences indicated that the NA proteins of the N1 viruses from this study were found in a clade with the Colombian, Brazilian and Guatemala H1N1 swine influenza viruses isolated between 2009 and 2013 (Figure 3). As shown in Figure 4, the NA2 gene of the specimens sequenced in this study were clustered with the swine H3N2 clade IV (Table 2). The majority of the NA proteins of the N2 viruses from this study were found in a clade with the swine human-like H3N2 influenza viruses isolated from 2005, but one specimen (M10721) clustered with the human H1N2 influenza viruses isolated from 2003. None of the viruses clustered with the triple reassorting swine virus (TRIG H3N2).

Table 2. Classification of hemagglutinin and neuraminidase genes of SIV isolates obtained from this study.

Subtype	Lineage	Clade	N° sequences
H1	Classical	Alpha	0
		Beta	0
		Gamma	0
	Human-like	Delta 1	0
		Delta 2	0
	Pandemic	-	140
H3	Cluster	I	0
		II	1
		III	0
		IV	0
NA	N1	-	146
	N2	huH3N2	7
		huH1N2	11
		TRIGH3N2	0

After phylogenetic analysis of all gene segments of the sequences obtained for the H3N2 and H1N2 SIV isolated from pigs in this study, we found reassortment events between classical swine viruses with human viruses. The reassorting H3N2 (rH3N2) virus contained the HA gene genetically related to a novel reassorting human virus and the NA gene genetically related to a seasonal human virus, while the reassorting H1N2 (rH1N2) contained the NA gene genetically related to a seasonal human virus.

Table 3. Swine influenza A viruses identified by whole genome or partial sequencing, distributed by year and place of sample collection.

Subtype	Number of samples (percent)	Year of collection (n)	Place of collection –State (n)
ALL	170 (100%)	2011 (16)	Meta (16)
		2013 (14)	Antioquia (6); Caldas (8)
		2015 (8)	Antioquia (5); Caldas (3)
		2016 (92)	Antioquia (42); Atlántico (3); Caldas (4); Cundinamarca (11); Huila (3); Quindío (14); Meta (8); Risaralda (4); Santander (3)
		2017 (40)	Antioquia (3); Cauca (12); Cundinamarca (2); Quindío (3); Valle del cauca (20)
H1N1	140 (82,4%)	2011 (15)	Meta (15)
		2013 (5)	Caldas (5)
		2015 (6)	Antioquia (4); Caldas (2)
		2016 (81)	Antioquia (37); Atlántico (2); Caldas (3); Cundinamarca (10); Huila (3); Meta (5); Quindío (1); Risaralda (4); Santander (3)
		2017 (33)	Antioquia (3); Quindío (2); Valle del cauca (17); Cauca (10); Cundinamarca (1);
H1N2	10 (5,9%)	2016 (7)	Antioquia (2); Atlántico (1); Cundinamarca (1); Meta (3)
		2017 (3)	Cauca (1); Valle del cauca (2)
H3N2	2 (1,2%)	2017 (2)	Cauca (1); Cundinamarca (1)
HxN1	6 (3,5%)	2016 (4)	Antioquia (3); Caldas (1)
		2017 (2)	Cauca (1); Cundinamarca (1)
H1Nx	12 (7,1%)	2011 (1)	Meta (1)
		2013 (9)	Antioquia (6); Caldas (3)
		2015 (2)	Antioquia (1); Caldas (1)

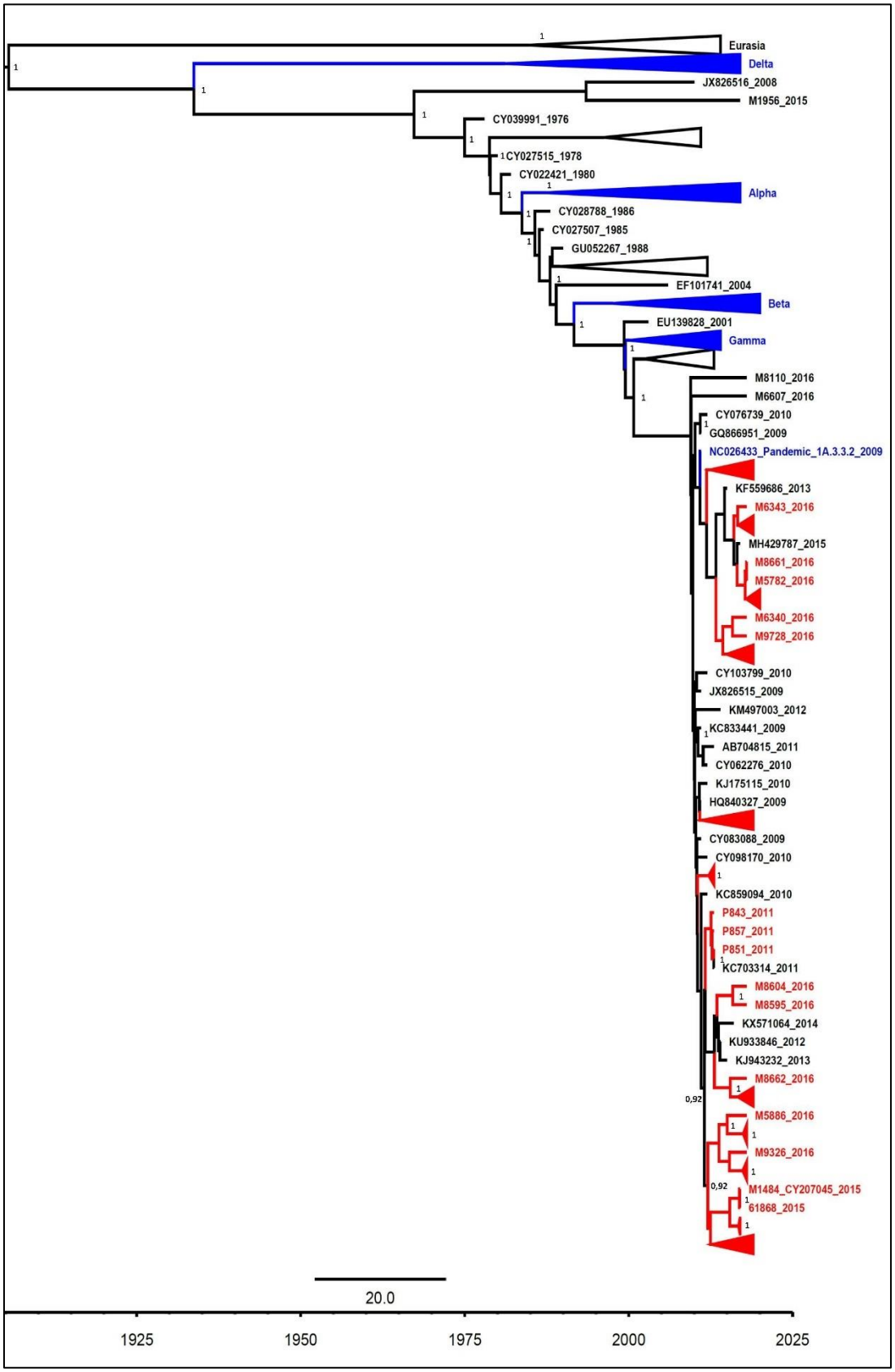
Eurasia
Delta
JX826516_2008
M1956_2015
CY039991_1976
CY027515_1978
CY022421_1980
Alpha
CY028788_1986
CY027507_1985
GU052267_1988
EF101741_2004
Beta
EU139828_2001
Gamma
M8110_2016
M6607_2016
CY076739_2010
GQ866951_2009
NC026433_Pandemic_1A.3.3.2_2009
KF559686_2013
M6343_2016
MH429787_2015
M8661_2016
M5782_2016
M6340_2016
M9728_2016
CY103799_2010
JX826515_2009
KM497003_2012
KC833441_2009
AB704815_2011
CY062276_2010
KJ175115_2010
HQ840327_2009
CY083088_2009
CY098170_2010
KC859094_2010
P843_2011
P857_2011
P851_2011
KC703314_2011
M8604_2016
M8595_2016
KX571064_2014
KU933846_2012
KJ943232_2013
M8662_2016
M5886_2016
M9326_2016
M1484_CY207045_2015
61868_2015
0,92
0,92
20.0
1925
1950
1975
2000
2025

Figure 1. Phylogeny of HA1 gene of SIV isolated in Colombia inferred using Bayesian analysis. Taxa sequenced obtained in this study are highlighted in red, while those in black and blue were obtained from the Influenza Virus Database. Highlighted in blue are referece sequences for clasification of the subtype. The phylogenetic tree was constructed using Beast program by Bayesian Markov-Chain Monte Carlo (BMCMC) with 50,000,000 generations and an average standard deviation of split frequencies < 0.10. Posterior probability and times of most recent common ancestor among H1-IAV are shown in the branch nodes. Sequences from this study sharing >95% similarity with other sequences were collapsed.

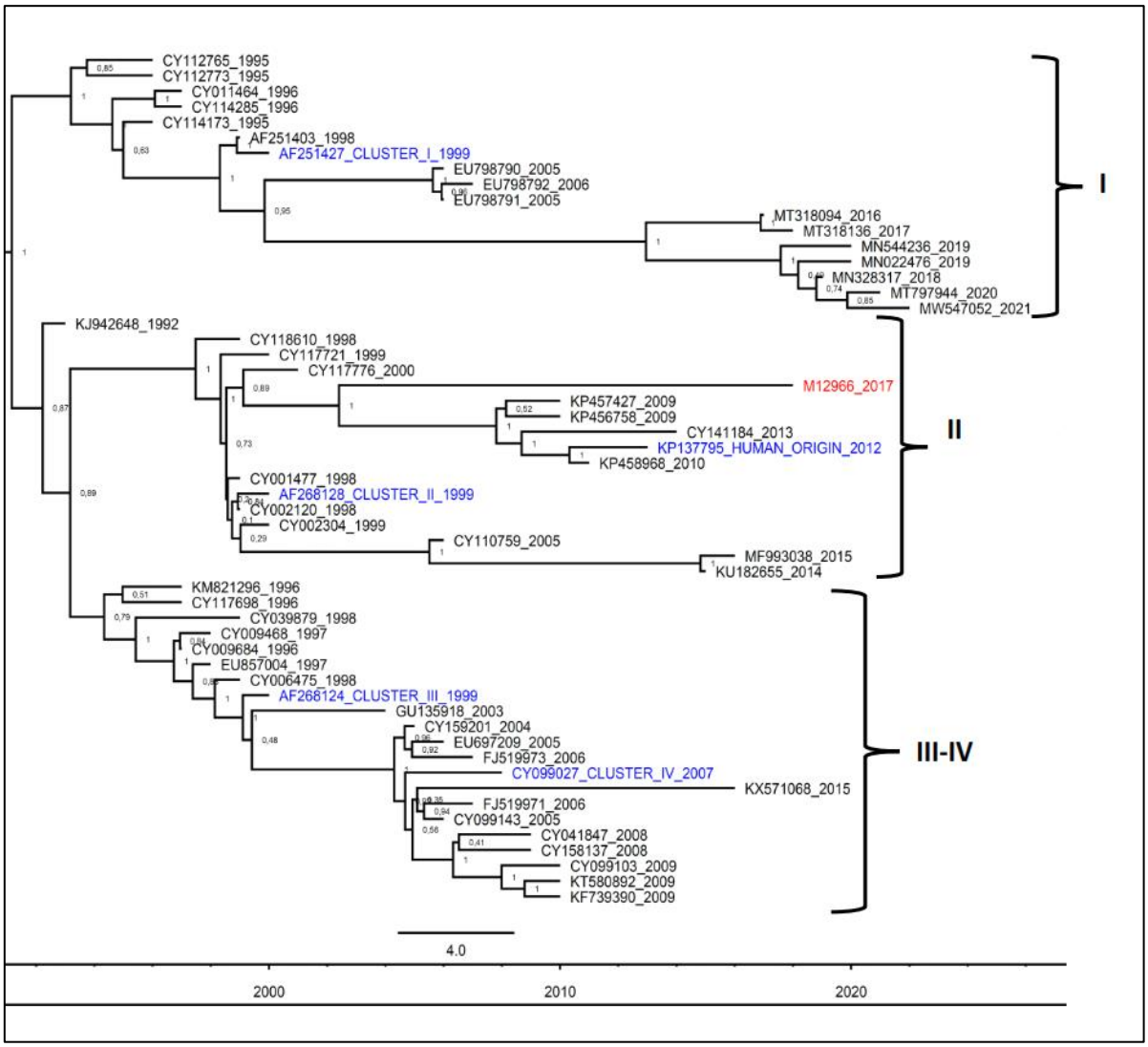

Figure 2. Phylogeny of HA3 gene of SIV isolated in Colombia inferred using Bayesian analysis. Taxa sequenced obtained in this study are highlighted in red, while those in black

and blue were obtained from the Influenza Virus Database. Highlighted in blue are referece sequences for clasification of the subtype. The phylogenetic tree was constructed using Beast program by Bayesian Markov-Chain Monte Carlo (BMCMC) with 50,000,000 generations and an average standard deviation of split frequencies < 0.10. Posterior probability and times of most recent common ancestor among H3-IAV are shown in branch nodes.

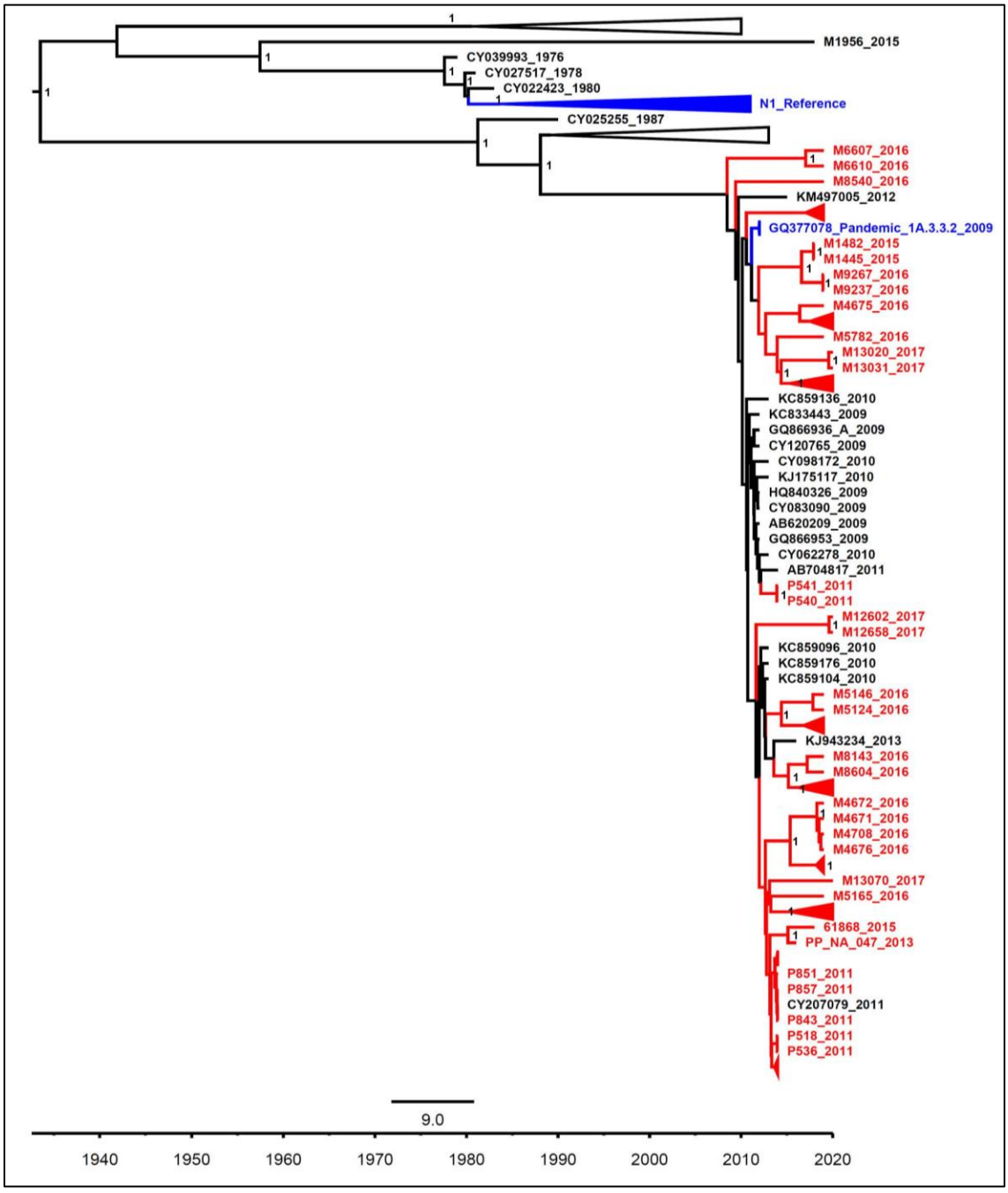

Figure 3. Phylogeny of NA1 gene of SIV isolated in Colombia inferred using Bayesian analysis. Taxa sequenced obtained in this study are highlighted in red, while those in black and blue were obtained from the Influenza Virus Database. Highlighted in blue are referece sequences for clasification of the subtype. The phylogenetic tree was constructed using Beast program by Bayesian Markov-Chain Monte Carlo (BMCMC) with 50,000,000 generations and an average standard deviation of split frequencies < 0.10. Posterior probability and times of most recent common ancestor among NA-IAV are shown in branch nodes. Sequences from this study sharing >95% similarity with other sequences were collapsed.

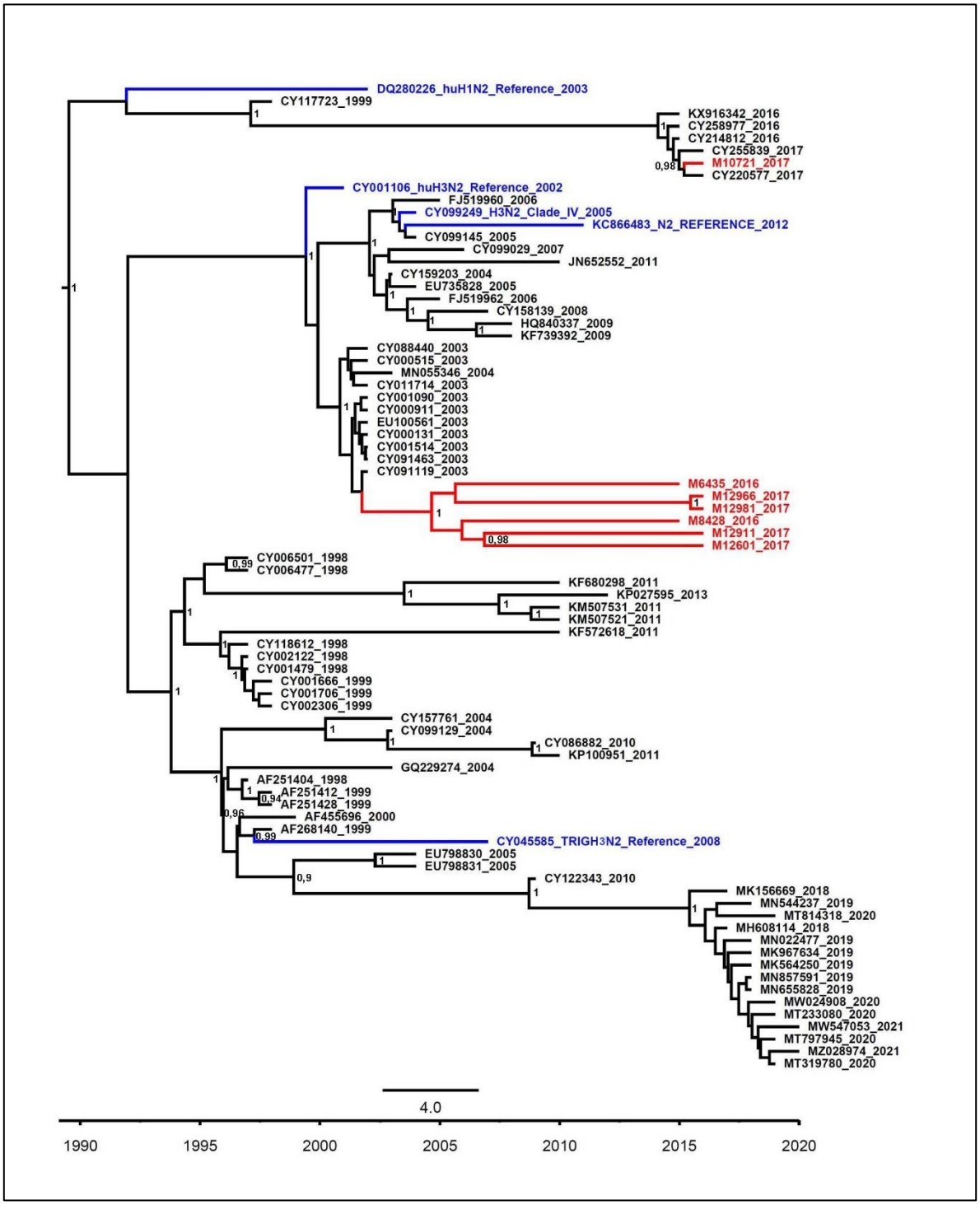

Figure 4. Phylogeny of NA2 gene of SIV isolated in Colombia inferred using Bayesian analysis. Taxa sequenced obtained in this study are highlighted in red, while those in black and blue were obtained from the Influenza Virus Database. Highlighted in blue are reference sequences for clasification of the subtype. The phylogenetic tree was constructed using Beast

program by Bayesian Markov-Chain Monte Carlo (BMCMC) with 50,000,000 generations and an average standard deviation of split frequencies < 0.10. Posterior probability and times of most recent common ancestor among NA-IAV are shown in branch nodes.

Genotypic diversity of the isolated SIVs

Gene constellation of the SIAVs was identified by using the combination of eight gene segments of the viruses isolated from the pigs. From the sequencing data, different genotypes (A to G) were observed across Colombian farms (Figure 4). We observed that most of the genotypes (A to C), contained all internal genes derived from A(H1N1)pdm09, while only few genotypes (B and C) contained either HA or NA genes derived from human lineages (Table 1). Overall genotypes identified were: (1) H1N1 contained all eight genes from CS pdm virus; (2) rH3N2 (pdm+2) contained six internal genes from CS pdm virus and HA3 and NA2 from human-like virus; (3) rH1N2 (pdm+1) contained NA2 from human-like virus and the other seven genes from pdm virus; Our results revealed that internal genes of pdm virus persist and become predominant lineage in the Colombian SIV, and reassorting events between human and classical swine viruses are occurring.

Genotype	PB2	PB1	PA	HA	NP	NA	M	NS	Total count	Count per year of collection 2011	2013	2015	2016	2017
A									133	10	4	5	80	33
B									1	0	0	1	0	0
C									8	0	0	0	6	2
D									7	4	2	0	0	1
E									1	0	0	0	1	0
F									11	1	7	2	0	1
G									1	0	0	0	1	0
H									6	0	0	0	4	2
I									1	1	0	0	0	0
J									1	0	1	0	0	0
Total									170	16	14	8	92	40

Pandemic 09 | Human-like H1N2 | Human-like H3N2

Figure 5. Genotypes of genome sequences of viruses isolated from Colombian swine. The origin of each gene segment is color coded according to the key. Abbreviations: PB2,

polymerase basic 2; PB1, polymerase basic 1; PA, polymerase acidic; HA, hemagglutinin; NP, nucleoprotein; NA, neuraminidase; M, matrix; NS, nonstructural. White boxes indicate no sequence was obtained for that gene segment.

5.6. Discussion

We conducted a surveillance study in several Colombian swine farms between January 2011 to June 2017 in different regions of the country. In this study a 6.14% SIV positivity of virus detection was observed by RT-PCR testing in the swine herds, which is consistent with percentages reported in other surveillance studies (Bakre et al., 2020; Corzo et al., 2013; Kyriakis et al., 2017). Three subtypes of SIVs (H1N1pdm, rH3N2 and rH1N2) were observed for the viruses isolated from pigs. Notably, SIV H3N2 and H1N2 had lower frequency of detection than SIV-H1N1 in our study. For the first time isolation of SIV rH1N2 and rH3N2 is reported for pigs in Colombia. Additionally, full genome analysis of H1N1pdm09, H3N2 and H1N2 isolates from pigs were reported in this study. H1N1 SIV from this study were most similar to IAV H1N1pdm09 sequences from human hosts, and phylogenetically supported with the close similarities to viral sequences from human and swine also isolated in Colombia. The full genome of H3N2 and H1N2 isolates from pigs that were analyzed in this study were most like to IAV sequences from human hosts.

To clarify the genetic relationships of the genes of the representative swine Colombian isolates with the counterparts of swine, human, and avian isolates, the determined gene sequences were deeper phylogenetically analyzed. The phylogenetic tree of the H1 HA genes (Fig. 1) revealed that most viruses are closely related to CS H1N1 lineage. Additionally, swine Colombian viruses are genetically similar from general swine H1 virus lineages, circulating in North, Central and South America. However, these viruses share in overall 95.7 to 99% homology with each other and 97.8 to 99% homology with the genetically closest isolates references of the HA gene sequences. These findings suggest that the ancestor of the HA1 genes of these viruses was introduced from human populations after 2009 and have circulated and stablished in the pig populations since then.

We observed a similar efficiency of the WGS for both technologies applied, while reduced cost and laboratory work was observed for the pipeline applied using ONT. However, statistical comparison of these two methods was not conducted since that was not the main goal of this work. On the other hand, studies have documented the use of these two different sequencing platforms to generate full length virus sequences from clinical samples. For example, McNaughton and col. (McNaughton et al., 2019) compared these two WGS methods for Hepatitis B virus, reporting that the Illumina protocol allowed the determination of diversity and detection of minor variants, while in contrast, the Nanopore protocol allowed to obtain an accurate full-length consensus sequence for diagnostic purposes. Additionally, Wang and col. (Wang et al., 2015) compared these methods for H3N2 Influenza virus WGS, reporting that coverage was similarly achieved with both technologies, however, the coverage for the Illumina protocol was higher than for the ONT MinION, due to the higher number of reads generated in the Illumina MiSeq run. In our work the Illumina MiSeq dataset showed 100% identity with the ONT MinION sequence genome of five selected influenza virus samples.

Whole genome phylogenetic analysis of Colombian SIV showed that H3 and N2 genes of these viruses were clustered with human-like viruses that introduced in pigs during 2012. Co-circulation of H1N1pdm09, H3N2 and H1N2 subtypes facilitated exchange of viral segments. In our study H3N2 and H1N2 viruses were still clustered with human-like viruses but had a combination of surface genes (HA and NA) from endemic IAVs and internal genes (backbone) from pdm-H1N1-09. The phylogenetic tree of the H3 HA genes revealed that swine Colombian viruses are genetically similar from human H3 virus lineages, circulating in North America in 2012. These findings suggest that the ancestor of the HA3 gene of these viruses was introduced from human populations around the end of the 20th century and has circulated and evolved in pig populations in Colombia for more than a decade. Interestingly, co-circulation of H1N1, H1N2 and H3N2 subtypes of IAV was observed in at least one region evaluated in this study, which may create an increased potential risk for more reassortment events and emergence of novel IAV viruses.

Phylogenetic analysis of HA genes showed that Colombian SIVs belonged to distinct lineages, including Classical swine pdm clade, H1N2 huma-like and novel H3 II clade of human origin. Based on the MCC phylogenetic tree, the H1 gene of SIV in this study have not separated yet from pdmH1N1 since 2009, while N1 gene of SIVs in this study was closely related to pdm lineage, and N2 gene of SIVs was closely related to human-like lineage. This result suggested that pdmH1N1-2009 have circulated in swine herds for a long period of time or was repletely introduced into the pig farms as reported for other areas of the world (Cador et al., 2017). The repeated transmission of human seasonal viruses to pigs has resulted in the establishment of several human-origin virus lineages globally, adding to the antigenic diversity of swine viruses. In this sense, global antigenic diversity characterization of H1 and H3 viruses circulating in pigs has revealed that it is a result of the frequent introductions of human-origin IAV into swine (Lewis et al., 2016). This finding is in contrast, with other reports for swine-producing areas in the world such as USA, where more lineages including classical alpha and beta H1 HAs had dominance and prevail over others (Bakre et al., 2020).

The genetic makeup of the pandemic H1 HAs analyzed in this study and their close relationship with seasonal human IAVs, may suggest that these viruses do not continuously circulate within the swine population, but are rather a result of a single or multiple reverse zoonotic events as it has been observed before (Nelson et al., 2015b). However, in several locations have been confirmed persistency of H1N1pdm09 infections in swine, and we observed that after four years of the pandemic 2009, many swine viruses have persisted as whole H1N1pdm09 in the Colombian pig populations. Thus, to avoid the constant reintroductions of pdmH1N1 viruses from humans to swine and more reassortment between human and swine IAVs, it is crucial that the Colombian pork industry to strength preventive biosecurity measures such as avoiding workers with influenza-like symptoms to get in close contact with animals and vaccination of swine workers.

The NA2 gene of swine IAV diverged into various clades, which resulted from reassortments events with seasonal human viruses (Nelson et al., 2011). The reassortment event in 1998 introduced human genes into the swine viruses leading the emergence of the TRIG H3N2 lineage in pigs (Zhou et al., 1999). Around 2000´s occurred a reassortment of TRIG H3N2

viruses with the NA of human H3N2 viruses which established another NA swine lineage, named clade IV (Nelson et al., 2011). In our phylogenetic tree of the NA genes, a similar pattern was observed; evolved from the NA of the human virus, most NAs were included in the clade of H3N2 human viruses, but they were more closely related to the N2 lineage of swine human-like H3N2 clade IV viruses, while one specimen was included in the clade the N2 lineage of human H1N2 viruses. All of the Colombian isolates from 2016–2017 were rooted in the N2 lineage of the human virus. These results suggest that the NA genes of the Colombian swine IAV obtained in this study are phylogenetically distant from those of the other contemporary isolates.

Reassortment events between H1N1pdm09 viruses and endemic swine IAV have been reported worldwide (Everett et al., 2020; Mon et al., 2020), and viruses containing different constellations of H1N1pdm09 internal genes are now predominant among IAV circulating in swine in several regions (Liang et al., 2014; Nasamran et al., 2020; Rajao et al., 2019). The detection in pigs of reassorting H1N2 and H3N2 containing genes from the H1N1pdm09 virus in Colombia, increases the awareness because persistence of pdm-09 genes for long periods in swine farms could contribute to the generation of novel viruses with higher infectivity and transmissibility for both pigs and humans (Chastagner et al., 2019). Influenza reassortment is an ongoing process, that both swine and humans can become infected with novel viruses, and that these viruses may have the potential to cause future pandemics (Komadina et al., 2014). For example, reassortment of the matrix gene of pdm-09, has been associated with high transmission efficiency (Chou et al., 2011). A genotype of particular note is the North American A(H3N2)v strain that has been associated with multiple swine-to-human zoonoses (Wong et al., 2012); which contain human-derived H3 and N2 glycoproteins that have since evolved within swine, and both contain internal gene cassettes with an acquired pdm09 matrix protein. A recent experimental study conducted in China showed also how an avian-like rH1N1 SIV (with pdm-H1N1-09 backbone) increased infectivity and pathogenicity in an animal model (Sun et al., 2020). In this study, there were 3 genotypes identified (H1N1 pdm; rH1N2; rH3N2). rH1N2 and rH3N2 genotypes identified in this study have been reported in the world, indicating that these genotypes are stable and continuously circulating in pig farms. On the other hand, rH3N2 and H1N2 has never been

reported in Colombia. In Latin America genetic reassorting events between lineages have been detected in swine viruses. rH1N1 y rH1N2 subtypes were identified in swine in Argentina, Chile and Brazil, which had a backbone from pdm while HA and NA had similarities to seasonal human viruses (Pereda et al., 2011; Schaefer et al., 2015; Tapia et al., 2020). Since most of the SIVs sequenced and reported in this study contained backbone (internal genes) from pdm-H1N1-09, it could be speculated that the virus persisted and became a predominant lineage of SIVs in Colombia, which is a great concern because it could increase the chance of the virus to expand diversity and evolve rapidly. Therefore, these Colombian constellations therefore poses an increased public health risk, particularly as its antigenic proteins have been evolving within swine for many years, so humans will likely be immunologically naive against these viruses (Watson et al., 2015). Because of the possible public health interest in these genotypes, it is highly recommended to follow up on the epidemiology of these viruses in both human and swine populations in the country.

The findings of our study are significant for future proper vaccine selection. Swine in Colombia are not currently immunized with IAV vaccines; however, for future vaccines it is crucial that they contain the HA lineage strains that are circulating in the region. Commercial swine IAV vaccines contain between two and six inactivated strains (Van Reeth & Ma, 2012), which offer protection against viruses within the same HA lineage. Additionally, possible mismatch between vaccine and wild type HA may lead to vaccine-associated enhanced respiratory disease (Rajão et al., 2016). Thus, long-term, and active IAV surveillance studies in swine are needed for monitoring viruses that can rapidly spread on a regional level, and also for selecting adequate biosecurity measures to prevent and control virus spread.

5.7. Conclusions

Active surveillance for IAV in swine in Colombia revealed that H1N1pdm09 viruses are seem to become endemic in swine farms in the country, where reassorting events and occurring with other endemic IAV human viruses. After genetic characterization of circulating swine IAV, reassortment events were identified where higher prevalence of H1N1

over H3N2 and H1N2 viruses was observed. Thus, these findings have key implications for influenza surveillance and public health, as well as for future vaccine selection, highlighting the importance of biosecurity, even more in places such as Colombia because there are not influenza vaccine programs currently implemented in the swine farms.

5.8. Declarations

Author contributions

First author performed study design, sample collection, laboratory testing, data analysis and was responsible for the first written draft of the manuscript alongside further editing and figure work. AC, SD, SV and ML produced, analyzed, and curated the sequencing data alongside editing process of the manuscript and figure work. LP produced, analyzed laboratory data alongside editing process of the manuscript and figure work. JO participated in the conceptualization, in funding acquisition and supervision and was a major contributor in the editing of the manuscript. JH contributed to the formal data analyses, funding acquisition alongside editing process of the manuscript. All authors performed data analysis, reviewed, and approved the manuscript.

Competing interests

The authors declare no conflict or competing interests.

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CAPÍTULO VI. CONCLUSIONES Y RECOMENDACIONES

6.1. Conclusiones

De las actividades de investigación de este trabajo se logró demostrar la importancia de la vigilancia del virus influenza en poblaciones de porcinos como una base fundamental y estratégica para determinar las características epidemiológicas y genéticas de las variantes o cepas virales que circulan en nuestro país y que pueden representar una amenaza para la salud animal y la salud pública.

Los hallazgos de estos estudios han proporcionado una información fundamental sobre el uso de fluidos orales como una herramienta útil para la detección molecular y el monitoreo del virus influenza en granjas porcinas sobre todo en las principales regiones productoras de cerdos en Colombia. Con esta técnica se demostró la aplicación de un método de muestreo simple basado en grupos de animales, como una herramienta rutinaria, eficiente y rentable para la vigilancia activa de enfermedades virales porcinas en la región. Los resultados adicionalmente sugirieron que el virus podría estar representando una causa común de enfermedad respiratoria en las granjas porcinas y que en algunas granjas la infección puede estar ocurriendo de forma endémica, sin embargo, muchos otros factores no investigados, como la coinfección con otros patógenos, podrían estar jugando un papel importante en la epidemiología de esta enfermedad en estas granjas. Por lo tanto, el uso rutinario de esta técnica de muestreo facilita la detección de patógenos a nivel de hato y contribuye de forma eficiente en el monitoreo y control de enfermedades virales porcinas de gran importancia para el país.

Por otra parte, la aplicación de encuestas epidemiológicas y de técnicas de análisis multivariadas en los datos obtenidos, son herramientas muy útiles para analizar conjuntos de datos complejos y grandes obtenidos de granjas porcinas, lo que permite identificar perfiles y caracterizar los sistemas de producción porcina basados en las variables de interés, para el caso particular, medidas de bioseguridad y prácticas de manejo pecuario. Además, estos perfiles de granjas y su estructura se pueden utilizar más para estimar las probabilidades de detección de patógenos e identificar elementos clave para diseñar estrategias apropiadas para

el monitoreo y control de enfermedades en los cerdos. Con los resultados de este estudio se incrementó por tanto el conocimiento de las diferentes características de las explotaciones porcinas del país, su manejo tradicional y las prácticas de bioseguridad aplicadas, que describen y se asocian con la estructura de los principales sistemas de producción porcina en Colombia. Además, el estudio reveló y destacó posibles patrones asociados con la detección del virus influenza en cerdos. En consecuencia, la metodología y el enfoque aplicado para la recopilación y el análisis de datos complejos de granjas porcinas puede ser una herramienta prometedora y de fácil uso para identificar brechas y evaluar la bioseguridad en las granjas porcinas colombianas.

Posterior al análisis de las secuencias genómicas completas de la gran parte de los aislados virales obtenidos en este trabajo, se encontró que la mayoría estaban filogenéticamente relacionados con el virus pandémico H1N1, sin embargo, se demostró la detección y aislamiento de virus que pertenecían a los subtipos H3N2 y H1N2. Adicionalmente, se detectaron posibles eventos de recombinación genética en algunos de los virus examinados. Algo importante que se observó es que la mayoría de los virus analizados mantienen genes internos de origen pandémico y este hecho resalta la importancia de vigilar muy de cerca la evolución de estas cepas pues la presencia de estos segmentos facilita en gran medida el salto inter-especie y aumenta el riesgo de aparición de nuevas variantes con potencial pandémico. Por otra parte, se analizó a detalle que existen algunas granjas con circulación de más de un subtipo, hecho que aumenta las posibilidades de recombinación genética de los segmentos virales y emergencia de nuevas variantes frente a las cuales no existe memoria inmune.

La recombinación genética entre virus humanos, de aves o porcinos en cerdos han sido documentados repetidamente en el pasado. Estos virus recombinantes han fundamentalmente alterado la epidemiología de la influenza porcina en muchas partes del mundo. Además, las pandemias humanas de 1957 y 1968 fueron originadas por recombinación del virus, aunque no puede ser probado que dicha recombinación ocurrió en cerdos. La más recientemente pandemia del 2009 se originó como resultado de recombinación de virus porcinos de diferentes linajes, aunque tampoco puede probarse que este evento de recombinación ocurrió en cerdos o personas.

Los datos epidemiológicos son fundamentales para comprender la distribución y variación regional de IAV y ayudan a determinar una respuesta adecuada ante los brotes y permiten comprender la naturaleza de las epidemias y zoonosis de IAV porcina. Con los hallazgos obtenidos de este trabajo de doctorado, junto con las evidencias dadas por otros estudios previos, puede considerarse que la IP es una enfermedad prevalente en la producción de porcina colombiana. Con este trabajo de investigación se permitió detectar los tres subtipos (H1N1, H1N2 y H3N2) comúnmente identificados en cerdos en el mundo y conocer su composición genómica. Entre estos los H1N1 pandémico, fue el de mayor frecuencia de detección o aislamiento en estas poblaciones de cerdos evaluadas. Es importante resaltar también que es el primer reporte de aislamiento de virus reordenados H1N2 y H3N2 en cerdos en el país. A la actualidad también se sabe que, desde la emergencia y aparición del virus influenza pandémico H1N1 en la población porcina de nuestro país, se ha convertido en el subtipo más predominante por detección molecular, esto resalta mucho la importancia de la vigilancia de estos virus en cerdos, sobre todo por la facilidad de mutación y emergencia de nuevas cepas con potencial pandémico. La circulación de esta cepa en cerdos favorece las altas tasas de mutación y evolución del virus y su presencia en la población porcina debe considerarse como un llamado para incrementar los esfuerzos de investigación epidemiológica para prevenir los próximos brotes.

El hecho de detectar el IAV en porcinos en distintas regiones del país demuestra la continua y amplia circulación de estos virus en la población porcina colombiana, de notable importancia sobre todo en las tres principales regiones productoras de cerdo (Cundinamarca, Valle y Antioquia). Con base en los análisis genómicos y filogenéticos, se podría decir que se han producido eventos de reordenamiento entre cepas porcinas endémicas y a cepa pandémica, considerando que la mayoría de los genes internos de los aislamientos colombianos un alto grado de identidad con el virus pandémico, pero los genes de las proteínas de superficie como HA y NA estaban relacionados con virus de influenza porcina clásica u otros subtipos.

La forma más básica de control de IAV en granjas porcinas es a través de la implementación de buenas prácticas de producción. Para controlar la transmisión entre granjas, las medidas de bioseguridad, como la cuarentena de los cerdos y la implementación de sistemas robustos de salud y seguridad para los trabajadores, ayudan en gran medida a reducir la probabilidad de presentación endémica de IAV porcina. Esto acompañado de programas de vigilancia de IAV robustos que complementan estrechamente las prácticas físicas en las granjas.

Entre las medidas de intervención sanitaria en los cerdos se encuentra la vacunación, una medida que enfrenta grandes retos intrínsecos para garantizar una respuesta inmune contra un virus altamente mutable. A la actualidad es la mejor herramienta disponible para controlar IAV en cerdos, sin embargo, se debe tener muy presente que las vacunas usadas sean homologas a los virus que circulan regionalmente. Debido a que la IAV per se generalmente tiene bajas tasas de mortalidad en cerdos, existe una resistencia a la aceptación de la vacunación.

6.2. Recomendaciones

Los hallazgos de esta investigación en conjunto permiten demostrar la necesidad de un esquema robusto de vigilancia activa del IAV en los sistemas de producción porcina en Colombia, que ayude a anticiparse a la emergencia y diseminación de variantes virales con potencial pandémico. Por otra parte, el hecho de que exista un establecimiento de virus de origen humano en las poblaciones de cerdos también implica que es necesario vigilar la epidemiologia de este patógeno en las personas con algún tipo de contacto (directo o indirecto) con estos animales, pues está estrechamente relacionada.

Por otra parte, el virus influenza ha sido asociado como uno de los patógenos que hace parte del complejo respiratorio porcino aumentado sus implicaciones en la salud animal y que a su vez por su carácter zoonótico tiene grandes repercusiones en la salud pública. Como hemos evidenciado los patógenos con potencial pandémico no tienen grandes barreras que le impidan causar efectos nocivos a nivel global, y en este sentido los directores técnicos y gerentes de las granjas porcinas están obligados a tener una visión más amplia en el manejo de las enfermedades, y no solo desde un entorno local sino hacia uno regional, nacional y

mundial que les permita atender oportunamente los retos sanitarios a los que se enfrentan y a partir de esto planear una mejora continua que garantice la competitividad sanitaria e inocuidad de la producción. Adicionalmente, la sanidad de una granja debe ir bajo un enfoque integral que involucre a los demás pilares de la producción porcina como son la alimentación, el manejo, las instalaciones, etc. No se puede desbalancear ninguno de estos factores ya que de nada sirve cumplir con todas las normas de sanidad y bioseguridad, si por ejemplo se falla en la alimentación del animal. Por tanto, basado en los hallazgos y resultados de este trabajo de investigación se recomienda a los productores porcinos colombianos avocarse a una mirada más integral de la sanidad de las granjas porcinas que abarque no solo el contexto de la salud animal sino su interrelación con la salud humana y del ecosistema, y la unión de otros factores en la producción, de modo que se identifiquen puntos críticos en cada uno de estos aspectos para robustecer la bioseguridad en esta triada de salud y reducir así el impacto de las enfermedades.

Adicionalmente, este trabajo demuestra como la bioseguridad es un tema prioritario y que, aunque parece ser usualmente abordado por los productores porcinos, se detectan aun fallas en estas prácticas de producción que permiten que aun exista transmisión y diseminación de la influenza porcina entre las granjas. Por tanto, es muy recomendable ampliar las campañas de educación y sensibilización a los productores porcinos sobre las implicaciones y beneficios en el control y prevención de IAV en sus granjas porcinas. De igual manera es también altamente recomendable explorar estrategias de prevención más eficaces como lo es la vacunación, sin embargo, cabe resaltar que ésta debe ir acorde y ser homologa a las cepas circulantes en el país para que garantice su efectividad y eficacia.

6.3. Perspectivas futuras

De este trabajo surgen nuevas preguntas de investigación y aspectos acerca de la enfermedad que conllevan a ideas para abordar en un futuro. Estas incluyen el desarrollo de una vacuna universal contra IAV en cerdos, el análisis de la diversidad de epítopos antigénicos, analizar las características antigénicas y de patogenicidad de estas cepas circulantes, empleando modelos *in vitro* o *in vivo*, el análisis filogeográfico de los virus en cerdos. También entender el papel de IAV en la presentación conjunta con otros patógenos del complejo respiratorio

porcino, y el impacto que puede tener en salud publica en Colombia, entre otros muchos estudios.

ANEXOS

Aval institucional y de ética

Anexos del capítulo IV:

S1 Base datos usada para el MCA de la encuesta (XLSX)

S2 Base datos usada para el HCA de la encuesta (XLSX)

S3 Base datos usada para el GLM de la encuesta (XLSX)

S1 Formulario de la encuesta (PDF)

Anexos del capítulo V:

Tabla S1. Base de datos de los especímenes secuenciados (XLSX)

Figura suplementaria 1. Filogenia del gen HA1 de los aislados virales obtenidos. Árbol extendido (PDF)

Figura suplementaria 2. Filogenia del gen NA1 de los aislados virales obtenidos. Árbol extendido (PDF)

Figura suplementaria 3. Filogenia del gen M de los aislados virales obtenidos. Árbol extendido (PDF)

Figura suplementaria 4. Filogenia del gen PB1 de los aislados virales obtenidos. Árbol extendido (PDF)

Figura suplementaria 5. Filogenia del gen PB2 de los aislados virales obtenidos. Árbol extendido (PDF)

Figura suplementaria 6. Filogenia del gen NS de los aislados virales obtenidos. Árbol extendido (PDF)

Figura suplementaria 7. Filogenia del gen PA de los aislados virales obtenidos. Árbol extendido (PDF)

Figura suplementaria 8. Filogenia del gen NP de los aislados virales obtenidos. Árbol extendido (PDF)

Figura suplementaria 9. Filogenia del gen NA2 de los aislados virales obtenidos. Árbol extendido (PDF)

Bogotá, 02 de marzo de 2017

Señores Convenio
WISCOL COLOMBIA/ UNIVERSIDAD WISCONSIN/ CORPOTROPICA
Atn Jorge Osorio / Karl Ciuoderis
Medellín

Reciban un cordial saludo:

La Asociación Porkcolombia – Fondo Nacional de la Porcicultura, se encuentra en ejecución del CONVENIO PARA EL DESARROLLO DE ACTIVIDADES CIENTÍFICAS Y TECNOLÓGICAS No. 2016-258, suscrito con el INSTITUTO WISCONSIN COLOMBIA y cuyo objeto es aunar esfuerzos técnicos, científicos, operativos y académicos entre la Asociación Colombiana de Porcicultores - FNP y el Instituto Wisconsin Colombia/ Universidad de Wisconsin, para desarrollar estudios con los que se fortalezca el conocimiento sanitario de las enfermedades infecciosas, tales como el virus de Influenza y otras enfermedades de importancia en porcinos en Colombia.

Teniendo en cuenta lo anterior, autorizamos al grupo de investigación, en representación de la Universidad de Wisconsin/ Instituto WISCONSIN COLOMBIA, el acceso al banco de muestras colectadas dentro del marco del Convenio antes mencionado, según lo establecido en la **CLÁUSULA SEGUNDA. — OBLIGACIONES DE EL INSTITUTO** y la **CLÁUSULA TERCERA. — OBLIGACIONES DE ASOCIACIÓN COLOMBIANA DE PORCICULTORES – FNP**, así como lo estipulado según **PARÁGRAFO** de la **CLÁUSULA DÉCIMA.** — **NATURALEZA DEL CONVENIO.** Esta autorización permite a su vez el almacenamiento, uso, procesamiento y posterior análisis de muestras procedentes del convenio para estudios de investigación con base en la aprobación de los propietarios de los animales (consentimientos informados), así como en las consideraciones bioéticas y de buenas prácticas de laboratorio que den a lugar.

Cordialmente,

CLEMENCIA MEJIA GARCIA
Directora Área de Erradicación PPC y Sanidad

Elaborado por: Wilmer Silva

Medellín, 02 de septiembre de 2021

Doctores
Carlos Muskus
Jorge Osorio
Grupo de Investigación PECET
Universidad de Antioquia

Proyecto*: "CARACTERIZACION MOLECULAR Y EPIDEMIOLOGIA DEL VIRUS INFLUENZA TIPO A EN PORCINOS DE COLOMBIA"*

Resultado de la revisión: Otorgar aval[1]

Cordial saludo.

Luego de estudiada su solicitud al proyecto de la referencia, el **Comité de Ética para la Experimentación con Animales** le expresa que, se otorga el aval ético solicitado, tal y como constará en el acta de sesión N° 142 del próximo 05 de octubre de 2021.

Con toda atención.

JOSÉ IGNACIO CALLE POSADA

JOSÉ IGNACIO CALLE POSADA
Coordinador
Comité de Ética para la Experimentación con Animales
Universidad de Antioquia

[1] *El aval otorgado hace referencia única y exclusivamente al proyecto y/o a los procedimientos que se mencionan, además será válido solamente por el tiempo que dure (n) este (os).*

Comité de Ética para la Experimentación con Animales (CEEA)| [57+4] **2196612** |
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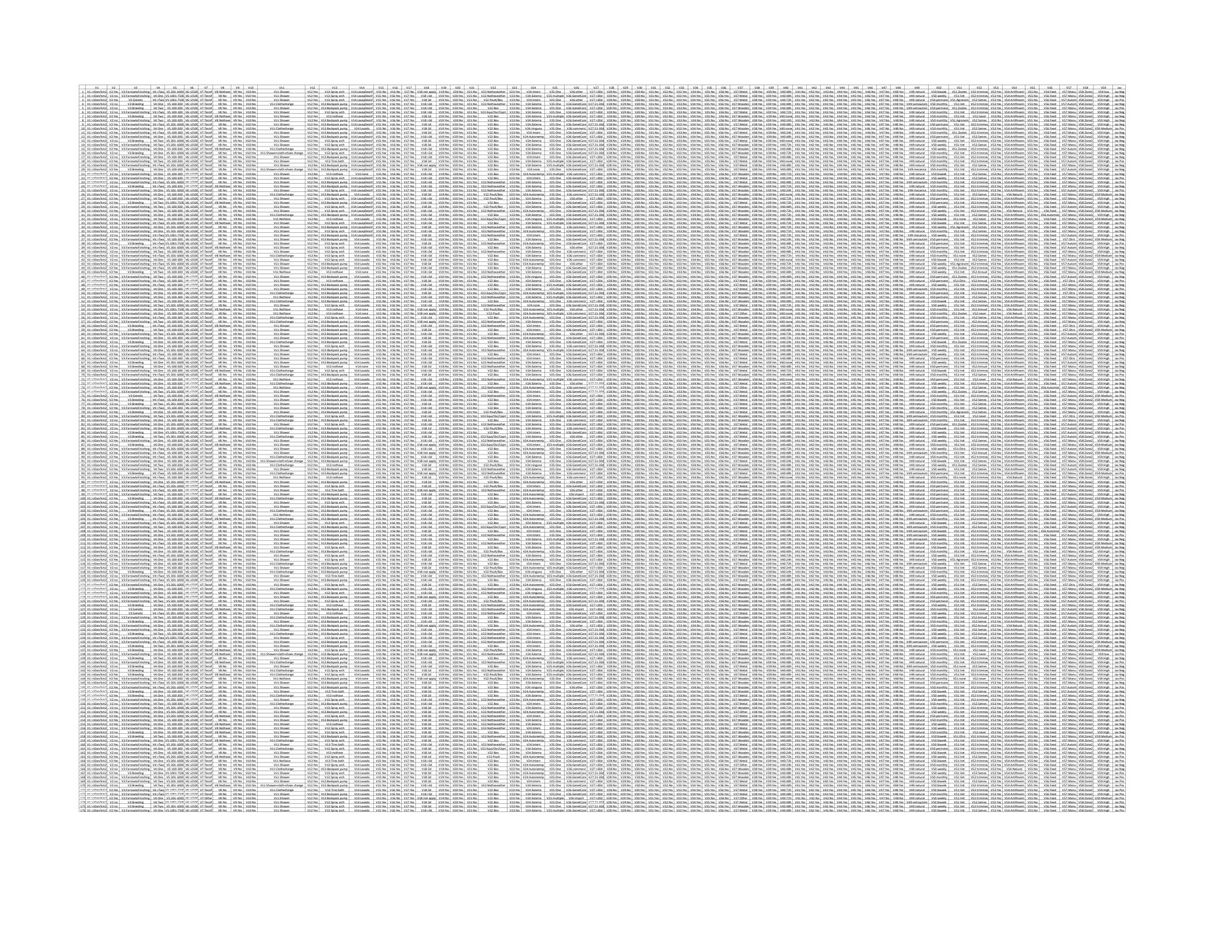

	V6	V11	V12	V14	V17	V18	V20	V23	V34	V35	V36	V39	V40	V41	V42	V44	V46	V54	V28	V29	V30	iav
1	V6:<2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:No	V18:none	V20:No	V23:Yes	V34:No	V35:No	V36:No	V39:No	V40:48h	V41:No	V42:Yes	V44:Yes	V46:No	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
2	V6:>2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
3	V6:>2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Pos
4	V6:<2500	V11:Cloth change	V12:Yes	V14:Wash & disinf	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
5	V6:<2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Pos
6	V6:<2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:Yes	V18:>3d	V20:No	V23:No	V34:Yes	V35:No	V36:No	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
7	V6:<2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:Yes	V18:1d	V20:Yes	V23:No	V34:Yes	V35:No	V36:No	V39:No	V40:none	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
8	V6:<2500	V11:Shower	V12:No	V14:Wash & disinf	V17:Yes	V18:>3d	V20:No	V23:No	V34:No	V35:No	V36:No	V39:Yes	V40:24h	V41:No	V42:No	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:Yes	V30:Yes	iav:Neg
9	V6:>2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:Yes	V18:1d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Neg
10	V6:<2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:2d	V20:Yes	V23:No	V34:Yes	V35:No	V36:No	V39:Yes	V40:none	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Pos
11	V6:>2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:Yes	V18:3d	V20:Yes	V23:No	V34:No	V35:Yes	V36:Yes	V39:No	V40:24h	V41:Yes	V42:Yes	V44:Yes	V46:No	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
12	V6:<2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:No	V36:No	V39:No	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
13	V6:<2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:none	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
14	V6:>2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
15	V6:<2500	V11:Cloth change	V12:Yes	V14:Wash & disinf	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:Yes	V30:Yes	iav:Neg
16	V6:>2500	V11:Danish	V12:Yes	V14:Wash & disinf	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:No	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
17	V6:<2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:Yes	V30:No	iav:Neg
18	V6:>2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:No	V36:No	V39:Yes	V40:none	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:Yes	iav:Pos
19	V6:>2500	V11:Shower	V12:No	V14:Wash & disinf	V17:Yes	V18:none	V20:Yes	V23:Yes	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:No	iav:Neg
20	V6:<2500	V11:Danish	V12:Yes	V14:Wash & disinf	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:No	V36:No	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
21	V6:<2500	V11:Shower	V12:No	V14:None	V17:Yes	V18:>3d	V20:No	V23:Yes	V34:Yes	V35:Yes	V36:No	V39:No	V40:72h	V41:Yes	V42:Yes	V44:No	V46:No	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
22	V6:>2500	V11:Shower	V12:No	V14:Wash & disinf	V17:Yes	V18:>3d	V20:Yes	V23:Yes	V34:Yes	V35:No	V36:No	V39:Yes	V40:24h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Pos
23	V6:>2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:Yes	V30:Yes	iav:Pos
24	V6:>2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
25	V6:>2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:24h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Pos
26	V6:<2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:Yes	V34:No	V35:No	V36:No	V39:No	V40:none	V41:No	V42:Yes	V44:Yes	V46:No	V54:Natural	V28:No	V29:No	V30:Yes	iav:Neg
27	V6:>2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:Yes	iav:Neg
28	V6:>2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:Yes	V30:No	iav:Pos
29	V6:>2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:Yes	V18:3d	V20:Yes	V23:Yes	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:No	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
30	V6:<2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:No	V54:Natural	V28:No	V29:No	V30:Yes	iav:Neg
31	V6:<2500	V11:Cloth change	V12:Yes	V14:Wash & disinf	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:No	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:No	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Neg
32	V6:<2500	V11:None	V12:No	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:Yes	V34:No	V35:No	V36:No	V39:Yes	V40:48h	V41:No	V42:No	V44:No	V46:No	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Neg
33	V6:>2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Pos
34	V6:>2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:Yes	V18:3d	V20:Yes	V23:Yes	V34:Yes	V35:No	V36:No	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:Yes	iav:Neg
35	V6:>2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:No	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:No	V42:Yes	V44:No	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
36	V6:>2500	V11:Shower	V12:Yes	V14:Wash & disinf	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:Yes	iav:Pos
37	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:24h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
38	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:2d	V20:Yes	V23:No	V34:Yes	V35:No	V36:No	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Pos
39	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
40	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
41	V6:<2500	V11:Cloth change	V12:No	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:No	V35:No	V36:Yes	V39:Yes	V40:72h	V41:No	V42:No	V44:Yes	V46:No	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
42	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:Yes	V34:No	V35:Yes	V36:Yes	V39:Yes	V40:none	V41:No	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
43	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:No	V36:No	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Pos
44	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:No	V36:No	V39:Yes	V40:24h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
45	V6:<2500	V11:None	V12:No	V14:None	V17:Yes	V18:>3d	V20:Yes	V23:Yes	V34:No	V35:No	V36:No	V39:Yes	V40:48h	V41:No	V42:No	V44:Yes	V46:No	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Neg
46	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Neg
47	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:Yes	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:24h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Pos
48	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:No	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:24h	V41:Yes	V42:No	V44:Yes	V46:No	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
49	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:Yes	V34:Yes	V35:Yes	V36:Yes	V39:No	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Pos
50	V6:<2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:Yes	V34:No	V35:Yes	V36:Yes	V39:No	V40:24h	V41:No	V42:Yes	V44:No	V46:No	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Neg
51	V6:>2500	V11:None	V12:No	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:Yes	V34:No	V35:No	V36:No	V39:Yes	V40:24h	V41:No	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:No	iav:Neg
52	V6:<2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:Yes	V34:No	V35:No	V36:No	V39:No	V40:48h	V41:No	V42:Yes	V44:Yes	V46:No	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
53	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:Yes	iav:Neg
54	V6:<2500	V11:None	V12:No	V14:None	V17:No	V18:none	V20:No	V23:Yes	V34:No	V35:No	V36:No	V39:No	V40:none	V41:No	V42:No	V44:No	V46:No	V54:Natural	V28:No	V29:No	V30:No	iav:Neg
55	V6:<2500	V11:None	V12:No	V14:None	V17:No	V18:none	V20:No	V23:Yes	V34:No	V35:No	V36:No	V39:No	V40:none	V41:No	V42:No	V44:No	V46:No	V54:Natural	V28:No	V29:No	V30:No	iav:Neg
56	V6:>2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:Yes	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:24h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg

57	V6:<2500	V11:Cloth change	V12:No	V14:Wash	V17:Yes	V18:none	V20:Yes	V23:Yes	V34:No	V35:No	V36:No	V39:No	V40:none	V41:No	V42:No	V44:No	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Neg
58	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Pos
59	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:2d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
60	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:No	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:No	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Pos
61	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:Yes	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:Yes	V30:Yes	iav:Pos
62	V6:<2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:2d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:24h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
63	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Neg
64	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:No	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Neg
65	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:No	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
66	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Pos
67	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:No	V39:Yes	V40:none	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
68	V6:<2500	V11:Shower	V12:Yes	V14:None	V17:Yes	V18:3d	V20:Yes	V23:Yes	V34:No	V35:No	V36:No	V39:No	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Neg
69	V6:<2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:Yes	V34:No	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Neg
70	V6:>2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Pos
71	V6:<2500	V11:None	V12:Yes	V14:Wash	V17:No	V18:>3d	V20:No	V23:Yes	V34:No	V35:Yes	V36:Yes	V39:No	V40:none	V41:No	V42:No	V44:Yes	V46:No	V54:Natural	V28:Yes	V29:Yes	V30:Yes	iav:Neg
72	V6:<2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:No	V35:Yes	V36:No	V39:Yes	V40:72h	V41:No	V42:Yes	V44:Yes	V46:No	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
73	V6:<2500	V11:None	V12:Yes	V14:None	V17:Yes	V18:none	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:No	iav:Neg
74	V6:<2500	V11:Shower	V12:No	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:Yes	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
75	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:Yes	V34:Yes	V35:No	V36:No	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Pos
76	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Neg
77	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:1d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:Yes	iav:Neg
78	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:No	V39:Yes	V40:72h	V41:No	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Neg
79	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:2d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Neg
80	V6:>2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:No	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Pos
81	V6:<2500	V11:Cloth change	V12:No	V14:Wash	V17:No	V18:3d	V20:Yes	V23:Yes	V34:No	V35:No	V36:Yes	V39:No	V40:48h	V41:No	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:No	iav:Neg
82	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Pos
83	V6:<2500	V11:Cloth change	V12:No	V14:Wash	V17:Yes	V18:1d	V20:Yes	V23:No	V34:No	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:No	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:Yes	V30:Yes	iav:Pos
84	V6:>2500	V11:Shower	V12:No	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:Yes	iav:Pos
85	V6:<2500	V11:Cloth change	V12:No	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:No	V34:No	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:No	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:Yes	V30:Yes	iav:Pos
86	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:Yes	iav:Neg
87	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:none	V20:No	V23:No	V34:No	V35:No	V36:No	V39:Yes	V40:48h	V41:No	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
88	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Neg
89	V6:<2500	V11:Shower	V12:No	V14:Wash	V17:Yes	V18:none	V20:Yes	V23:No	V34:Yes	V35:No	V36:No	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Pos
90	V6:<2500	V11:Cloth change	V12:No	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:No	V36:No	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:No	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Neg
91	V6:<2500	V11:Danish	V12:Yes	V14:Wash	V17:Yes	V18:none	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:24h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
92	V6:<2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:Yes	V34:No	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
93	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:Yes	V30:Yes	iav:Neg
94	V6:<2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:none	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:No	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Pos
95	V6:<2500	V11:None	V12:No	V14:Wash	V17:Yes	V18:1d	V20:Yes	V23:No	V34:No	V35:No	V36:No	V39:Yes	V40:none	V41:No	V42:Yes	V44:Yes	V46:Yes	V54:Natural	V28:No	V29:No	V30:No	iav:Neg
96	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:No	V36:No	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
97	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:2d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:Yes	iav:Pos
98	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:none	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Pos
99	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:Yes	iav:Neg
100	V6:<2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:24h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
101	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:Yes	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Neg
102	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Pos
103	V6:<2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:No	V23:Yes	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
104	V6:<2500	V11:None	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:No	V35:Yes	V36:Yes	V39:Yes	V40:24h	V41:No	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:No	iav:Neg
105	V6:<2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Pos
106	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:No	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Neg
107	V6:<2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:No	V23:Yes	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
108	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Neg
109	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:Yes	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Pos
110	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
111	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Neg
112	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:No	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Neg
113	V6:<2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:No	V40:48h	V41:Yes	V42:Yes	V44:No	V46:No	V54:Natural	V28:No	V29:No	V30:No	iav:Neg

114	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Pos
115	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:2d	V20:Yes	V23:Yes	V34:No	V35:Yes	V36:Yes	V39:Yes	V40:24h	V41:No	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
116	V6:<2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:Yes	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
117	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:2d	V20:Yes	V23:Yes	V34:No	V35:Yes	V36:Yes	V39:Yes	V40:24h	V41:No	V42:Yes	V44:Yes	V46:No	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
118	V6:<2500	V11:Cloth change	V12:No	V14:Wash	V17:Yes	V18:none	V20:No	V23:Yes	V34:No	V35:No	V36:Yes	V39:No	V40:24h	V41:No	V42:Yes	V44:No	V46:No	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
119	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:No	V54:Artificial	V28:Yes	V29:No	V30:Yes	iav:Neg
120	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Pos
121	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:2d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Neg
122	V6:<2500	V11:Cloth change	V12:No	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:Yes	V34:Yes	V35:No	V36:No	V39:No	V40:48h	V41:Yes	V42:No	V44:No	V46:No	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
123	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:No	V39:Yes	V40:48h	V41:No	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:Yes	iav:Neg
124	V6:<2500	V11:Shower	V12:No	V14:Wash	V17:Yes	V18:none	V20:Yes	V23:Yes	V34:No	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:No	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Neg
125	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:Yes	V34:Yes	V35:Yes	V36:No	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Pos
126	V6:<2500	V11:Cloth change	V12:No	V14:Wash	V17:Yes	V18:2d	V20:Yes	V23:Yes	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
127	V6:<2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:No	V36:No	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Neg
128	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:Yes	V30:Yes	iav:Neg
129	V6:<2500	V11:Cloth change	V12:No	V14:Wash	V17:No	V18:2d	V20:No	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:No	iav:Neg
130	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:No	V35:Yes	V36:Yes	V39:No	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Neg
131	V6:<2500	V11:Cloth change	V12:No	V14:Wash	V17:Yes	V18:none	V20:Yes	V23:Yes	V34:No	V35:No	V36:No	V39:Yes	V40:48h	V41:No	V42:Yes	V44:Yes	V46:No	V54:Natural	V28:No	V29:No	V30:No	iav:Neg
132	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:Yes	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:24h	V41:Yes	V42:Yes	V44:Yes	V46:No	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
133	V6:<2500	V11:Cloth change	V12:No	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:Yes	V34:No	V35:No	V36:No	V39:Yes	V40:72h	V41:No	V42:No	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
134	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
135	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
136	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
137	V6:<2500	V11:Shower	V12:No	V14:Wash	V17:Yes	V18:none	V20:No	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:24h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
138	V6:<2500	V11:Danish	V12:No	V14:Wash	V17:Yes	V18:none	V20:Yes	V23:Yes	V34:Yes	V35:Yes	V36:Yes	V39:No	V40:none	V41:No	V42:Yes	V44:Yes	V46:No	V54:Artificial	V28:No	V29:No	V30:No	iav:Pos
139	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
140	V6:<2500	V11:Cloth change	V12:No	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:Yes	V34:Yes	V35:No	V36:No	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Pos
141	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:No	V36:No	V39:No	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:No	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
142	V6:<2500	V11:Cloth change	V12:No	V14:Wash	V17:Yes	V18:1d	V20:No	V23:Yes	V34:Yes	V35:Yes	V36:Yes	V39:No	V40:48h	V41:No	V42:Yes	V44:Yes	V46:No	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
143	V6:<2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:2d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:No	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:No	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
144	V6:<2500	V11:None	V12:No	V14:None	V17:Yes	V18:none	V20:Yes	V23:No	V34:No	V35:No	V36:No	V39:No	V40:none	V41:No	V42:Yes	V44:Yes	V46:No	V54:Artificial	V28:No	V29:No	V30:No	iav:Pos
145	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Neg
146	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:No	V34:No	V35:Yes	V36:Yes	V39:Yes	V40:24h	V41:No	V42:Yes	V44:Yes	V46:No	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Pos
147	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Neg
148	V6:<2500	V11:Cloth change	V12:No	V14:Wash	V17:Yes	V18:2d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:No	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:No	V54:Artificial	V28:No	V29:No	V30:No	iav:Pos
149	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Neg
150	V6:<2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:Yes	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Neg
151	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:No	iav:Pos
152	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:24h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Pos
153	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Pos
154	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:No	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Pos
155	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:Yes	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:Yes	iav:Neg
156	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:No	V36:No	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Neg
157	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Neg
158	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:24h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Pos
159	V6:<2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Pos
160	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Pos
161	V6:<2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:Yes	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:24h	V41:Yes	V42:Yes	V44:Yes	V46:No	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Neg
162	V6:<2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:1d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:24h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Pos
163	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:1d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:Yes	iav:Pos
164	V6:>2500	V11:None	V12:No	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Pos
165	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:24h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:No	iav:Neg
166	V6:>2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:Yes	V34:Yes	V35:Yes	V36:No	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Natural	V28:No	V29:No	V30:Yes	iav:Neg
167	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Pos
168	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:No	iav:Pos
169	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:Yes	iav:Pos
170	V6:<2500	V11:Danish	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:No	iav:Pos

171	V6:>2500	V11:Cloth change	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:Yes	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:72h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:No	V30:Yes	iav:Pos
172	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Pos
173	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:none	V20:Yes	V23:Yes	V34:Yes	V35:No	V36:No	V39:Yes	V40:72h	V41:No	V42:No	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:Yes	V30:Yes	iav:Pos
174	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:Yes	iav:Pos
175	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:No	V29:Yes	V30:Yes	iav:Neg
176	V6:>2500	V11:Shower	V12:Yes	V14:Wash	V17:Yes	V18:>3d	V20:Yes	V23:No	V34:Yes	V35:Yes	V36:Yes	V39:Yes	V40:48h	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V54:Artificial	V28:Yes	V29:No	V30:Yes	iav:Pos

V1	V4	V5	V6	V9	V13	V15	V16	V17	V19	V20	V23	V27	V29	V33	V34	V36	V37	V39	V41	V42	V44	V46	V47	V48	V50	V52	V54	V55	V57	iav	clust
V1:>10an/km2	V4:>Two	V5:301-1000	V6:<2500	V9:Yes	V13:Spray Arch	V15:No	V16:No	V17:No	V19:No	V20:No	V23:Yes	V27:>30d	V29:No	V33:Yes	V34:No	V36:No	V37:Carroc_Alum	V39:No	V41:No	V42:Yes	V44:Yes	V46:No	V47:No	V48:Yes	V50:quince	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:2
V1:>10an/km2	V4:One	V5:1001-7500	V6:>2500	V9:Yes	V13:Spray Arch	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:>Two	V5:1001-7500	V6:>2500	V9:Yes	V13:Spray Arch	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:Biannual	V54:InsemArtif	V55:No	V57:Autom	iav:Pos	clust:1
V1:>10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:No	V20:Yes	V23:No	V27:21-30d	V29:No	V33:No	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:trimmes	V54:InsemArtif	V55:No	V57:Autom	iav:Neg	clust:1
V1:>10an/km2	V4:Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:<20d	V29:Yes	V33:No	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Pos	clust:1
V1:>10an/km2	V4:>Two	V5:100-300	V6:<2500	V9:Yes	V13:Spray Arch	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:No	V23:No	V27:>30d	V29:No	V33:No	V34:Yes	V36:No	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:Two	V5:100-300	V6:<2500	V9:Yes	V13:Not apply	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:No	V34:Yes	V36:No	V37:Carroc_Made	V39:No	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:never	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:No	V16:No	V17:Yes	V19:Yes	V20:No	V23:No	V27:<20d	V29:Yes	V33:No	V34:No	V36:No	V37:Carroc_Made	V39:Yes	V41:No	V42:No	V44:Yes	V46:Yes	V47:No	V48:No	V50:mensu	V52:Biannual	V54:InsemArtif	V55:Yes	V57:Manu	iav:Neg	clust:2
V1:>10an/km2	V4:>Two	V5:301-1000	V6:>2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:No	V16:No	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:Yes	V34:Yes	V36:No	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:No	V48:No	V50:mensu	V52:Biannual	V54:InsemArtif	V55:No	V57:Manu	iav:Pos	clust:1
V1:>10an/km2	V4:>Two	V5:100-300	V6:>2500	V9:Yes	V13:Backpack spray	V15:No	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:No	V34:No	V36:Yes	V37:Carroc_Alum	V39:No	V41:Yes	V42:Yes	V44:Yes	V46:No	V47:No	V48:No	V50:mensu	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:No	V34:Yes	V36:No	V37:Carroc_Made	V39:No	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:Biannual	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:Two	V5:301-1000	V6:<2500	V9:Yes	V13:Spray Arch	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:No	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:trimmes	V54:InsemArtif	V55:Yes	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:Two	V5:301-1000	V6:>2500	V9:Yes	V13:Spray Arch	V15:No	V16:No	V17:Yes	V19:No	V20:Yes	V23:No	V27:21-30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:No	V48:No	V50:seman	V52:Biannual	V54:InsemArtif	V55:Yes	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:One	V5:100-300	V6:<2500	V9:No	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:No	V20:Yes	V23:No	V27:21-30d	V29:Yes	V33:No	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:trimmes	V54:InsemArtif	V55:No	V57:Autom	iav:Neg	clust:1
V1:<10an/km2	V4:One	V5:301-1000	V6:>2500	V9:Yes	V13:Spray Arch	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:No	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:trimmes	V54:InsemArtif	V55:Yes	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:<20d	V29:Yes	V33:No	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:Two	V5:100-300	V6:>2500	V9:Yes	V13:Tire bath	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:No	V34:Yes	V36:No	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:trimmes	V54:InsemArtif	V55:No	V57:Autom	iav:Pos	clust:1
V1:>10an/km2	V4:>Two	V5:301-1000	V6:>2500	V9:Yes	V13:Backpack spray	V15:No	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:Yes	V27:21-30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:No	V50:mensu	V52:Biannual	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:No	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:trimmes	V54:InsemArtif	V55:No	V57:Autom	iav:Neg	clust:1
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Not apply	V15:No	V16:No	V17:Yes	V19:No	V20:No	V23:Yes	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:No	V37:Carroc_Made	V39:No	V41:Yes	V42:Yes	V44:No	V46:No	V47:No	V48:No	V50:quince	V52:Biannual	V54:InsemArtif	V55:Yes	V57:Manu	iav:Neg	clust:2
V1:>10an/km2	V4:One	V5:100-300	V6:>2500	V9:Yes	V13:Spray Arch	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:Yes	V27:>30d	V29:Yes	V33:No	V34:Yes	V36:No	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:Biannual	V54:InsemArtif	V55:No	V57:Manu	iav:Pos	clust:1
V1:>10an/km2	V4:>Two	V5:301-1000	V6:>2500	V9:Yes	V13:Backpack spray	V15:No	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:Yes	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Pos	clust:1
V1:>10an/km2	V4:>Two	V5:100-300	V6:>2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:No	V17:Yes	V19:No	V20:Yes	V23:No	V27:>30d	V29:No	V33:No	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:trimmes	V54:InsemArtif	V55:Yes	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:One	V5:301-1000	V6:>2500	V9:Yes	V13:Spray Arch	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:trimmes	V54:InsemArtif	V55:No	V57:Autom	iav:Pos	clust:1
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:No	V16:No	V17:Yes	V19:No	V20:Yes	V23:Yes	V27:21-30d	V29:No	V33:No	V34:No	V36:No	V37:Carroc_Made	V39:No	V41:No	V42:Yes	V44:Yes	V46:No	V47:Yes	V48:No	V50:mensu	V52:Biannual	V54:Monta	V55:Yes	V57:Manu	iav:Neg	clust:2
V1:<10an/km2	V4:Two	V5:100-300	V6:>2500	V9:Yes	V13:Spray Arch	V15:Yes	V16:Yes	V17:Yes	V19:No	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:trimmes	V54:InsemArtif	V55:Yes	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:Two	V5:1001-7500	V6:>2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:No	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:Yes	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:trimmes	V54:InsemArtif	V55:Yes	V57:Manu	iav:Pos	clust:1
V1:<10an/km2	V4:Two	V5:100-300	V6:>2500	V9:Yes	V13:Spray Arch	V15:Yes	V16:No	V17:Yes	V19:No	V20:Yes	V23:Yes	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:No	V47:Yes	V48:Yes	V50:seman	V52:trimmes	V54:InsemArtif	V55:Yes	V57:Autom	iav:Neg	clust:1
V1:<10an/km2	V4:Two	V5:100-300	V6:<2500	V9:Yes	V13:Spray Arch	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:No	V47:Yes	V48:Yes	V50:seman	V52:trimmes	V54:Monta	V55:Yes	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:No	V13:Backpack spray	V15:No	V16:No	V17:Yes	V19:No	V20:Yes	V23:No	V27:21-30d	V29:No	V33:Yes	V34:No	V36:Yes	V37:Carroc_Made	V39:Yes	V41:No	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:No	V50:seman	V52:Biannual	V54:InsemArtif	V55:Yes	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:Two	V5:100-300	V6:<2500	V9:No	V13:Not apply	V15:No	V16:No	V17:Yes	V19:Yes	V20:Yes	V23:Yes	V27:>30d	V29:No	V33:Yes	V34:No	V36:No	V37:Carroc_Made	V39:Yes	V41:No	V42:No	V44:No	V46:No	V47:No	V48:No	V50:quince	V52:never	V54:InsemArtif	V55:Yes	V57:Manu	iav:Neg	clust:2
V1:>10an/km2	V4:>Two	V5:301-1000	V6:>2500	V9:Yes	V13:Spray Arch	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:No	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:quince	V52:Biannual	V54:InsemArtif	V55:No	V57:Manu	iav:Pos	clust:1
V1:<10an/km2	V4:One	V5:301-1000	V6:>2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:Yes	V27:>30d	V29:No	V33:No	V34:Yes	V36:No	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:Biannual	V54:InsemArtif	V55:Yes	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:One	V5:301-1000	V6:>2500	V9:Yes	V13:Spray Arch	V15:No	V16:No	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:No	V34:No	V36:Yes	V37:Carroc_Made	V39:Yes	V41:No	V42:Yes	V44:No	V46:Yes	V47:No	V48:No	V50:mensu	V52:Biannual	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:>Two	V5:1001-7500	V6:>2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:trimmes	V54:InsemArtif	V55:No	V57:Autom	iav:Pos	clust:1
V1:>10an/km2	V4:>Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:No	V16:No	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:No	V48:No	V50:permane	V52:trimmes	V54:InsemArtif	V55:No	V57:Otro	iav:Neg	clust:1
V1:>10an/km2	V4:>Two	V5:1001-7500	V6:>2500	V9:Yes	V13:Spray Arch	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:No	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:trimmes	V54:InsemArtif	V55:No	V57:Autom	iav:Pos	clust:1
V1:<10an/km2	V4:>Two	V5:301-1000	V6:>2500	V9:Yes	V13:Spray Arch	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:No	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:trimmes	V54:InsemArtif	V55:No	V57:Autom	iav:Neg	clust:1
V1:<10an/km2	V4:Two	V5:100-300	V6:<2500	V9:No	V13:Spray Arch	V15:Yes	V16:Yes	V17:Yes	V19:No	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:>Two	V5:301-1000	V6:<2500	V9:Yes	V13:Spray Arch	V15:No	V16:No	V17:Yes	V19:No	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:No	V36:Yes	V37:Carroc_Made	V39:Yes	V41:No	V42:No	V44:Yes	V46:No	V47:No	V48:No	V50:mensu	V52:Biannual	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:2
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Spray Arch	V15:Yes	V16:No	V17:Yes	V19:Yes	V20:Yes	V23:Yes	V27:>30d	V29:No	V33:Yes	V34:No	V36:Yes	V37:Carroc_Made	V39:Yes	V41:No	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:quince	V52:Biannual	V54:InsemArtif	V55:No	V57:Autom	iav:Neg	clust:1
V1:<10an/km2	V4:>Two	V5:301-1000	V6:>2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:No	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:trimmes	V54:InsemArtif	V55:No	V57:Autom	iav:Pos	clust:1
V1:>10an/km2	V4:>Two	V5:100-300	V6:>2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:No	V20:Yes	V23:No	V27:>30d	V29:No	V33:No	V34:Yes	V36:No	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:Two	V5:100-300	V6:<2500	V9:No	V13:Not apply	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:Yes	V27:>30d	V29:No	V33:No	V34:No	V36:No	V37:Carroc_Made	V39:Yes	V41:No	V42:No	V44:Yes	V46:No	V47:Yes	V48:No	V50:seman	V52:Annual	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:2
V1:<10an/km2	V4:Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:Yes	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:trimmes	V54:InsemArtif	V55:Yes	V57:Autom	iav:Neg	clust:1
V1:>10an/km2	V4:>Two	V5:100-300	V6:>2500	V9:Yes	V13:Not apply	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:Yes	V27:<20d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:Annual	V54:InsemArtif	V55:No	V57:Otro	iav:Pos	clust:1
V1:>10an/km2	V4:>Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:No	V19:No	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:No	V44:Yes	V46:No	V47:Yes	V48:Yes	V50:seman	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:Yes	V27:>30d	V29:No	V33:No	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:No	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Pos	clust:1
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:No	V20:Yes	V23:Yes	V27:21-30d	V29:No	V33:No	V34:No	V36:Yes	V37:Carroc_Made	V39:No	V41:No	V42:Yes	V44:No	V46:No	V47:Yes	V48:Yes	V50:permane	V52:Annual	V54:InsemArtif	V55:No	V57:Otro	iav:Neg	clust:2
V1:<10an/km2	V4:One	V5:100-300	V6:>2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:Yes	V27:>30d	V29:No	V33:No	V34:No	V36:No	V37:Carroc_Made	V39:Yes	V41:No	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:No	V50:permane	V52:Biannual	V54:InsemArtif	V55:Yes	V57:Manu	iav:Neg	clust:2
V1:>10an/km2	V4:Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:No	V16:No	V17:Yes	V19:No	V20:No	V23:Yes	V27:>30d	V29:No	V33:No	V34:No	V36:No	V37:Carroc_Made	V39:No	V41:No	V42:Yes	V44:Yes	V46:No	V47:No	V48:No	V50:quince	V52:Biannual	V54:InsemArtif	V55:Yes	V57:Manu	iav:Neg	clust:2
V1:<10an/km2	V4:Two	V5:301-1000	V6:>2500	V9:Yes	V13:Spray Arch	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:No	V50:seman	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:One	V5:1001-7500	V6:<2500	V9:Yes	V13:Not apply	V15:No	V16:No	V17:No	V19:No	V20:No	V23:Yes	V27:21-30d	V29:No	V33:No	V34:No	V36:No	V37:Carroc_Made	V39:No	V41:No	V42:No	V44:No	V46:No	V47:No	V48:No	V50:mensu	V52:Annual	V54:Monta	V55:Yes	V57:Manu	iav:Neg	clust:2
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Not apply	V15:No	V16:No	V17:No	V19:No	V20:No	V23:Yes	V27:21-30d	V29:No	V33:No	V34:No	V36:No	V37:Otro	V39:No	V41:No	V42:No	V44:No	V46:No	V47:No	V48:No	V50:mensu	V52:never	V54:Monta	V55:Yes	V57:Manu	iav:Neg	clust:2
V1:>10an/km2	V4:>Two	V5:100-300	V6:>2500	V9:Yes	V13:Spray Arch	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:Yes	V27:21-30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:Biannual	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:Yes	V27:>30d	V29:No	V33:No	V34:No	V36:No	V37:Carroc_Made	V39:No	V41:No	V42:No	V44:No	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:Annual	V54:InsemArtif	V55:Yes	V57:Manu	iav:Neg	clust:2
V1:>10an/km2	V4:>Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:trimmes	V54:InsemArtif	V55:No	V57:Otro	iav:Pos	clust:1
V1:>10an/km2	V4:Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:trimmes	V54:InsemArtif	V55:No	V57:Otro	iav:Neg	clust:1
V1:<10an/km2	V4:Two	V5:301-1000	V6:>2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:Yes	V34:No	V36:Yes	V37:Carroc_Made	V39:Yes	V41:No	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:Annual	V54:InsemArtif	V55:No	V57:Autom	iav:Pos	clust:1
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:Yes	V27:>30d	V29:Yes	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:trimmes	V54:InsemArtif	V55:No	V57:Autom	iav:Pos	clust:1
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:No	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:quince	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:No	V13:Spray Arch	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:Yes	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:One	V5:301-1000	V6:>2500	V9:No	V13:Backpack spray	V15:No	V16:No	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:Yes	V34:Yes	V36:No	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:No	V48:No	V50:seman	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:>Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:No	V16:No	V17:Yes	V19:Yes	V20:No	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:No	V48:No	V50:seman	V52:trimmes	V54:InsemArtif	V55:No	V57:Autom	iav:Neg	clust:1
V1:>10an/km2	V4:>Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:trimmes	V54:InsemArtif	V55:No	V57:Otro	iav:Pos	clust:1
V1:>10an/km2	V4:One	V5:301-1000	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:No	V34:Yes	V36:No	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:trimmes	V54:InsemArtif	V55:No	V57:Autom	iav:Neg	clust:1
V1:>10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Not apply	V15:Yes	V16:Yes	V17:Yes	V19:No	V20:Yes	V23:Yes	V27:>30d	V29:Yes	V33:No	V34:No	V36:No	V37:Carroc_Made	V39:No	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:Annual	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:2
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:No	V13:Spray Arch	V15:Yes	V16:No	V17:Yes	V19:Yes	V20:Yes	V23:Yes	V27:>30d	V29:No	V33:No	V34:No	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:No	V48:No	V50:quince	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:>Two	V5:301-1000	V6:>2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:<20d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:Biannual	V54:InsemArtif	V55:No	V57:Manu	iav:Pos	clust:1
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Not apply	V15:No	V16:No	V17:No	V19:No	V20:No	V23:Yes	V27:<20d	V29:Yes	V33:No	V34:No	V36:Yes	V37:Carroc_Made	V39:No	V41:No	V42:No	V44:Yes	V46:No	V47:No	V48:No	V50:permane	V52:never	V54:Monta	V55:Yes	V57:Manu	iav:Neg	clust:2
V1:>10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:No	V16:No	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:No	V34:No	V36:No	V37:Carroc_Alum	V39:Yes	V41:No	V42:Yes	V44:Yes	V46:No	V47:No	V48:No	V50:seman	V52:trimmes	V54:InsemArtif	V55:No	V57:Autom	iav:Neg	clust:1
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:No	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:No	V34:Yes	V36:Yes	V37:Otro	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:Biannual	V54:InsemArtif	V55:Yes	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:Two	V5:301-1000	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:Yes	V27:21-30d	V29:No	V33:No	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:Biannual	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:Yes	V27:>30d	V29:No	V33:No	V34:Yes	V36:No	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:trimmes	V54:InsemArtif	V55:Yes	V57:Autom	iav:Pos	clust:1
V1:>10an/km2	V4:>Two	V5:100-300	V6:>2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:<20d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:quince	V52:Biannual	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:Two	V5:301-1000	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:Biannual	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:>Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:No	V37:Carroc_Alum	V39:Yes	V41:No	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:Biannual	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:Yes	V33:No	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:No	V50:mensu	V52:Biannual	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:Two	V5:301-1000	V6:>2500	V9:Yes	V13:Spray Arch	V15:No	V16:No	V17:Yes	V19:No	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:never	V54:InsemArtif	V55:No	V57:Otro	iav:Pos	clust:1
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:No	V19:Yes	V20:Yes	V23:Yes	V27:>30d	V29:No	V33:Yes	V34:No	V36:Yes	V37:Carroc_Made	V39:No	V41:No	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:2
V1:>10an/km2	V4:One	V5:301-1000	V6:>2500	V9:Yes	V13:Spray Arch	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:Yes	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:trimmes	V54:InsemArtif	V55:No	V57:Autom	iav:Pos	clust:1
V1:<10an/km2	V4:One	V5:301-1000	V6:<2500	V9:Yes	V13:Backpack spray	V15:No	V16:No	V17:Yes	V19:No	V20:Yes	V23:No	V27:<20d	V29:Yes	V33:No	V34:No	V36:Yes	V37:Carroc_Made	V39:Yes	V41:No	V42:Yes	V44:Yes	V46:Yes	V47:No	V48:No	V50:quince	V52:trimmes	V54:InsemArtif	V55:No	V57:Autom	iav:Pos	clust:1
V1:>10an/km2	V4:One	V5:1001-7500	V6:>2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:trimmes	V54:InsemArtif	V55:No	V57:Autom	iav:Pos	clust:1
V1:>10an/km2	V4:Two	V5:301-1000	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:Yes	V33:Yes	V34:No	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:No	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:trimmes	V54:InsemArtif	V55:No	V57:Autom	iav:Pos	clust:1
V1:>10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:Biannual	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:No	V16:No	V17:Yes	V19:Yes	V20:No	V23:No	V27:>30d	V29:No	V33:No	V34:No	V36:No	V37:Carroc_Alum	V39:Yes	V41:No	V42:Yes	V44:Yes	V46:Yes	V47:No	V48:No	V50:mensu	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:2
V1:>10an/km2	V4:>Two	V5:301-1000	V6:>2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:Yes	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:trimmes	V54:InsemArtif	V55:No	V57:Autom	iav:Neg	clust:1
V1:>10an/km2	V4:>Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:No	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:Yes	V34:Yes	V36:No	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:Annual	V54:InsemArtif	V55:Yes	V57:Manu	iav:Pos	clust:1
V1:>10an/km2	V4:>Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:No	V17:Yes	V19:No	V20:Yes	V23:No	V27:>30d	V29:No	V33:No	V34:Yes	V36:No	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:No	V47:Yes	V48:Yes	V50:mensu	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:quince	V52:Biannual	V54:InsemArtif	V55:Yes	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:Two	V5:100-300	V6:<2500	V9:No	V13:Not apply	V15:Yes	V16:Yes	V17:Yes	V19:No	V20:Yes	V23:Yes	V27:21-30d	V29:No	V33:No	V34:No	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:Biannual	V54:InsemArtif	V55:No	V57:Autom	iav:Neg	clust:1

V1:>10an/km2	V4:Two	V5:301-1000	V6:>2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:No	V20:Yes	V23:No	V27:>30d	V29:Yes	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:Biannual	V54:InsemArtif	V55:No	V57:Autom	iav:Neg	clust:1
V1:>10an/km2	V4:>Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:No	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Pos	clust:1
V1:<10an/km2	V4:>Two	V5:100-300	V6:<2500	V9:Yes	V13:Not apply	V15:No	V16:No	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:<20d	V29:No	V33:Yes	V34:No	V36:No	V37:Carroc_Made	V39:Yes	V41:No	V42:Yes	V44:Yes	V46:Yes	V47:No	V48:No	V50:mensu	V52:trimmes	V54:Monta	V55:Yes	V57:Manu	iav:Neg	clust:2
V1:>10an/km2	V4:One	V5:301-1000	V6:>2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:No	V17:Yes	V19:No	V20:Yes	V23:No	V27:<20d	V29:No	V33:No	V34:Yes	V36:No	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:No	V50:mensu	V52:trimmes	V54:InsemArtif	V55:Yes	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:>Two	V5:301-1000	V6:>2500	V9:Yes	V13:Spray Arch	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Pos	clust:1
V1:>10an/km2	V4:>Two	V5:100-300	V6:>2500	V9:Yes	V13:Tire bath	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:<20d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:trimmes	V54:InsemArtif	V55:Yes	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:trimmes	V54:InsemArtif	V55:No	V57:Autom	iav:Neg	clust:1
V1:>10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:>Two	V5:100-300	V6:<2500	V9:Yes	V13:Spray Arch	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:Yes	V27:21-30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:>Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:Yes	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:trimmes	V54:InsemArtif	V55:No	V57:Autom	iav:Pos	clust:1
V1:>10an/km2	V4:>Two	V5:301-1000	V6:<2500	V9:Yes	V13:Backpack spray	V15:No	V16:No	V17:Yes	V19:Yes	V20:No	V23:Yes	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:No	V50:permane	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:>Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:No	V16:No	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:No	V34:No	V36:Yes	V37:Carroc_Made	V39:Yes	V41:No	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:No	V50:mensu	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:>Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Pos	clust:1
V1:>10an/km2	V4:>Two	V5:301-1000	V6:<2500	V9:Yes	V13:Spray Arch	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:No	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:No	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:quince	V52:Biannual	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:No	V23:Yes	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:Annual	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:No	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:quince	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Pos	clust:1
V1:>10an/km2	V4:One	V5:301-1000	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:Yes	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:trimmes	V54:InsemArtif	V55:No	V57:Autom	iav:Pos	clust:1
V1:>10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:Biannual	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:>Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:No	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:No	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:Biannual	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:>Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:No	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:Annual	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:No	V20:No	V23:No	V27:>30d	V29:No	V33:No	V34:No	V36:No	V37:Carroc_Made	V39:Yes	V41:No	V42:Yes	V44:Yes	V46:No	V47:No	V48:No	V50:mensu	V52:Annual	V54:Monta	V55:No	V57:Autom	iav:Neg	clust:2
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:No	V16:No	V17:Yes	V19:Yes	V20:Yes	V23:Yes	V27:>30d	V29:No	V33:No	V34:Yes	V36:No	V37:Carroc_Made	V39:Yes	V41:No	V42:No	V44:Yes	V46:Yes	V47:Yes	V48:No	V50:seman	V52:Biannual	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:>Two	V5:301-1000	V6:<2500	V9:Yes	V13:Not apply	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:<20d	V29:No	V33:No	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:trimmes	V54:InsemArtif	V55:No	V57:Autom	iav:Neg	clust:1
V1:<10an/km2	V4:Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:No	V19:No	V20:No	V23:No	V27:21-30d	V29:No	V33:No	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:Annual	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:No	V20:No	V23:No	V27:>30d	V29:Yes	V33:Yes	V34:No	V36:No	V37:Carroc_Made	V39:No	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:Biannual	V54:InsemArtif	V55:Yes	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:No	V13:Backpack spray	V15:No	V16:No	V17:Yes	V19:Yes	V20:Yes	V23:Yes	V27:>30d	V29:No	V33:No	V34:No	V36:No	V37:Carroc_Made	V39:Yes	V41:No	V42:Yes	V44:No	V46:No	V47:No	V48:No	V50:mensu	V52:Annual	V54:Monta	V55:No	V57:Autom	iav:Neg	clust:2
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:No	V13:Backpack spray	V15:No	V16:No	V17:Yes	V19:Yes	V20:Yes	V23:Yes	V27:>30d	V29:No	V33:Yes	V34:No	V36:No	V37:Carroc_Made	V39:No	V41:No	V42:Yes	V44:No	V46:No	V47:No	V48:No	V50:seman	V52:Biannual	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:No	V13:Backpack spray	V15:No	V16:No	V17:Yes	V19:Yes	V20:Yes	V23:Yes	V27:21-30d	V29:No	V33:Yes	V34:No	V36:No	V37:Carroc_Made	V39:No	V41:No	V42:Yes	V44:No	V46:No	V47:No	V48:No	V50:mensu	V52:Annual	V54:Monta	V55:No	V57:Autom	iav:Neg	clust:2
V1:<10an/km2	V4:Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:No	V42:No	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:Annual	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:Biannual	V54:InsemArtif	V55:Yes	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:No	V16:No	V17:Yes	V19:Yes	V20:Yes	V23:Yes	V27:>30d	V29:No	V33:Yes	V34:No	V36:No	V37:Carroc_Made	V39:No	V41:No	V42:Yes	V44:No	V46:No	V47:No	V48:No	V50:mensu	V52:never	V54:InsemArtif	V55:No	V57:Manu	iav:Pos	clust:2
V1:>10an/km2	V4:Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:No	V16:No	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:No	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:No	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:No	V41:No	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:mensu	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Pos	clust:1
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:No	V16:No	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:No	V41:No	V42:Yes	V44:Yes	V46:No	V47:No	V48:No	V50:mensu	V52:never	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:2
V1:>10an/km2	V4:>Two	V5:301-1000	V6:>2500	V9:Yes	V13:Backpack spray	V15:No	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:Yes	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:permane	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Neg	clust:1
V1:>10an/km2	V4:>Two	V5:301-1000	V6:>2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:No	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:<20d	V29:Yes	V33:Yes	V34:No	V36:Yes	V37:Carroc_Made	V39:Yes	V41:No	V42:Yes	V44:Yes	V46:No	V47:Yes	V48:Yes	V50:mensu	V52:trimmes	V54:InsemArtif	V55:Yes	V57:Autom	iav:Pos	clust:1
V1:>10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Tire bath	V15:Yes	V16:Yes	V17:Yes	V19:No	V20:Yes	V23:No	V27:>30d	V29:No	V33:No	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:No	V47:Yes	V48:Yes	V50:quince	V52:trimmes	V54:InsemArtif	V55:Yes	V57:Manu	iav:Neg	clust:1
V1:<10an/km2	V4:>Two	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:<20d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Made	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:seman	V52:trimmes	V54:InsemArtif	V55:No	V57:Autom	iav:Neg	clust:1
V1:<10an/km2	V4:One	V5:100-300	V6:<2500	V9:Yes	V13:Backpack spray	V15:No	V16:No	V17:Yes	V19:No	V20:Yes	V23:Yes	V27:<20d	V29:Yes	V33:Yes	V34:Yes	V36:No	V37:Otro	V39:Yes	V41:No	V42:No	V44:Yes	V46:Yes	V47:No	V48:No	V50:seman	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Pos	clust:1
V1:>10an/km2	V4:Two	V5:1001-7500	V6:>2500	V9:Yes	V13:Spray Arch	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:quince	V52:Biannual	V54:InsemArtif	V55:No	V57:Manu	iav:Pos	clust:1
V1:<10an/km2	V4:Two	V5:301-1000	V6:>2500	V9:Yes	V13:Spray Arch	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:21-30d	V29:No	V33:Yes	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:quince	V52:trimmes	V54:InsemArtif	V55:No	V57:Manu	iav:Pos	clust:1
V1:<10an/km2	V4:One	V5:1001-7500	V6:>2500	V9:Yes	V13:Spray Arch	V15:Yes	V16:Yes	V17:Yes	V19:Yes	V20:Yes	V23:No	V27:>30d	V29:No	V33:No	V34:Yes	V36:Yes	V37:Carroc_Alum	V39:Yes	V41:Yes	V42:Yes	V44:Yes	V46:Yes	V47:Yes	V48:Yes	V50:quince	V52:Biannual	V54:InsemArtif	V55:No	V57:Manu	iav:Pos	clust:1

Código del predio

Fecha

Latitud ,

Longitud

Departamento

Minucipio

Vereda

Nombre Predio

Propietario ó Empresa

Teléfono

Correo Electrónico

INVENTARIO DE ANIMALES

Hembras activas Hembras de reemplazo Machos reproductores Lechones lactantes

Precebos Levante Ceba Total Inventario ,

1. INFRAESTRUCTURA Y SISTEMA DE PRODUCCIÓN DE LA GRANJA

1.1 Tipo de producción

☐ Cría ☐ Levante - Ceba ☐ Ciclo completo ☐ Genénita

1.3 Tipo de sistema de ventilación

☐ Natural ☐ Mecanico ☐ Otro Cual

1.2 Tipo de sistema de alimentación

○ Manual ○ Automatico ○ Otro Cual

1.4 La Granja está:

Registrada ante el ICA ○ SI ○ NO

Certificada en resolución 2640 ○ SI ○ NO

Fecha

1.2.1 Numero de sitios de producción

○ 1 ○ 2 ○ 3

Mas sitios ¿Cuantos?

1.5 Numero de animales por comedero

1.3 Caracteristicas delpiso

○ Traspatio ○ Tecnificado ○ Otro Cual

1.6 Numero de animales por bebedero en precebo

2 BIOSEGURIDAD.

2.1.1 ¿ Cuál es el principal tipo de cerco perimetral o de barrera física que aisla el predio?

☐ cerca alambre ☐ Cerca viva ☐ Cerca madera ☐ Barrera natural

☐ No tiene ☐ Otro Cuál

2.1.5 La granja se encuentra ubicada cerca de otras granjas

Cuantas

Cual es al distancia aproximada en metros

○ SI ○ NO

2.1.2 en la granja se lleva registro de ingreso a vehiculos

○ SI ○ NO

2.1.2 En la granja se llevan registros de ingreso a personas

○ SI ○ NO

2.1.6 la granja se encuentra cerca a vias principales

○ SI ○ NO

Cual es la distancia aproximada en metros

2.1.3 Cuál es el tipo de fitro sanitario para el ingreso del personal

○ Baño danes ○ Otro
○ Ducha cambio de ropa y calzado ○ No tiene
○ Cambio de ropa y calzado

Cuál

2.1.7 La granja se encuantra ubicada cerca de plantas de benefecio y/o ferias

○ SI ○ NO

2.1.7.1 Cual es la distancia aproximada en metros

2.1.8 Con qué lava las instalaciones

☐ Manguera ☐ agua caliente ☐ Hidrolavadora

☐ No las lava ☐ Otro Cuál

2.1.4 El aréa de caurentena es independiente de la zona productiva

○ SI ○ NO ○ No tiene

2.1.4.1 Tiempo de caurentena

○ ≤ 20 días ○ 21 A 30 días ○ > 30 días

2.1.4.2 Realiza monitoreo durante la cuarentena

○ SI ○ NO

A cuáles enfermedades

2.1.8.1 Para el lavado de las instalaciones tiene en cuenta:

Uso del jabón
○ SI ○ NO

Uso de desinfectante
○ SI ○ NO

Secado antes de usarlo de nuevo
○ SI ○ NO

Cuál

2.2 TRANSPORTE

2.2.1 Cuál es le principal sistema de desinfeción de vehículos para su ingreso

○ Arco ○ Rodiluvio ○ Bomba espalda ○ No tiene

2.2.5 A cuáles sitios el personal vinculado con el transporte de animles o alimento tiene acceso dentro del predio:

☐ Oficina ☐ Bodega de alimento ☐ Bodega herramientas ☐ Zona cuarentena ☐ Zona de cría ☐ Zona gestación
☐ Zona lev/Ceba ☐ Sin restricción ☐ Tiene prohibido el ingreso

2.2.2 La granja cuanta con vehículos especializados para animales
○ SI ○ NO

2.2.2 La granja cuenta con vehículos especializados para alimento
○ SI ○ NO

2.2.6 Entre cada carga de animales, la granja tiene establecido para el vehículo:

Lavado
○ SI ○ NO

Desinfección
○ SI ○ NO

Secado
○ SI ○ NO

2.2.3 Los vehículos son de uso exclusivo para la granja
○ SI ○ NO

2.2.4 Cuál es la principal clase de vehículo empleado para el transporte de cerdos en pie

○ Carroceria de madera ○ No moviliza
○ Motocarro ○ Otro
○ Carroceria de aluminio

Cuál

2.2.7 De las siguienjtes partes de los vehículos, a cuáles se les verifica el lavado para el ingreso a la granja

☐ Carrocería ☐ Chasis ☐ cabina ☐ Llantas ☐ Guarbarros ☐ Ninguna

2.3 PERSONAL Y EQUIPOS

2.3.1 ¿ Se tiene establecido que el personal de granja debe pasar por el filtro sanitario para el ingreso a la granja y se cumple?
○ SI ○ NO

2.3.5 ¿ se limita el número de visitas a la granja?

○ SI ○ NO

2.3.2 ¿ La granja cuenta con el personal exclusivo para cada área?

○ SI ○ NO

2.3.6 Número de personas que residen en la granja

2.3.3 ¿De cáunto tiempo se exige periodo de cuarentena a visitantes?

○ 24 Horas ○ 48 Horas ○ 72 Horas ○ No exige

2.3.7 Número de nuevos operarios en la granja el ultimo año

2.3.4 ¿La granja cuenta con dotación para uso exclusivo del personal y visitantes? (botas, oevroles, etc)

○ SI ○ NO

2.3.8 ¿ Cuenta con transporte independiente para animales y alimento?

○ SI ○ NO

2.4 FLUJO DE ANIMALES

2.4.1 ¿Que tipo de reposición de animles tuvo en el ultimo semestre?

○ Autoremplazo ○ No hubo reposición
○ Externa

2.4.2 ¿Cuál fue la procedencia de los animales que ingresaron a la granja?

○ Un origen ○ Multiples orígernes
○ Dos orígenes

Nombre de la finca

M/pio de procedencia

2.4.3 Cada cuántos días ingresan animales

2.4.7 ¿ En cuál de las siguentes etápas utiliza el todo adentro y todo afuera?

☐ Curentena ☐ Gestación ☐ Lactancia ☐ Precebo ☐ Levante y Ceba ☐ No lo usa

2.4.8 Tipo de sistema reproductivo

○ Inseminación artificial
○ Monta directa

2.4.4 los animales que ingresan a la granja proceden de:

- ○ Núcle genético
- ○ Granjas comerciales
- ○ Animales importados
- ○ Paraderos
- ○ Plazas de ferias
- ○ Otro

Cuál

2.4.8.1 El semen procede de:

- ☐ Nucle genetico
- ☐ Granja comercial
- ☐ Colecta en misma granja
- ☐ Semen importado

2.4.7.2 El reproductor proviene de:

- ○ Fincas vecinas
- ○ Mismas fincas
- ○ Otra

Cuál

2.4.5 Se mezclan cerdos de diferentes procedencias

- ○ SI
- ○ NO

2.4.5 Se realizan atetes

- ○ SI
- ○ NO

2.4.8 ¿De cuánto tiempo es el vacío sanitario en las secciones productivas?

- ○ 1 Día
- ○ 2 Días
- ○ 3 Días
- ○ > 3 Días
- ○ No se realiza

2.5 OTRAS ESPECIES DE ANIMALES

2.5.1 ¿Cuál de las siguentes especies existen en el predio?

☐ Aves ☐ Bobina ☐ Ovina/Caprina ☐ Equina ☐ Otro ☐ Ninguno Cuál

2.5.1.1 Son independientes de la producción porcina

- ○ SI
- ○ NO

2.5.3 Los animales domesticos tienen acceso al sitio de producción

- ○ SI
- ○ NO

2.5.2 Que tipo de mascotas hay en la granja

☐ Perros ☐ Gatos ☐ No tiene ☐ Otro Cuál

2.5.4 Realiza acciones de control para las siguentes plagas: Moscas

- ○ SI
- ○ NO

2.5.4 Realiza acciones de control para las siguentes plagas: Roedores

- ○ SI
- ○ NO

2.5.5 Utiliza mayas antipajaros en la zona de producción

- ○ SI
- ○ NO

3 SANIDAD

3.1.1 Princiapelmente, ¿Qué profesional le brinda asesoría técnica?

- ○ Médico Veterinario
- ○ MVZ
- ○ Zootecnista
- ○ Agrónomo
- ○ No tiene
- ○ Otro

Cuál

experiencia en años

3.1.4 Cuál de las sigueintes actividades de la granja están documentadas

- ☐ Programas de bioseguridad
- ☐ Plan sanitario
- ☐ Desinfección de vehículos
- ☐ Desinfección instalaciones
- ☐ Caurentena - Ingreso de animlaes
- ☐ Control integrando de plagas
- ☐ Alimentación animales
- ☐ Asesorias técnicas (seguimiento)
- ☐ Ninguna

3.1.1.1 Con qué frecuencia el asesor técnico asiste a la granja

- ○ Permanente
- ○ quincenal
- ○ Semanal
- ○ Mensual

3.1.1.2 Realiza capacitación a técnicos y operarios

- ○ Nunca
- ○ Semestral
- ○ Trimestral
- ○ Anual

3.1.5 Se garantiza la calidad del agua sumistrada a los animales mediante exámenes microbiológicos, fisicoquímicos y tratamiento

- ○ SI
- ○ NO

3.1.1.3 En la asesoría técnica se incluye seguimiento en:

- ☐ Aclimatación de hembras
- ☐ Monitoreos serológicos
- ☐ Protocolos de desinfección
- ☐ alimentación y nutrición
- ☐ Plan sanitario
- ☐ Rendimiento reproductivo
- ☐ Medicación
- ☐ Plan vacunal

3.1.6 Qué tipo de alimentación suministra a los cerdo

- ○ Concentrado
- ○ Granos
- ○ Desechos de alimentción humana
- ○ Residuos industriales
- ○ Automezclas
- ○ Suero de leche
- ○ Otro

Cúal

3.1.2 Ha realizado auditorias en bioseguridad el ultimo año

- ○ SI
- ○ NO

3.1.3 realiza cortes de cola

- ○ SI
- ○ NO

3.1.1.5.1 Realiza castraciones

- ○ SI
- ○ NO

3.1.7 Cuentas con protocolos de medicación y vermufigación

- ○ SI
- ○ NO

3.1.8 Durante los últimos 6 meses, cúal ha sido la actividad sanitaria adelantada en la granja para el diagnóstico y/o prevención de las sigueints enfermedades

*Peste porcina clásica ☐ Pos ☐ Neg *PRRS ☐ Pos ☐ Neg *Circo virus ☐ Pos ☐ Neg

*Erisipelia ☐ Pos ☐ Neg *Parvovirus ☐ Pos ☐ Neg *Leptospirosis ☐ Pos ☐ Neg

*Pleuroneumonía contagiosa porcina APP ☐ Pos ☐ Neg *Colibacilosis ☐ Pos ☐ Neg

*Clostridiosis ☐ Pos ☐ Neg *Ileítis ☐ Pos ☐ Neg *Salmonelosis ☐ Pos ☐ Neg

*Estreptococosis ☐ Pos ☐ Neg *Micoplasmosis ☐ Pos ☐ Neg

*Enfermedad de Glaser-H parasuis ☐ Pos ☐ Neg

3.1.9 Durante los último 6 meses, ¿cuáles de los siguientes cuadros clínicos han sido detectados en la granja y en que etapa productiva?

*Digestivos ☐ Hembras de cría ☐ Hembras de reemplazo ☐ Machos reproductores ☐ Lechones lactantes ☐ Precebos ☐ Levante - ceba

*Respiratorios ☐ Hembras de cría ☐ Hembras de reemplazo ☐ Machos reproductores ☐ Lechones lactantes ☐ Precebos ☐ Levante - ceba

*Nervioso ☐ Hembras de cría ☐ Hembras de reemplazo ☐ Machos reproductores ☐ Lechones lactantes ☐ Precebos ☐ Levante - ceba

*Sistémico ☐ Hembras de cría ☐ Hembras de reemplazo ☐ Machos reproductores ☐ Lechones lactantes ☐ Precebos ☐ Levante - ceba

*Disminución crecimiento ☐ Hembras de cría ☐ Hembras de reemplazo ☐ Machos reproductores ☐ Lechones lactantes ☐ Precebos ☐ Levante - ceba

*Otro ☐ Hembras de cría ☐ Hembras de reemplazo ☐ Machos reproductores ☐ Lechones lactantes ☐ Precebos ☐ Levante - ceba Cuál

4 PARAMETROS PRODUCTIVOS EN ETAPA DE PRECEBO

Ganancia de peso semanal del grupo del lote anterior Sem 3 , Sem 5 , Sem 7 ,

Sem 10 ,

Ganancia de peso semanal del grupo del lote actual Sem 3 , Sem 5 , Sem 7 ,

Sem 10 ,

Consumo promedio lote anterior , Consumo promedio lote actual ,

Edad promedio para alcanzar 35Kg

Consumo promedio desde destete hasta la última semana precebo , Costo alimento en precebo

5 COMERCIALIZACIÓN

5.1 Cúal es la modalidad de venta de los animales de la granja

☐ En pie ☐ En pie y en canal ☐ En canal ☐ Autoconsumo

5.2 Dónde se comercializan los animales de la granja

☐ En la granja ☐ Plaza de feria ☐ Planta de beneficio ☐ Otro Cúal

6 MANEJO DE PORQUINAZA Y MORTALIDAD

6.1 Cúal manejo de la porquinaza liquida utiliza

☐ Tanque estercolero ☐ Laguna de oxidación ☐ Biodigestor ☐ Cama profunda ☐ Ninguna

6.1.1 Cúal es la disposición final de la porquinaza liquida dentro de la granja

☐ Riego directo al suelo ☐ Fertilización de potreros ☐ Alcatarillado ☐ Fuentes hidricas (rios, caños, lagos y lagunas)

6.2 Cúal manejo de la porquinaza solida utiliza

☐ Lecho de secado ☐ Compostaje ☐ Cama profunda ☐ Ninguno

6.2.1 Cúal es la disposición final de la porquinaza sólida

☐ Uso dentro de la granja ☐ Uso en otros predios ☐ Venta

5.3 Cúal es el manejo de la mortalidad en granja

☐ Entierra ☐ Incinera ☐ Composta ☐ Empresa de residuos ☐ Otro Cúal _____

INFORMACIÓN DE MUESTREO

En cúantos presios tomó el total de muestras _____ Numero total de muestras tomadas _____

Nombre encuestador _____

Specimen ID	Gene segments sequenced								Virus subtype classification (Ha/Na)	Place of collection	Year of collection
	HA	NA	M	PB1	PB2	NS	PA	NP			
LC-017	X	X	X						H1N1	caldas	2013
LC-020	X	X	X						H1N1	caldas	2013
M10209		X							HxN1	Cundinamarca	2017
M10489		X							HxN1	Antioquia	2017
M10491		X							HxN1	Antioquia	2017
M10721		X							HxN2	Cundinamarca	2017
M11541	X	X	X	X	X	X	X	X	H1N1	Quindio	2017
M11545		X							HxN1	Quindio	2017
M11548	X	X	X	X	X	X	X	X	H1N1	Quindio	2017
M11913	X	X	X	X	X	X	X	X	H1N1	Valle del cauca	2017
M11915	X	X	X	X	X	X	X	X	H1N1	Valle del cauca	2017
M12601		X							HxN2	Valle del cauca	2017
M12602	X	X	X	X	X	X	X	X	H1N1	Valle del cauca	2017
M12658	X	X	X	X	X	X	X	X	H1N1	Valle del cauca	2017
M12720		X							HxN1	Valle del cauca	2017
M12731	X	X	X	X	X	X	X	X	H1N1	Valle del cauca	2017
M12736	X	X	X	X	X	X	X	X	H1N1	Valle del cauca	2017
M12747	X	X	X	X	X	X	X	X	H1N1	Valle del cauca	2017
M12748	X	X	X	X	X	X	X	X	H1N1	Valle del cauca	2017
M12751	X	X	X	X	X	X	X	X	H1N1	Valle del cauca	2017
M12756	X	X	X	X	X	X	X	X	H1N1	Valle del cauca	2017
M12775	X	X	X	X	X	X	X	X	H1N1	Cauca	2017
M12910	X	X	X	X	X	X	X	X	H1N1	Valle del cauca	2017
M12911		X							HxN2	Valle del cauca	2017
M12916	X	X	X	X	X	X	X	X	H1N1	Valle del cauca	2017
M12957	X	X	X	X	X	X	X	X	H1N1	Cauca	2017
M12960	X	X	X	X	X	X	X	X	H1N1	Cauca	2017
M12963	X	X	X	X	X	X	X	X	H1N1	Cauca	2017
M12966	X	X	X	X	X	X	X	X	H3N2	Cauca	2017
M12978	X	X	X	X	X	X	X	X	H1N1	Cauca	2017
M12981	X	X	X	X	X	X	X	X	H1N2	Cauca	2017
M13020	X	X	X	X	X	X	X	X	H1N1	Cauca	2017
M13070	X	X	X	X	X	X	X	X	H1N1	Valle del cauca	2017
M13152	X	X	X	X	X	X	X	X	H1N1	Valle del cauca	2017
M13154	X	X	X	X	X	X	X	X	H1N1	Valle del cauca	2017
M13159	X	X	X	X	X	X	X	X	H1N1	Valle del cauca	2017
M1482	X	X	X	X	X	X	X	X	H1N1	Caldas	2015
M1484	X	X	X	X	X	X	X	X	H1N1	Caldas	2015
M1492	X								H1Nx	Caldas	2015
M1695	X								H1Nx	Antioquia	2015
M1698	X								H1Nx	Antioquia	2015
M1707	X								H1Nx	Antioquia	2015
M1859	X								H1Nx	Antioquia	2015
M1956	X	X	X	X	X	X	X	X	H1N1	Antioquia	2015
M4671	X	X	X	X	X	X	X	X	H1N1	Quindio	2016
M4672	X	X	X	X	X	X	X	X	H1N1	Quindio	2016
M4675	X	X	X	X	X	X	X	X	H1N1	Quindio	2016
M4676	X	X	X	X	X	X	X	X	H1N1	Quindio	2016
M4708	X	X	X	X	X	X	X	X	H1N1	Quindio	2016
M4709	X	X	X	X	X	X	X	X	H1N1	Quindio	2016
M4869	X	X	X	X	X	X	X	X	H1N1	Antioquia	2016
M4907	X	X	X	X	X	X	X	X	H1N1	Antioquia	2016

M5120	X	X	X	X	X	X	X	X	H1N1	Cundinamarca	2016
M5126		X							HxN1	Cundinamarca	2016
M5129	X	X	X	X	X	X	X	X	H1N1	Cundinamarca	2016
M5144	X	X	X	X	X	X	X	X	H1N1	Cundinamarca	2016
M5772		X							HxN1	Antioquia	2016
M5782	X	X	X	X	X	X	X	X	H1N1	Antioquia	2016
M5885	X	X	X	X	X	X	X	X	H1N1	Antioquia	2016
M5886	X	X	X	X	X	X	X	X	H1N1	Antioquia	2016
M6406	X	X	X	X	X	X	X	X	H1N1	Meta	2016
M6413	X	X	X	X	X	X	X	X	H1N1	Meta	2016
M6435		X							HxN2	Meta	2016
M6438	X	X	X	X	X	X	X	X	H1N1	Meta	2016
M6607	X	X	X	X	X	X	X	X	H1N1	Santander	2016
M6610		X							HxN1	Santander	2016
M6613		X							HxN1	Santander	2016
M6656	X	X	X	X	X	X	X	X	H1N1	Antioquia	2016
M7020	X	X	X	X	X	X	X	X	H1N1	Huila	2016
M7063	X	X	X	X	X	X	X	X	H1N1	Huila	2016
M7128	X	X	X	X	X	X	X	X	H1N1	Quindio	2016
M7129		X							HxN1	Quindio	2016
M7130	X	X	X	X	X	X	X	X	H1N1	Quindio	2016
M7132	X	X	X	X	X	X	X	X	H1N1	Quindio	2016
M7133	X	X	X	X	X	X	X	X	H1N1	Quindio	2016
M7134		X							HxN1	Quindio	2016
M7137		X							HxN1	Quindio	2016
M7181		X							HxN1	Antioquia	2016
M7186		X							HxN1	Antioquia	2016
M7191		X							HxN1	Antioquia	2016
M7192		X							HxN1	Antioquia	2016
M7208	X	X	X	X	X	X	X	X	H1N1	Antioquia	2016
M7303		X							HxN1	Caldas	2016
M8110	X	X	X	X	X	X	X	X	H1N1	Cundinamarca	2016
M8114		X							HxN1	Cundinamarca	2016
M8140		X							HxN1	Antioquia	2016
M8428		X							HxN2	Antioquia	2016
M8540	X	X	X	X	X	X	X	X	H1N1	Risaralda	2016
M8594		X							HxN1	Caldas	2016
M8595	X	X	X	X	X	X	X	X	H1N1	Caldas	2016
M8661	X	X	X	X	X	X	X	X	H1N1	Antioquia	2016
M8662	X	X	X	X	X	X	X	X	H1N1	Antioquia	2016
M9237		X							HxN1	Risaralda	2016
M9267	X	X	X	X	X	X	X	X	H1N1	Risaralda	2016
M9326	X	X	X	X	X	X	X	X	H1N1	Antioquia	2016
M9364		X							HxN1	Antioquia	2016
M9719		X							HxN1	Antioquia	2016
M9725	X	X	X	X	X	X	X	X	H1N1	Antioquia	2016
M9737		X							HxN1	Antioquia	2016
M9739	X	X	X	X	X	X	X	X	H1N1	Antioquia	2016
M9947		X							HxN1	Antioquia	2016
M9951		X							HxN1	Antioquia	2016
M9952		X							HxN1	Antioquia	2016
M9953		X							HxN1	Antioquia	2016
P503	X	X	X	X	X	X	X	X	H1N1	Meta	2011
P508	X	X	X						H1N1	Meta	2011
P511	X	X	X						H1N1	Meta	2011

P513	X	X	X						H1N1	Meta	2011
P517	X	X	X	X	X	X	X	X	H1N1	Meta	2011
P518	X	X	X	X	X	X	X	X	H1N1	Meta	2011
P519	X	X	X	X	X	X	X	X	H1N1	Meta	2011
P521	X	X	X	X	X	X	X	X	H1N1	Meta	2011
P533	X	X	X	X	X	X	X	X	H1N1	Meta	2011
P536	X	X	X	X	X	X	X	X	H1N1	Meta	2011
P540	X	X	X	X	X	X	X	X	H1N1	Meta	2011
P541	X	X	X	X	X	X	X	X	H1N1	Meta	2011
P843	X	X	X	X	X	X		X	H1N1	Meta	2011
P851	X	X	X						H1N1	Meta	2011
P858	X								H1Nx	Meta	2011
P857	X	X	X	X	X	X	X	X	H1N1	Meta	2011
PP-NA-045	X								H1Nx	Caldas	2013
PP-NA-047	X	X	X	X	X	X	X	X	H1N1	Caldas	2013
PP-NA-048	X		X						H1Nx	Caldas	2013
PP-NA-060	X								H1Nx	Caldas	2013
S17	X								H1Nx	Antioquia	2013
S20	X								H1Nx	Antioquia	2013
S21	X								H1Nx	Antioquia	2013
S30	X								H1Nx	Antioquia	2013
S32	X								H1Nx	Antioquia	2013
S33	X								H1Nx	Antioquia	2013
M1494	X								H1Nx	Caldas	2013
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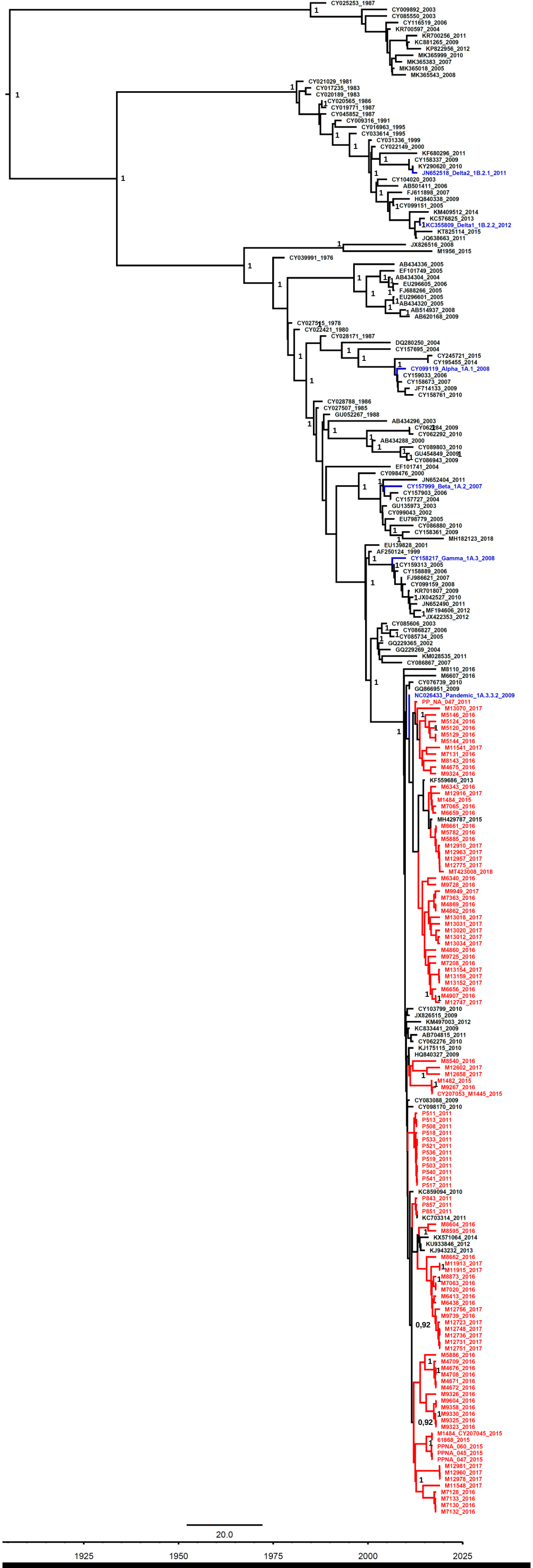
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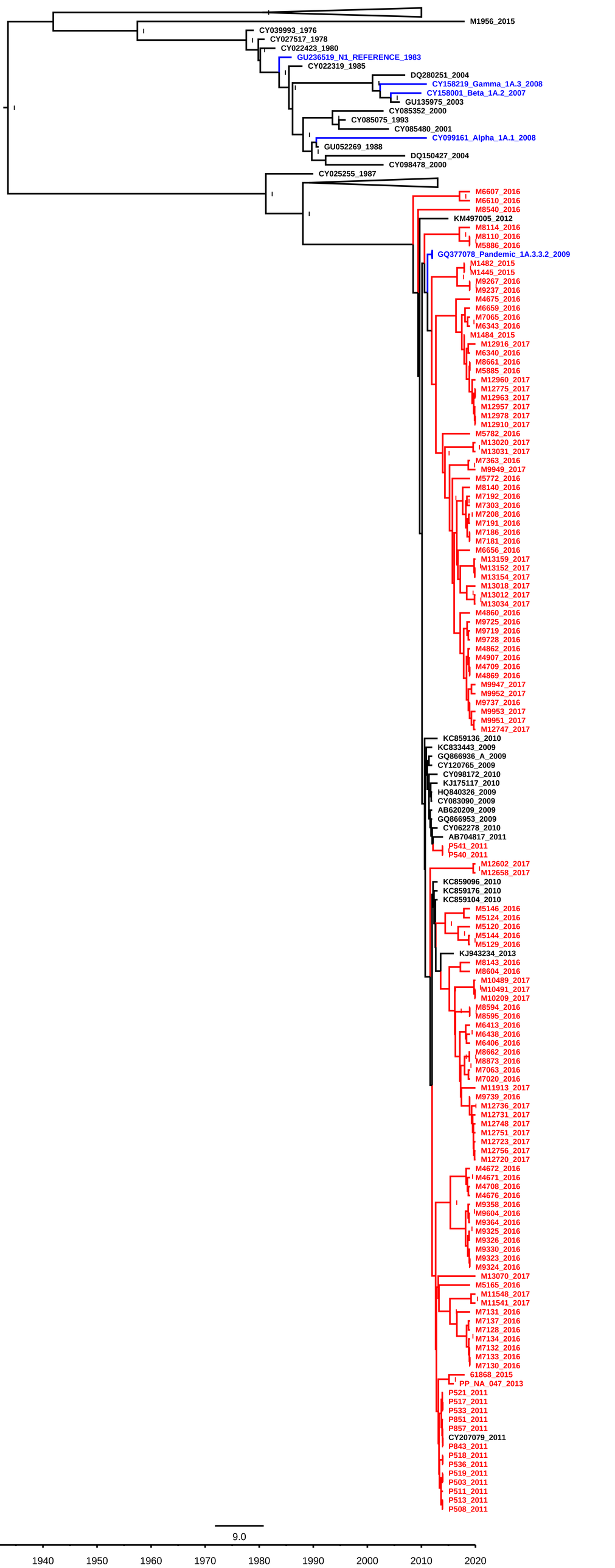
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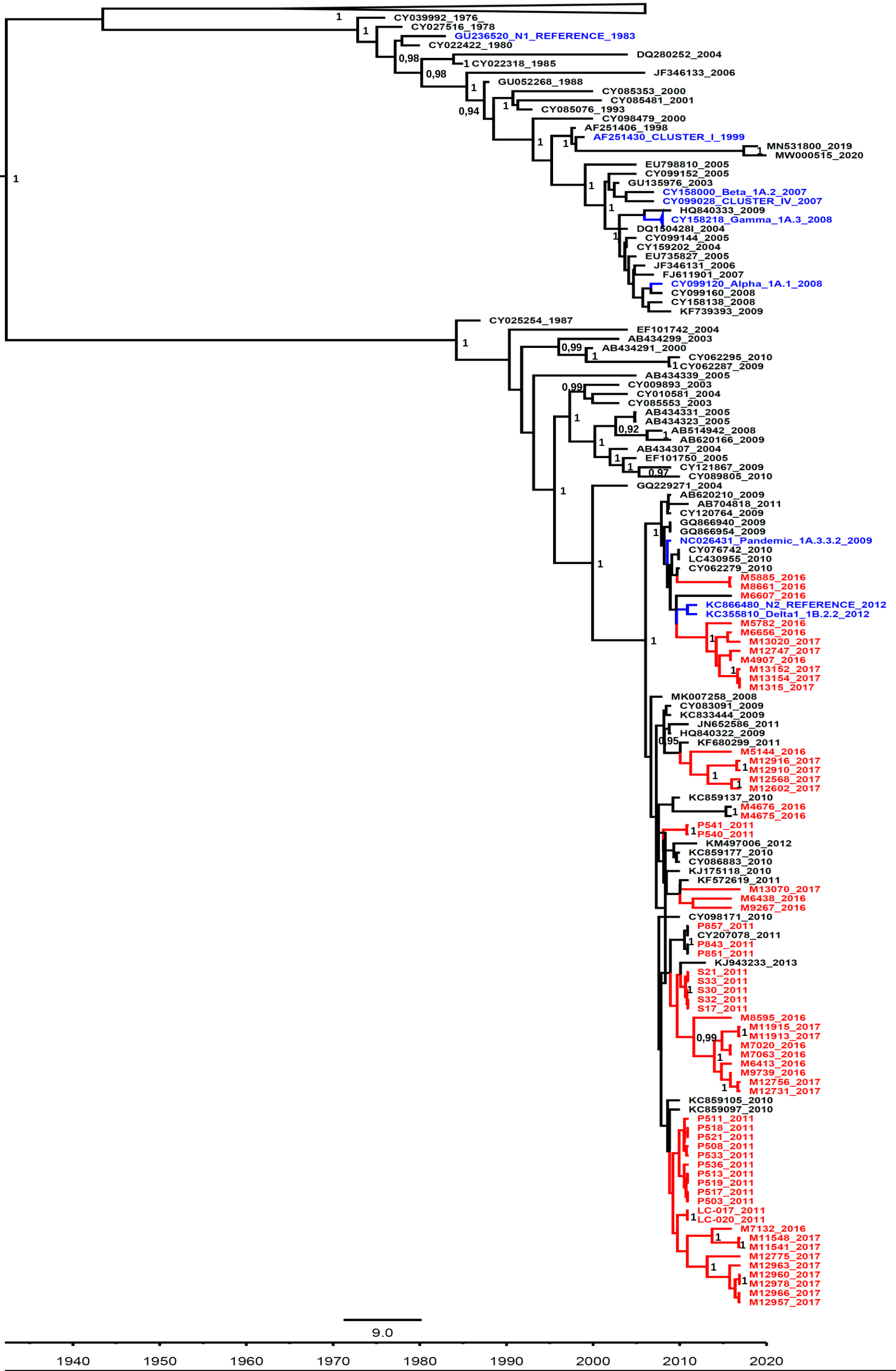
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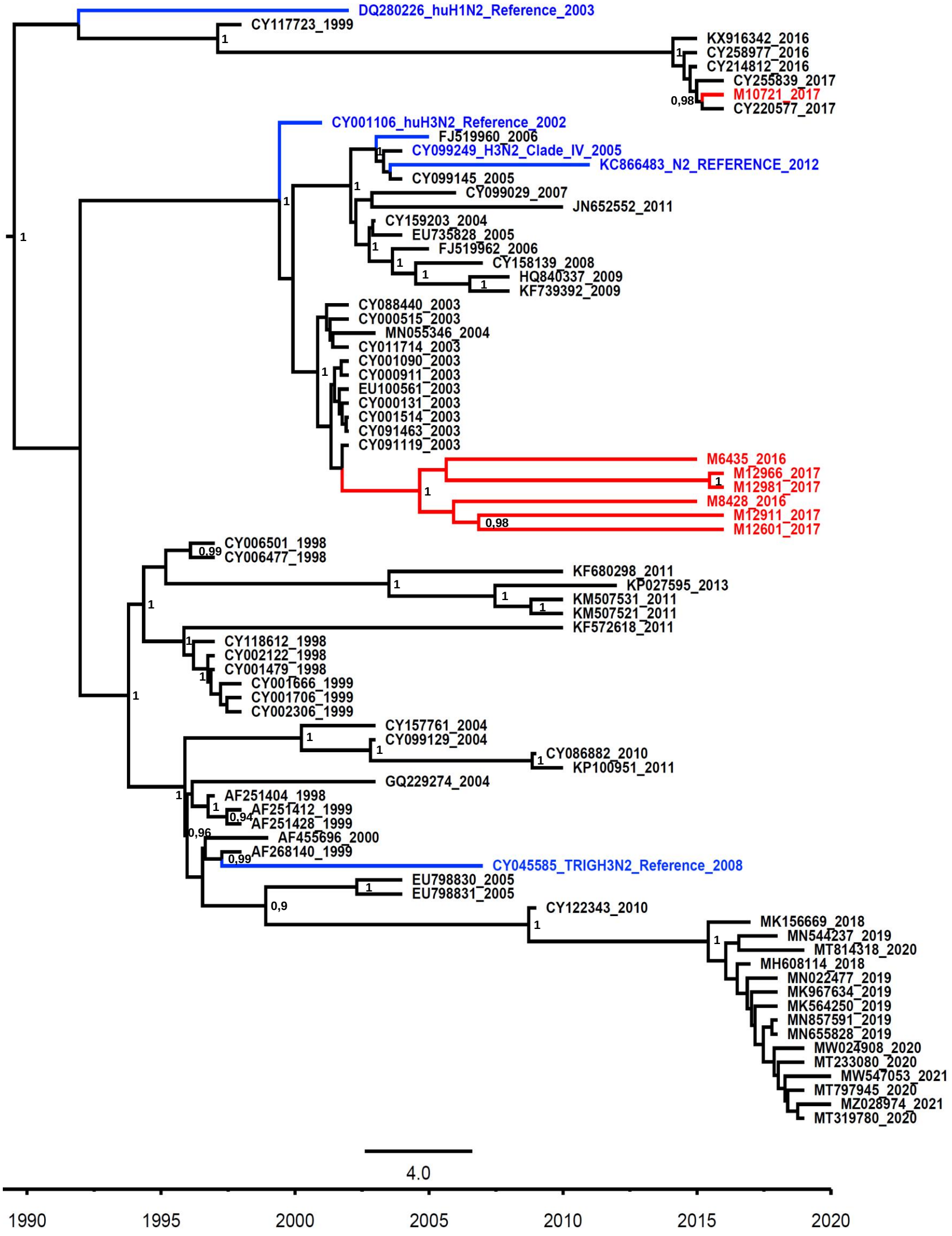
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