UNIVERSIDAD DE ANTIOQUIA

Facultad de Ciencias Agrarias

Caracterización de las especies de *Eimeria* presentes en granjas de pollo de engorde de 4 regiones de importancia avícola de Colombia y realización de una prueba de sensibilidad anticoccidial *in vivo.*

Trabajo de grado en coccidiosis aviar presentado por:

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Maestría en Ciencias Animales

Línea parasitología

Facultad de Ciencias Agrarias

Universidad de Antioquia

2021

UNIVERSIDAD DE ANTIOQUIA
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LISTA DE ABREVIATURAS

ac	*Eimeria acervulina*
Ant	Antioquia
BW	Body weight/ Peso final
BWG	Body weigh gain/ Ganancia de peso
CA	Conversión alimenticia
CA/RS	Cascarilla de arroz/Rice hulls
CE	Comisión Europea
Cun	Cundinamarca
DE/SD	Desviación estándar/Standar Deviation
DIC	Diclazuril
DNA	Ácido Desoxirribonucléico
dNTps	Nucleósido trifosfato -nucleótidos
FAO	Organización de las Naciones Unidas para la Alimentación y la Agricultura
FCR/CA	Feed Conversion Ratio/Conversión alimenticia
Fenavi	Federación Nacional de Avicultores de Colombia
g	Gramo
GALT	Gut associated lymphoid tissue/ Tejido linfoide asociado a intestino
GI	Global index/Indice global
Giadj	Global index adjusted/índice global de resistencia ajustado
h	Horas
H_2O	Agua
ICA	Instituto Colombiano Agropecuario
IgY	Immunoglobulin Y (IgY)/ Inmunoglobulina Y (IgY)
INC	Infectado no medicado
ITS	Internal transcribed spaces /Espaciador Transcrito Interno
KCl	Cloruro de potasio
K2Cr2O7	Dicromato de potasio
Log	Logaritmo natural
LS	Score de lesiones
m^2	Metro cuadrado
MAPS	Mapas Avícolas para la Productividad y Sostenibilidad
Max	Máximo
Met. Clo	Metilbenzocuato Clopidol
MgCl2	Cloruro de Magnesio
Mín	Mínimo
ml	Mililitros
TMLS	Sumatory of the average score of Eimeria species intestinal lesions/ Sumatoria total del promedio del Score de Lesiones intestinales
mm	Milímetros
MR	Mortalidad

msnm	metros sobre el nivel de mar
mt	*Eimeria mitis*
mx	*Eimeria máxima*
nc	*Eimeria necatrix*
NNC	No infectado no medicado
OI	Oocyst index/Índice de ooquistes
OPG	Ooquistes por gramo de heces/cama
pb	Pares de bases
PBS	Phosphate buffered saline/ Buffer fosfato salino
PCR	Polymerase chain reaction/ Reacción en cadena de la polimerasa
ppm	partes por millón
pr	*Eimeria praecox*
RAM	Resistencia a los antimicrobianos
RCF	Relative Centrifugal Force/ fuerza centrífuga relativa
rpm	Revoluciones por minuto
SAL	Salinomicina
San	Santander
SO_4	Sulfato
tn	*Eimeria tenella*
USDA	Departamento de Agricultura de los Estados Unidos
Vall	Valle del Cauca
VM/WS	Viruta de madera/wood shavings
WAAVP	World Association for the Advancement of Veterinary Parasitology
WGNNC	Weight gain non-infected, non-medicated control/Ganancia de peso grupo no medicado no infectado
µl	Microlitro
µM	Micromolar
µm	Micrómetro
%	Porcentaje
°C	Grados centígrados

RESUMEN

Capítulo I. Se presenta una revisión de literatura sobre el estado actual de la coccidiosis aviar, haciendo enfásis en los principios básicos de dicha enfermedad, como el ciclo bilógico del parásito, especies de *Eimeria* que pueden infectar al *Gallus gallus,* tipos de anticoccidiales, vacunas y otras estrategias de control alternativo o de tipo natural, que se han promovido durante el tiempo para combatir una de las enfermedades más costosas para el sector avícola.

Capítulo II. Teniendo en cuenta la importancia y las pérdidas económicas generadas en la industria avícola mundial por enfermedades parasitarias como la coccidiosis aviar, desarrollamos el primer estudio epidemiológico en cuatro de las principales zonas avícolas de Colombia, evaluando muestras de camas recolectadas en los galpones para estimar la prevalencia y distribución de especies de *Eimeria* en granjas de pollos de engorde. Se recolectaron un total de 245 muestras de cama de 194 granjas en Antioquia (28), Santander (72), Valle del Cauca (57) y Cundinamarca (37) entre marzo y agosto de 2019. Las muestras se procesaron en el laboratorio de Parasitología de la Universidad de Antioquia para determinar la carga parasitaria mediante el recuento de ooquistes a través de la técnica de McMaster, seguido de un proceso de esporulación en dicromato de potasio al 2.5%, para la determinación de las especies *Eimeria.* Se estandarizó la técnica de PCR punto final, para confirmar la presencia de especies de *Eimeria* en las áreas muestreadas y con las cepas de *Eimeria* recolectadas en la zona de Cundinamarca, en una granja experimental se realizó una prueba de resistencia anticoccidial a tres medicamentos de uso frecuente para el control de la coccidia, utilizando 160 machos Ross AP de un día de vida para formar 5 grupos experimentales (NNC, INC, SAL, DIC, MET.CLO). Como resultados encontramos *Eimeria spp*. en 236 (96,3%) de 245 galpones individuales, lo que representa 180 (92,8%) de las 194 granjas muestreadas. *E. acervulina* fue la especie más prevalente (35,0%) seguida de *E. tenella* (30,9%), *E. maxima* (20,4%) y otras *Eimeria* spp. (13,6%). Sin embargo, las infecciones de especies mixtas con dos, tres y más infecciones fueron comunes, siendo la combinación más prevalente las mezclas de *E. acervulina, E. maxima, E. tenella* y otras especies en 74 de las 236 muestras positivas. El análisis de PCR reportó las especies *de Eimeria acervulina, maxima, tenella, necatrix, mitis y praecox* con variación de especies en cada área muestreada. Se encontró resistencia parcial de las especies de *Eimeria* para la salinomicina (SAL) y el clopidol metilbenzocuate (MET.CLO) y resistencia a diclazuril. Los resultados indican la naturaleza ubicua de *Eimeria* spp. en las granjas de pollos de engorde de Colombia. Se necesitan más pruebas de sensibilidad anticoccidial *in vivo* para ampliar el panorama del estado actual de la resistencia a los fármacos anticoccidiales en Colombia y de esta forma orientar los programas de control y manejo integrado de la coccidiosis aviar.

ABSTRACT

Chapter I. A review of literature on the current state of avian coccidiosis is presented, emphasizing the principles of this disease, such as the biological cycle of the parasite, *Eimeria* species that can infect *Gallus gallus*, types of anticoccidials, vaccines and other strategies of wild-type or alternative control, which have been promoted over time to combat a costly disease for the poultry industry.

Chapter II. Considering the importance and economic losses generated in the world poultry industry due to parasitic diseases such as avian coccidiosis, we developed the first epidemiological study in four of the main poultry areas of Colombia, examining samples of litter collected in the pens to estimate the prevalence and distribution of *Eimeria* species in broiler farms. A total of 245 litter samples were collected from 194 farms in Antioquia (28), Santander (72), Valle del Cauca (57) and Cundinamarca (37) between March and August 2019. The samples were processed in the parasitology laboratory of the "Universidad de Antioquia" to determine parasite load by the oocyst count through the McMaster technique, followed by a sporulation process in 2.5% potassium dichromate for the determination of *Eimeria* species. End-point PCR technique was standardized to confirm the presence of *Eimeria* species in the sampled areas and with the *Eimeria* strains collected in Cundinamarca, an experimental farm an anticoccidial resistance test to three drugs was carried out. Frequently used for the control of coccidia, using 160 one-day-old Ross AP males to form 5 experimental groups (NNC, INC, SAL, DIC, MET.CLO). We found *Eimeria* spp. in 236 (96.3%) of 245 individual houses, which represents 180 (92.8%) of the 194 sampled farms. *E. acervulina* was the most prevalent species (35.0%) followed by *E. tenella* (30.9%), *E. maxima* (20.4%) and other *Eimeria* spp. (13.6%). However, mixed species infections with two, three, and more infections were common, with the most prevalent combination being mixtures of *E. acervulina, E. maxima, E. tenella*, and other species in 74 of the 236 positive samples. PCR analysis reports *Eimeria* species *acervulina, maxima, tenella, necatrix, mitis* and *praecox* with species variation in each sampled area. Partial resistance was found for salinomycin (SAL) and clopidol methylbenzocuate (MET.CLO) and resistance to diclazuril. The results indicate the ubiquitous nature of *Eimeria* spp. in Colombian broiler farms. More *in vivo* anticoccidial sensitivity tests are needed to broaden the panorama of the current state of resistance to anticoccidial drugs in Colombia.

INTRODUCCIÓN GENERAL

La industria avícola es uno de los principales proveedores de alimentos a nivel mundial, siendo la carne de pollo y huevos una importante fuente de grasas y proteína de origen animal (Quiroz y Dantán, 2015). Para inicios del año 2020 el Departamento de Agricultura de los Estados Unidos (USDA), reportó una producción mundial de 100.5 millones de toneladas de carne de pollo (USDA, 2020), un incremento del 1% con respecto al mismo período del año anterior. Este aumento en la producción es importante, ya que se espera que para el año 2050 la población humana supere los nueve mil millones de habitantes, convirtiéndose la seguridad alimentaria sostenible en una preocupación mundial (O'neill et al*.,* 2010).

El sector avícola a nivel mundial en los últimos 20 años ha incrementado considerablemente, alcanzando una producción de 90 millones de toneladas de carne de pollo y 1.1 trillones de huevos por año (Prakashbabu et al*.,* 2017) y específicamente en Colombia, esta industria ha impulsado el desarrollo económico del campo en los últimos años, consolidándose como uno de los sectores determinantes para el crecimiento del PIB en el sector agropecuario que en el año 2018, tuvo un crecimiento del 4.5% con una producción de 14.6 millones de unidades de huevos y 1.6 toneladas de carne más (MADR, 2019); teniendo como principales zonas avícolas según el censo nacional agropecuario del Instituto Colombiano Agropecuario ICA 2019, Santander (24.0%), Cundinamarca (18.2%), Valle del Cauca (16.9%) y Antioquia (5.8%).

La necesidad de seguir con un crecimiento en la producción aviar ha llevado a tener sistemas de producciones más intensivos, que incluyen manejo de aves a altas densidades en espacios pequeños y con un tiempo de descanso corto entre lotes, facilitando la propagación de enfermedades; siendo esto un factor crítico, ya que cualquier patógeno que comprometa la producción puede representar una grave amenaza para el suministro mundial de alimentos (Godfray et al., 2010).

En estas condiciones en las granjas avícolas es frecuente encontrar patógenos como la *Eimeria* spp. quien se caracteriza por tener un ciclo de vida monoxeno y corto con un alto potencial reproductivo, facilitado por el manejo de aves en altas densidades de alojamiento, permitiendo su acumulación, transmisión y superviviencia (Morris y Gasser, 2006; Chapman y Jeffers, 2014); conviertiendo a la coccidiosis aviar en una de las principales enfermedades parasitarías que afectan a las aves de corral, ocasionando deficiencia en la absorción de alimento, disminución en la tasa de crecimiento, mortalidad y altos costos por medicamentos para prevención y tratamiento de la enfermedad (Dalloul y Lillehoj, 2006), causando grandes pérdidas económicas en la avicultura mundial (Williams, 1999; Blake et al., 2020).

El control de la coccidiosis aviar, se puede lograr mediante una combinación de varias estrategias, que incluyen buenas prácticas de manejo, estándares óptimos de bioseguridad en granja, en algunos casos la administración de vacunas vivas

de *Eimeria* (Ojimelukwe et al., 2018), pero el eje principal ha sido el uso de medicamentos anticoccidiales como una práctica tradicional de quimioprofilaxis (Abdisa et al., 2019; Ojimelukwe et al., 2018), que bajo las condiciones de crianza ya mencionadas, ha funcionado como un método de control eficaz y posible para combatir la coccidia (Chapman, 1997). Anteriormente, en Estados Unidos y algunas regiones de Europa reportaban una tasa de uso de anticoccidiales en granjas avícolas superior al 70% (Chapman et al., 2010), pero este uso extensivo ha llevado a que las especies de *Eimeria* adquieran resistencia a los medicamentos para su control (Blake and Tomley, 2014), problemática que ha sido ampliamente documentada (Chapman y Jeffers, 2015) y se cree que probablemente es el principal factor que causa la reducción de la eficacia de los medicamentos (McDougald et al.,1986).

Esta problemática de resistencia no solo a los anticoccidiales sino también a otros antimicrobianos ha llevado a que entidades como la Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO) desarrollen el Plan de Acción de la resistencia a los antimicrobianos 2016-2020, argumentando que el aumento global de la Resistencia a los Antimicrobianos (RAM) representa una importante amenaza para la salud humana y animal, pone en peligro la actual medicina humana y veterinaria y socava la seguridad de nuestros alimentos y el medio ambiente (FAO, 2016). Impulsando cada día más, producciones libres de antimicrobianos, siendo esto un reto adicional para que la industria avícola esté en la búsqueda constante de estrategias efectivas y económicamente viables para la prevención y control de la coccidiosis aviar (Abdisa et al., 2019).

Teniendo en cuenta la importancia del sector avícola para Colombia y considerando que no existe un estudio epidemiológico que nos indique ¿cuál es el estado actual de la coccidiosis aviar? y ¿cómo puede estar afectando el rendimiento productivo en los sistemas de pollo de engorde del país?, se hace necesario realizar un trabajo de investigación que pueda dar indicio del panorama general de la coccidiosis aviar, la diversidad de especies presentes en nuestros sistemas de producción y la respuesta a los medicamentos de uso frecuente para su control a través de pruebas de sensibilidad anticoccidial *in vivo.*

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OBJETIVOS

Objetivos Generales

Identificar las especies de *Eimeria* presentes en granjas avícolas de pollo de engorde en 4 regiones de Colombia y realizar una prueba de sensibilidad anticoccidial *in vivo*.

Objetivos Específicos

1. Realizar la caracterización general de las granjas avícolas de pollo de engorde de los departamentos de Antioquia, Santander, Valle del Cauca y Cundinamarca.
2. Determinar por características morfológicas las especies de *Eimeria* que infectan pollos de engorde en granjas avícolas de los departamentos de Antioquia, Santander, Valle del Cauca y Cundinamarca.
3. Validar por medio la prueba de PCR en punto final las especies de *Eimeria* presentes en las 4 zonas de muestreo.
4. Determinar la eficacia de 3 medicamentos comerciales de uso frecuente en los sistemas de producción de pollo de engorde para el control de la coccidia con una prueba de resistencia *in vivo*.

Capítulo I: Marco teórico

Con este capítulo se hace una revisión de los conceptos básicos del agente causal de la coccidiosis aviar, incluyendo ciclo de vida, especies, patología y algunas estrategias quimioprofilácticas y de manejo para su control.

Este mansucrito fue sometido al Journal of Applied Poultry Research el 8 de agosto de 2020 y actualmente se encuentra en segunda revión por parte de los evaluadores de la revista

REVIEW: GENERAL CONCEPTS ABOUT AVIAN COCCIDIOSIS: A CURRENT PROBLEM IN GLOBAL POULTRY FARMING.

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Primary Audience: Researchers, Nutritionists, Veterinarians, Animal husbandry.

SUMMARY

The poultry industry is one of the main sources of protein production for human consumption worldwide, but it faces great challenges such as avian coccidiosis, classified as one of the diseases with the greatest impact on the productive performance of this sector. This disease is caused by the protozoan *Eimeria*, which is a monoxenic obligate intracellular parasite. Seven species of this genus that can affect *Gallus gallus* have been recognized, each with different pathogenic characteristics and with a specific site of intestinal localization. *Eimeria* alters the correct functioning of the digestive tract, generating deficiencies in the absorption of nutrients and a low productive performance, which affects the economic

performance of the poultry production systems. The objective of this work was to evaluate the current knowledge of avian coccidiosis in aspects such as life cycle, pathophysiology, diagnosis, prevention and treatment.

Keywords: Chickens, *Eimeria,* oocysts, anticoccidials, diagnostic

INTRODUCTION

The poultry industry is one of the main suppliers of animal protein worldwide, both in chicken and eggs. (Bogosavljevic et al., 2010; Quiroz and Dantán, 2015). This is an industry in constant growth, as demonstrated by production reports from the United States Department of Agriculture (USDA). They reported a production of 102.9 million tons of chicken meat in January of 2020, which represent 3.9% more production compared to the same period of the previous year (USDA, 2020). This increase is significant, given that by 2050 the human population is expected to exceed nine billion, making sustainable food security a worldwide priority (O'neill et al 2010). Thus, any pathogen that compromises the efficiency of a poultry production system can pose a threat to food security worldwide (Godfray et al., 2010). Among the pathogens of great importance in the poultry industry, there are several species of *Eimeria,* that belong to the Apicomplexa phylum and that cause avian coccidiosis. These parasites are obligate intracellular with special organelles within the apical complex, necessary for the process of invasion of the host's intestinal cell (Quiroz and Dantán, 2015; Chapman, 2014). The following species are recognized in poultry: *E. acervulina, E. maxima, E. tenella, E. necatrix, E. mitis, E. praecox y E. brunetti*, each of them with a specific development site at the intestinal level and with different pathogenicity characteristics (Tewari and Maharana, 2011). The infection process in birds begins with the ingestion of sporulated oocysts (their infectious form); depending on the species, they could cause deficiencies in feed absorption, reduction in growth rates and, in the case of the most pathogenic species, mortality (Chapman, 2014).

Coccidiosis control has focused on several strategies including: management practices in farms, use of vaccines, natural additives and anticoccidials (Shivaramaiah et al., 2014) as a preventive measure through food, the latter being the most successful and frequent (Peek and Landman, 2011) for parasite control (Abbas et al., 2011b). However, the constant and indiscriminate use of anticoccidials has led to the selection of strains of resistant parasites (Abbas et al., 2011b; Chapman and Jeffers, 2015). As a result of drug resistance, diminished performance and increased mortality, avian coccidiosis is one of the main diseases that economically impact the productivity of the poultry industry worldwide (Berghiche et al., 2018).

Hence, the objective of this review is to present basic concepts about avian coccidiosis such as: species, biological cycle and ways to control in the presence of anticoccidial resistance and the tendency to reduce its use, which allow us to review the general concepts and to increase our understanding of the causal agent of avian coccidiosis and its sustainable and holistic management methods.

ETIOLOGICAL AGENT THAT CAUSES AVIAN COCCIDIOSIS

Coccidia consist of a wide variety of unicellular parasites in the protozoan subgroup of the phylum Apicomplexa. As a group, coccidia of the genus *Eimeria* (*Eimeridae* family) are species-specific, occurring in a single species of host or a group of closely related hosts (Shivaramaiah et al., 2014; Lopez et al., 2020). This phylum is characterized by grouping obligate intracellular parasites, which possess unique specialized organelles (López et., al 2020) that form the apical complex and include: micronemes, rhoptries, dense granules, conoid and polar rings (Figure 1.1) that provide the structural stability required during the process of invasion of the host cell (Chapman, 2014). Infection by a sufficiently large number of coccidia produces clinical manifestations of the “coccidiosis” disease, whereas a mild and subclinical infection is called “coccidiasis” (Amerah and Ravindran 2015; Conway and McKenzie, 2007). Once the parasite enters the host, different developmental stages of *Eimeria* spp. take place, invading the intestinal cells (enterocytes) to begin their replication process, resulting in pathological changes that range from the local destruction of the mucosal barrier and the underlying tissue, to the systemic effects such as blood loss and death (Ahmad et al., 2016).

LIFE CYCLE OF *Eimeria* spp.

The protozoans of the genus *Eimeria* spp. have a direct life cycle, which is characterized by a high tissue and host specificity, in addition to involving stages of asexual and sexual multiplication (Tewari and Maharana, 2011).

This cycle consists of three development stages: the formation of schizogony (agamogony/merogony), gametogony (gamete formation for sexual reproduction) and sporogony (Chapman, 2014).

As shown in Figure 1.A, the transmission happens via the fecal-oral route and the infection begins with the ingestion of sporulated oocysts containing 8 sporozoites (Sharman et al., 2010), which once inside the bird’s digestive system, will be in charge of starting the process of cellular infection, beginning with the life cycle stage called schizogony (Quiroz and Dantán 2015; Ahmad et al., 2016). The enzymatic microenvironment of the digestive tract and the mechanical action of the gizzard alter the structure and permeability of the oocyst wall (Quiroz and Dantán, 2015), the sporozoites contained within each sporocyst begin moving in order to remove the protein and carbohydrate plug called Stieda body, located in the sharp and narrow end of the sporocyst, thus allowing the sporozoites to exit into the oocyst cavity (excystation process), releasing them into the intestinal lumen through the oocyst micropyle (Kheysin, 1972) (Figure 1.B).

The sporozoites invade the intestinal cells and start a parasite feeding period that lasts approximately 12 to 48 hours, the parasitophorous vacuole is formed, the trophozoite begins to enlarge, and the parasite nucleus performs multiple asexual divisions (Walker et al., 2013), forming the schizont or meront, which is full of merozoites. Approximately three days post infection, the mature schizont breaks and releases the merozoites (Conway and McKenzie, 2007) (Figure 1.C), which are fusiform and have an apical complex (Figure 1.1) that allows them to move and infect intestinal epithelial cells in order to originate different schizont generations that reproduce asexually. The number of phases of asexual reproduction is

characteristic of each *Eimeria* species and is thought to be genetically programmed (Ahmad et al., 2016). Apparently, the main purpose of this phase is the amplification of the number of merozoites within the host as preparation for the sexual reproduction phase, which is an important characteristic of every apicomplexan life cycle (Walker at al., 2013). Later, the sexual reproduction stage or gametogony begins (Figure 1.D), which has three main events: gametocytogenesis, in which gametocytes are produced from merozoites; gametogenesis, from the gametocytes, haploid micro and macrogametes are differentiated; and the macrogametocyte fertilization process by microgametocytes (Figure 1.D), producing diploid zygotes with the consequent meiosis that marks the end of the sexual phase (Kheysin, 1972; Tewari and Maharana 2011; Walker et al., 2013).

The micro and macrogametocytes are morphologically different. The macrogametocyte grows and forms a single macrogamete. The male gamete matures, breaks up and releases a large number of small biflagellate microgametes (Price, 2012), after the fertilization, the oocyst is formed with an undifferentiated cytoplasmic mass which corresponds to the zygote and is protected by a double wall of proteins and fats that give it great resistance to mechanical and chemical damage in the environment (Quiroz and Dantán, 2015). The duration of the parasite's endogenous or internal phase development is determined by the time needed to complete the asexual and sexual reproduction and the formation of oocysts.

Once the oocyst is expelled along with the feces to the outside (Figure 1.E), the sporulation process begins (You, 2014a), this would be the third stage of the cycle. If the environmental conditions are adequate, the diploid oocyst initiates the sporogony formation, which happens in three stages (Kheysin, 1972): 1) Division of the zygote nucleus, preparation and reorganization of the cytoplasm. Division is performed twice to give rise to 4 nuclei; 2) Formation of 4 sporoblasts and their cytoplasmic reorganization, going through the pyramidal stage and the formation of the oval sporoblasts, which will give rise to 4 sporocysts in total. There is no nuclear division in this stage; 3) Sporozoite formation. A single nuclear division is produced in each sporocyst and the cytoplasm is divided in two longitudinal parts to form two sporozoites inside each sporocyst (Figure 1.F). For this process to occur, optimal conditions of oxygen, temperature and humidity are required. (Venkateswara et al., 2013; Quiroz and Dantán, 2015). The oxygen is necessary for the oocyst's respiration, since it would not be able to develop in anaerobic conditions (Kheysin, 1972). The temperature is another key factor, since the sensibility of the oocysts to high or very low temperatures has been demonstrated (Kheysin, 1972) for example, in the work done by You, Myung-Jo (2014), he evaluated the oocyst sporulation at different temperatures and found sporulation rates of 88.91%, 88.03% and 82.44% at 25, 20 and 30°C, respectively. The last necessary factor is humidity, a dry environment causes water loss, dehydration and deformation of the oocyst wall. As a result, the zygote is pressed by the collapsed walls and there can be no normal formation of sporogony (Kheysin, 1972). Awais et al., 2011 reported that the prevalence of coccidiosis was higher in the fall (60.02 ± 4.38) compared to other seasons, being this mainly related to the environmental conditions that promote sporulation and survival of the oocyst. Sporulation time could also be influenced by the *Eimeria* species. Venkateswara et al., 2013 evaluated

the sporulation dynamic of 6 *Eimeria* species subjected to a temperature range between 32 and 39°C and a relative humidity of 65% to 75%; including reports from other authors (Table 1).

Table 1. Comparative sporulation time (h) of *Eimeria* spp.

Species	Temp. 20°C (Edgar, 1954)	Temp. 29°C (Edgar, 1954)	Temp. 32-39°C (Venkateswara et al., 2013)	Levine, 1985	AbdulBasith et al., (1995)
E. acervulina	27	17	168	24	-
E. mitis	48	18	192	48	30
E. maxima	48	30	216	48	-
E. necatrix	48	18	96	48	24
E. tenella	48	18	96	48	-
E. brunetti	24-48	18	120	48	36

Modified from Venkateswara et al., 2013

The coccidia cycle is short, it has an approximate duration of 4-6 days, depending on the species (Walker et al., 2013). The infection occurs when the bird ingests sporulated oocysts, which once inside the microenvironment of the host's digestive tract, cause the stimulus of the excystation in the gizzards, and release sporozoites that invade and destroy the cells of the intestinal mucosa (Quiroz and Dantán, 2015). As a consequence, infected birds decrease the growth rate or egg production, possibly because of the intestinal damage, in many cases it is complicated by necrotic enteritis due to the proliferation of *Clostridium perfringens* and a high mortality rate, especially in young animals (Tewari and Maharana, 2011).

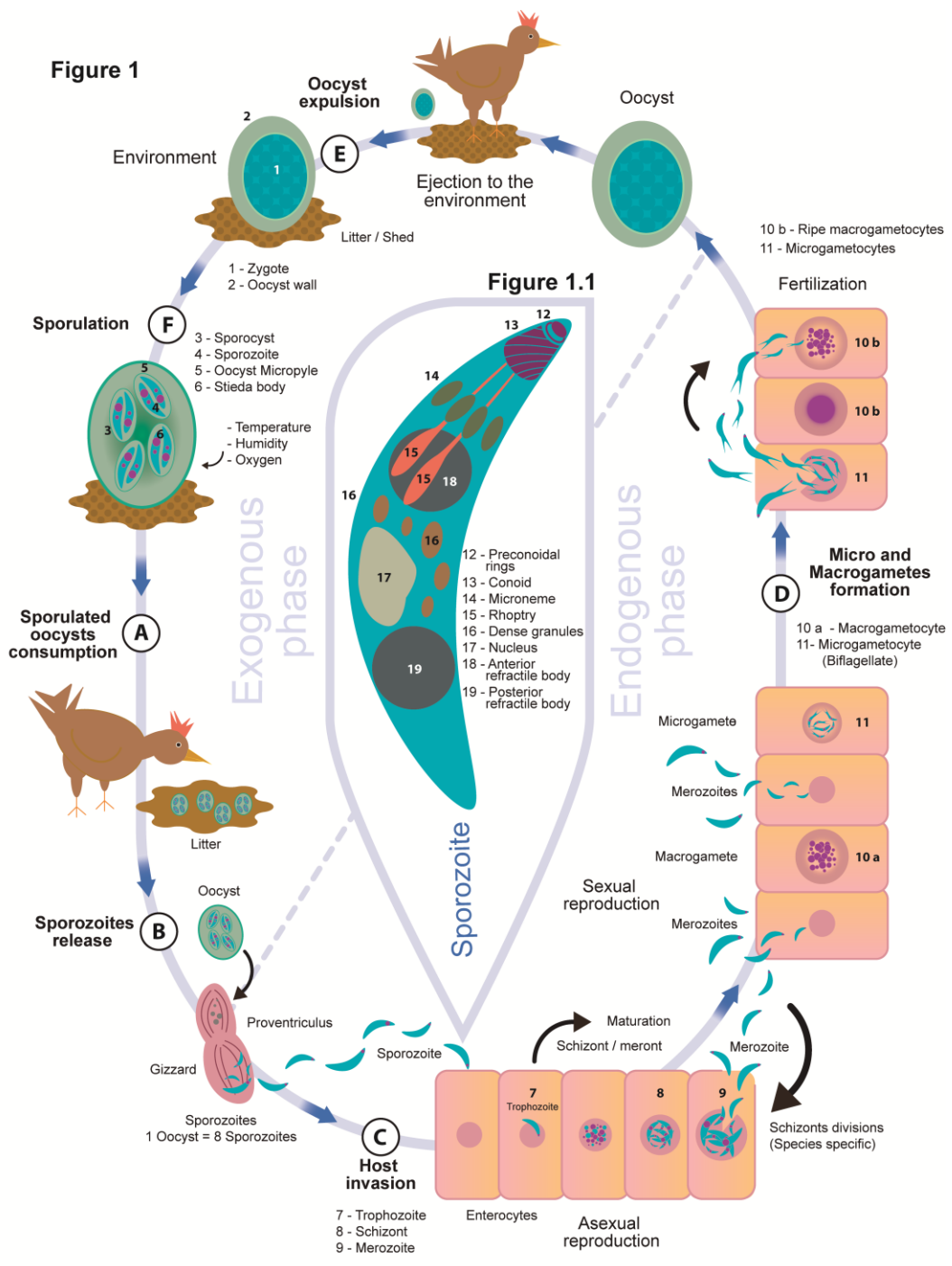

Figure 1. Life cycle of *Eimeria* spp.

Figure 1.1 Aplical complex

EIMERIA SPECIES THAT AFFECT THE _Gallus gallus_

Among poultry, 9 species of *Eimeria* that affect different sections of the intestine have been described (Table 2) (Ali et al., 2014), even though the pathogenicity data is in validation for two species that are frequently mentioned in literature, *E. hagani* and *E. mivati* (Conway and McKenzie, 2007). Each species of the parasite has a preference for a specific site of the gastrointestinal tract (Quiroz and Dantán, 2015), as well as a characteristic aspect of the macroscopic lesions, the morphology of the oocysts, the minimum sporulation time, the minimum prepatent period (time between the bird's infection with a sporulated oocyst and the expulsion of the first oocysts to the environment) (Arabkhazaeli et al., 2011), the size of the schizont and the location of the development of the parasite in the intestinal epithelium (You 2014a; Györke et al., 2013). Out of all the described species, 3 are the main cause of economic losses in broiler chicken, these are: *E. acervulina* which develops in the epithelial cells in the proximal region of the small intestine, mainly in the duodenum (Kant et al., 2013), *E. maxima* which is located in the intermediate region of the intestine and is easily recognizable due to the size of its oocysts, and *E. tenella* which infects the cecum (Conway and McKenzie, 2007).

Table 2. *Eimeria* species that affect poultry (*Gallus gallus)* and their main characteristics

Species	Development site	Pathogenicity *	Lesion scoring	Reference
E. praecox	Duodenum, jejunum	+	Intestinal water content, mucus and molten mucous material.	Long and Reid, (1982)
E. hagani	Duodenum, jejunum, ileum	+	Petechiae and white opacities in the upper small intestine. The intestinal content may be creamy or aqueous.	Long and Reid, (1982)
E. acervulina	Duodenum, jejunum	++	Limited enteritis, causing loss of fluids. Poor absorption of nutrients.	Chapman (2014)
E. mitis	Ileum	+	Limited enteritis, causing loss of fluids. Poor absorption of	Chapman (2014)

			nutrients.	
E. mivati	Duodenum, rectum	+	Red petechiae and round white spots. Severe mucosa denudation.	Chapman (2014)
E. maxima	Jejunum, ileum	++	Swelling of the intestinal wall with hemorrhagic points, detachment of the epithelium.	Chapman (2014)
E. brunetti	Cecum and rectum	+++	Swelling of the intestinal wall with hemorrhagic points, detachment of the epithelium.	Chapman (2014)
E. tenella	Cecum	+++	Thickening of the walls and blood content in the proximal end. Relaxation of the cecum. Destruction of villi, causing large hemorrhages and death. Intestine may be bloated.	Chapman (2014), Duffy et., al (2005)
E. necatrix	Jejunum, ileum, cecum	+++	Thickening of the mucosa and intestinal lumen filled with liquid, blood and the remains of tissue. Lesions in dead birds are observable as white and black sheets (salt and pepper appearance).	Chapman (2014)

** - nonpathogenic; + low pathogenicity; ++ moderate pathogenicity; +++ high pathogenicity*

Modified from Quiroz and Dantán, 2015

PATHOLOGY AND DIAGNOSIS OF AVIAN COCCIDIOSIS

It has been shown that the degree of infection and the pathognomonic signs of coccidiosis may vary and be influenced by multiple factors like: the *Eimeria* species; since some may cause loss of fluids and a decrease in nutrient absorption (*E. acervulina* and *E. mitis),* swelling of the intestinal wall with hemorrhagic spots and detachment of the epithelium (*E. brunetti* and *E. maxima)* or complete destruction of villi, producing hemorrhages and death (*E. necatrix and E. tenella)* (Chapman, 2014).

Other factors that may play a role are the genotype of the host, the amount of ingested oocysts, the sporulation percentage of the oocysts, the history of the animal's previous exposition (Blake, 2015) and management factors like: inadequate biosafety measures, equipment and facilities, presence of vectors such as rodents and insects, and other infectious agents like enteric viruses and/or bacteria, can collectively favor the propagation of the parasite and thus, perpetuate the infection in poultry production systems (Peek, 2010). Classical methods for the evaluation of *Eimeria* infections include the observation of the clinical signs in infected animals, the location and appearance of macroscopic lesions during the necropsy, the size and shape of the oocysts (Price, 2012) (Figures 2.E- 2.F) and sometimes the evaluation of other developmental stages in microscopic smears (Barrios et al., 2017).

Infected birds are seen with ruffled feathers and signs of depression or drowsiness (Figure 2.C). Also, the consumption of food and water is diminished, the feces may be aqueous, whitish and occasionally bloody (Figure 2.D) (Ali et al., 2014). This results in dehydration, decreased weight gain and in the absence of treatment, death (Blake, 2015).

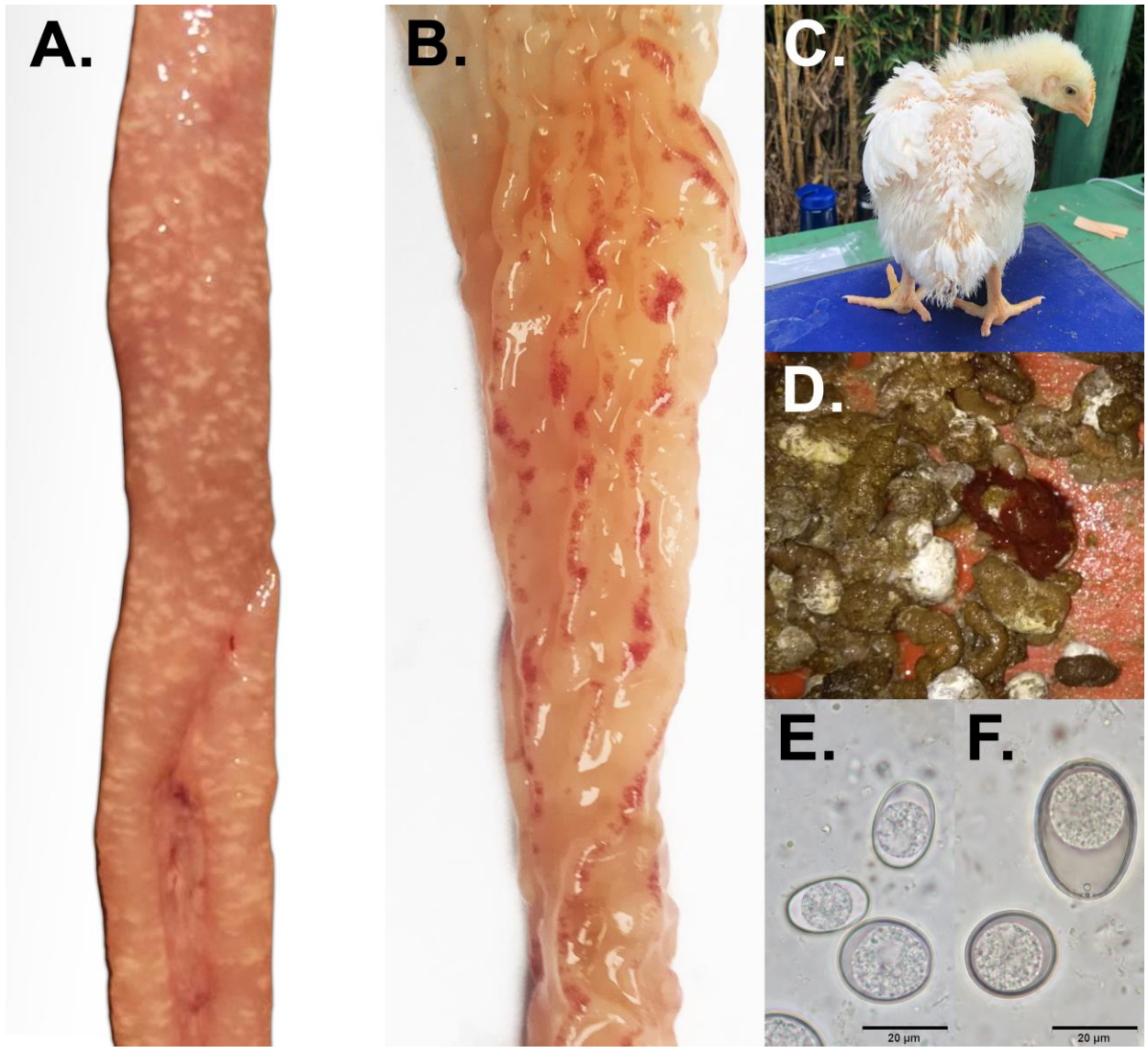

Figure 2. Strategies for coccidia diagnosis

Additionally, lesions in the intestinal tract generated by coccidiosis (figure 2.A - 2.B) may allow or promote secondary colonization by other pathogens, like *Clostridium perfringens* (Jenkins et al., 2017; Adhikari et al., 2020), Salmonella and certain viruses (Shivaramaiah et al., 2014), altering the intestinal health by causing problems with metabolism, nutrient absorption (Khater et al., 2020) and favoring the appearance of diseases such as necrotic enteritis (Adhikari et al., 2020).

For the evaluation of the macroscopic lesions, the intestinal lesion scoring technique is used (Johnson and Reid, 1970), which is based on giving a score in a scale of zero to four, with the goal of obtaining a numeric classification of the macroscopic lesions caused by each *Eimeria* species (Price, 2012; Barrios et al., 2017) (figure 2.A- 2.B).

For the scoring, the entire intestine of the bird must be evaluated, beginning with the duodenum, and mucous and serous membranes are examined in order to detect lesions, a good light source (solar or lamp) is needed for this (Conway and McKenzie, 2007). Generally, a set amount of birds per shed is examined (between 5 and 6, or 5% of the total number of birds) and the individual scores are grouped for all the *Eimeria* species (Raman et al., 2011). This is a laborious method, it may be subjective, and it needs experienced personnel in order to get a reliable result, but it is still the most widely used diagnostic method (Conway and McKenzie, 2007). This examination often needs to be complemented with counts of oocysts per gram of feces (OPG) or poultry litter through the McMaster technique (Bortoluzzi et al., 2018). It is believed that the correlation between lesion scoring and productive performance is stronger than the relation with the oocyst counts (OPG), but greater accuracy is needed to determine the level of lesions at which performance begins to be impacted, especially when subclinical conditions are present (Conway and McKenzie, 2007).

Recently, the Mini-FLOTAC was developed as a new method for qualitative and quantitative diagnosis of infections by helminths and protozoans, in several mammal hosts, being a useful technique to process large amounts of samples with a rapid analysis in a lab or in a farm (Cringoli et al., 2017). This technique has been seen as an alternative to the McMaster method, especially in cases where greater precision is needed (Bortoluzzi et al., 2018), having been used successfully in different species such as goats and horses (Silva et al., 2013; Noel et al., 2017). According to Bortoluzzi et al., (2018), who compared the precision of the McMaster technique and the Mini-FLOTAC to quantify *Eimeria maxima* oocysts, the Mini-FLOTAC is a reliable and precise method of quantification for this species.

Currently, molecular biology techniques are already available, like the polymerase chain reaction (PCR) based on the amplification of regions of the internal transcribed spaces 1 (ITS1) of the ribosomal DNA (Hamidinejat et al., 2010; Kumar et al., 2014; Tang et al., 2018); these methodologies are used for research and occasionally for the diagnosis of *Eimeria* spp., being more sensitive and less subjective (You, 2014b), making them ideal methods for the identification of species. However, they can be expensive and are not yet available in all poultry production regions of Colombia.

CONTROL METHODS FOR AVIAN COCCIDIOSIS

The control of coccidia has been focused on the use of vaccines, natural food additives, prophylaxis with anticoccidial medications, and improvement of handling practices in farms which includes cleaning and disinfection of facilities, adequate ventilation and water supply systems, which allow the maintenance of adequate conditions of the poultry litter and avoidance of the sporulation process of the oocysts (Peek and Landman, 2011). Prevention (prophylaxis) has been the pillar of production of broiler chicken (Chapman et al., 2010), greatly depending on anticoccidial medications in order to avoid the appearance of the disease (Peek, 2010).

Control with anticoccidial agents. Since the 1950s, almost all broiler chickens and turkeys were raised with an anticoccidial medication in their food. According to Agri Stats Inc. (Fort Wayne, IN), in the late 1990s, 99% of broiler chickens were raised with an anticoccidial drug in one or more foods, and this concept is still prevailing (Chapman, 2009).

Anticoccidials, based on their mode of action, may be divided into: coccidiostats, which halt the development of the parasite, impeding its replication and growth, its effect may be reversible and thus, its removal will lead to the end of the parasite's life cycle; and coccidicides, which are characterized by killing or causing irreversible damage to the parasite (Peek, 2010).

Anticoccidials may also be classified into three categories according to their origin (Peek and Landman, 2011; Chapman and Jeffers, 2014; Quiroz and Dantan 2015): 1) synthetic compounds, which are produced by chemical synthesis and have a specific mode of action against the metabolism of the parasite (Peek and Landman, 2011); 2) polyether or ionophore antibiotics, which are produced by the fermentation of *Streptomyces* spp*.* or *Actinomadura* spp. bacteria, they generally destroy coccidia by interfering with the passage of monovalent or divalent ions like sodium, potassium, calcium and magnesium, through the parasite's cell membrane (Peek and Landman 2011; Khater et al., 2020; Chapman 2009; Witcombe and Smith 2014). These may be classified into (Table 3).

Table 3. Anticoccidial agents classification

Category	**Anticoccidial agent**
Monovalent ionophores	Monensin, narasin, salinomycin
Monocyclic glycosidic ionophores	Maduramicin, semduramicin
Divalent ionophores	Lasalocid
Mixed: Synthetic with ionophore	Salinomycin/nicarbazin, Narasin/nicarbazin, Maduramicin/nicarbazin
Mixed: Synthetic with	Meticlorpindol/methylbenzoquate

synthetic

(Peek and Landman, 2011; Witcombe and Smith, 2014)

The extensive use of these medications prophylactically has resulted in a loss of efficacy of these compounds in controlling the parasite, causing resistance problems (Abbas et al., 2011b). In order to combat this situation, medication is used under programs called “Shuttle” or combined and direct rotation systems (“straight”). In the first program, two or more medications, usually with different modes of action, are used in the different foods throughout the bird’s life cycle; whereas in the second program, the type of drug used is changed after one or several lots or seasonally (Chapman, 2014; Quiroz and Dantán, 2015).

Vaccination for the control of coccidia. Infection with *Eimeria* spp. activates multiple facets of the host’s immune system, resulting in an effective, long-lasting, but species-specific immunity (Shivaramaiah et al., 2014).

The first study that showed the resistance of chickens against infection with *E. tenella*, was reported by Beach and Corl in 1925; but it was only 27 years later that the first live commercial vaccine against coccidiosis CocciVac® was released, registered in the United States (Peek, 2010; Witcombe and Smith 2014; Khater et al., 2020). Vaccination is included in coccidia control programs with the purpose of inducing an immune response, generating “resistance” to a subsequent challenge with *Eimeria* spp., decreasing the severity of coccidiosis (Peek and Landman, 2011; Price, 2012; Shivaramaiah et al., 2014).

Different vaccines are available on the market, such as live virulent, live attenuated, non-infectious derivatives of the parasite and vaccine subunits, which are genetically modified antigens (Shivaramaiah et al., 2014). The efficacy of live vaccines lies in the oral introduction of low doses of *Eimeria* oocysts, so that the ingested live antigens develop and initiate humoral and cellular responses against the different stages of the parasite (Chapman, 2014), with the cellular response being the most important one in terms of resistance to the disease (Shivaramaiah et al., 2014). These affirmations are supported by works like the one carried out by Lillehoj in 1987 on birds treated at different times with Cyclosporin A (drug that suppresses cellular immunity), without bursectomy and challenged with *Eimeria tenella*, finding that in the face of primary infection and the drug administered concomitantly, resistance to the parasite was high, but they were more susceptible to challenge in secondary infections.

Live vaccines can be divided into virulent and attenuated:

Virulent vaccines come from field strains without any manipulation in their pathogenicity (Peek, 2010). In mass vaccination, it is important to carefully standardize the dosage methods and conditions, so that the immunization is uniform among the lot, since the expulsion of oocysts into the environment increases and the birds will have a greater exposure to the parasite, by

means of fecal-oral transmission, causing subsequent infections that can provoke the appearance of serious reactions in those animals that did not receive the adequate vaccine dose, resulting in an asynchronous immunity, which may compromise the productive performance of the birds and cause greater susceptibility to the disease (Shivaramaiah et al., 2014).

Live attenuated vaccines. The attenuation process aims to decrease the pathogenicity of the parasite and can be performed by irradiation, chemical treatments, serial passage in chicken embryos or by precocity of the strains (Tewari and Maharana, 2011; Shivaramaiah et al., 2014). Precocity is characterized by a shortened endogenous life cycle, reducing the number of schizogonies, oocysts produced and therefore, intestinal damage in the host (Shivaramaiah et al., 2014; Witcombe and Smith, 2014). This causes a decrease in the pre-patent period, the reproductive potential of the strain, resulting in an attenuation of the virulence, but retaining its immunogenicity (Shivaramaiah et al., 2014).

Vaccine subunits. Identification of protective antigens is essential for the development of new vaccines against coccidia (Sharman et al., 2010). Separate and purified epitopes from the virulent organism have been used in anticoccidial vaccines, mainly native or in recombinant proteins expressed from the DNA of various stages of development (sporogonies, merogonies, gametogonies) of the *Eimeria* parasite (Peek, 2010). Vaccination links maternal immunization, stimulating the production of large amounts of immunoglobulin Y (IgY), to be transferred through the yolk of the egg, providing protective immunity to its offspring. This vaccination strategy has shown a decrease in the excretion of oocysts in birds challenged with *Eimeria maxima* (up to 83%), maternal transfer of protective antibodies and a possible cross-protection to heterologous species like *E. tenella* and *E. acervuline* (Sharman et al., 2010).

To improve the development of this type of vaccine, the immune response against the parasite must be understood, as well as the interaction between the parasite and the host (Peek, 2010).

Table 4 shows the available vaccines, considered a practical and important alternative, with a level of protection against coccidia for broiler chicken comparable to current anticoccidial programs (Chapman and Jeffers, 2015).

Table 4. Available commercial vaccines against avian coccidiosis

Name	***Eimeria* species**	**Type**	**Administration way**
Advent®	*E. acervulina, E. maxima, E. tenella*	Live non-attenuated	Oral

Coccivac-B®	*E. acervulina, E. maxima, E. mivati, E. tenella*	Live non-attenuated	Oral, spray, intraocular
Coccivac-D®	*E. acervulina, E. brunetti, E. hagani, E. maxima, E. mivati, E. necatrix, E. praecox, E. tenella*	Live non-attenuated	Oral, spray
Eimeriavax 4M®	*E. acervulina, E. maxima, E. necatrix, E. tenella*	Live non-attenuated	Intraocular
Inovocox®	*E. acervulina, E. maxima, E. tenella*	Live non-attenuated	*In ovo*
Immucox I®	*E. acervulina, E. maxima, E. necatrix, E. tenella*	Live non-attenuated	Oral, gel spray
Immucox II®	*E. acervulina, E. maxima, E.necatrix, E. tenella*,*E. brunetti*	Live non-attenuated	Oral, gel spray
Hatchpak Cocci III®	*E. acervulina, E. maxima, E. tenella*	Live non-attenuated	Spray
Paracox-5®	*E. acervulina, E. maxima, E. mitis, E. tenella*	Live attenuated	Oral
Paracox-8®	*E. acervulina, E. brunetti, E. mitis, E. maxima, E. necatrix, E. praecox, E. tenella*	Live attenuated	Oral
Livacox Q®	*E. acervulina, E. maxima, E. necatrix, E. tenella*	Live attenuated	Oral, Spray
Livacox T®	*E. acervulina, E. maxima, E. tenella*	Live attenuated	Oral, Spray
Coxabic®	Purified *E. maxima* antigens from microgametocyte stages	Vaccine subunit	Oral
Hipracox	*E. acervulina, E. maxima, E. mitis, E. praecox, E. tenella*	Live attenuated	Oral

Modified from Shivaramaiah et al., 2014.

Another important aspect when including live vaccines for the control of coccidia is the replacement of *Eimeria* populations in the facilities, thus restoring the susceptibility to anticoccidial methods, as evidenced in the work of Chapman and Jeffer (2015), who performed an anticoccidial resistance follow up to parasites obtained during 5 successive lots in broiler chicken, subjected to a rotation of different anticoccidial programs using salinomycin (ionophore) and diclazuril (chemical), including vaccination as an alternative method for restoring sensibility and concluding that the medication programs that were followed by vaccination improved the sensibility of the parasite to this type of medications.

Natural products for coccidiosis control. Currently, difficulties such as resistance and high cost of anticoccidials (Abbas et al., 2011a; Abbas et al., 2012), consumer pressure for antibiotic-free poultry products, restrictive legislation with the use of

these products in several countries (Witcombe and Smith, 2014) and problems associated to the pathogenicity of live vaccines, causes poultry producers around the world to increase their search for strategies that include safe, effective and economically viable alternative controls against avian coccidiosis (Abbas et al., 2011a; Abbas et al., 2012).

These alternatives include prebiotics, probiotics, essential oils, organic acids, antioxidants and nanobiotics (plant nanoparticles that have been used as antibacterial agents) (Khater et al., 2020), to reduce the problems caused by *Eimeria* spp. (Quiroz and Dantán 2015; Khater et al., 2020; Chapman et al., 2013). Many of these compounds are used as dietary supplements with various effects including immune system stimulation, anti-inflammatory and antioxidant action (Quiroz and Dantán 2015; Abbas et al., 2012).

Table 5 lists several of the natural compounds that have been used for control, their action mechanism and their results in controlling coccidiosis (Kadykalo et al., 2018).

Table 5. Alternative products with potential anticoccidial effect

Additive	Action mode	Effect	Reference
Artemisia (essential oil)	Induction of oxidative stress	Reduces the number of oocysts *in vitro*	Remmal *et al (2011)*
Clove (essential oil)	Unknown	Reduces the number of oocysts *in vitro*	Remmal *et al (2011)*
Turmeric combined with saponins and inulin	Stimulation of the system by inactivation of reactive nitrogenous radicals	Has no significant effect on lesion scoring	Sheurer et al (2013)
Oregano (essential oil)	Mucosal immunity stimulation	Has no significant effect on lesion scoring	Sheurer et al (2013)
Quillajacea (plant	Antiprotozoal activity (It binds to the protein	Has no significant effect on	Sheurer et

extract)	of the membrane of protozoal cells)	lesion scoring	al (2013)
S-nitrosoglutathione (GSNO)	Inhibits the sporulation process of *E. tenella* oocysts	Interrupts the sporulation process for 10 h after the initial sporulation; no effect after 12 h	Li et al (2010)
Lespedeza cuneata (plant extract)	Tannins have anticoccidial activity against the parasite	No significant difference in the number of oocysts	Rathinam et al (2014)
Tea tree (essential oil)	Unknown	Reduces the number of oocysts *in vitro*	Remmal *et al (2011)*
Thyme (essential oil)	Unknown	Reduces the number of oocysts *in vitro*	Remmal *et al (2011)*

Modified from Kadykalo et al., 2018

PERSPECTIVES

The growth of poultry production is essential to ensure food availability to the human population worldwide (food security), remembering that this topic not only covers the amount of the product, but also quality and safety characteristics for the final consumer. Additionally, the poultry industry faces challenges such as antibiotic-free production. As documented in the FAO Action Plan on antimicrobial resistance 2016-2020, the global increase in Antimicrobial Resistance (AMR) represents a major threat to human and animal health. The inappropriate use of

these drugs, associated with the appearance and spread of antimicrobial resistant microorganisms, puts everyone in a situation of great risk (FAO 2016).

Recently, the Colombian Agricultural Institute (ICA) released a mandatory regulation such as Resolution No. 22747 of April 09, 2018 (ICA 2018), which prohibits the use of additives containing polymyxin E (colistin) and polymyxin B as growth promoters in animal species that produce food for human consumption. The trend will be to ban more compounds, including some anticoccidials of the ionophore type, which have the property of inhibiting Gram-positive bacteria, exerting control over *Clostridium perfringes,* frequently being used as growth promoters (Peek and Landman, 2011). In Colombia, coccidiosis is not classified as an official control disease, there is no epidemiological information on the current problem and how it may be affecting the productive performance in the country's broiler chicken and laying hen systems; therefore, it is necessary to continue doing research that includes laboratory tests to make an evaluation in cell cultures in a fast and effective way, that may be extrapolated to field work at the national level in order to contribute to a better understanding of the problem of said disease, possible state of drug resistance and thus offer control alternatives that are sustainable, harmless and environmentally friendly.

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Capitulo II

Con el siguiente capitulo se busca dar cumplimiento a los 4 objetivos específicos de este trabajo de grado.

Survey of coccidia on commercial broiler farms in Colombia: frequency of *Eimeria* species, anticoccidial sensitivity, and histopathology

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ABSTRACT Avian coccidiosis continues to be one of the costliest diseases of commercial poultry. Understanding the epidemiology of *Eimeria* species in poultry flocks and the resistance profile to common anticoccidials is important to design effective disease prevention and control strategies. This study examined litter samples to estimate the prevalence and distribution of *Eimeria* species among broiler farms in 4 geographic regions of Colombia. A total of 245 litter samples were collected from 194 broiler farms across representative regions of poultry production between March and August 2019. The litter samples were processed for oocysts enumeration and speciation after sporulation. End-point polymerase chain reaction **(PCR)** analysis was conducted to confirm the presence of *Eimeria* species. Anticoccidial sensitivity was determined with 160 Ross AP males in 5 treatment groups: noninfected, nonmedicated control **(NNC)**, infected, nonmedicated control **(INC),** infected salinomycin treated **(SAL, dose: 66 ppm)**, infected diclazuril treated **(DIC, dose: 1 ppm),** and infected methylbenzocuate-Clopidol treated **(MET.CLO, dose: 100 ppm),** All birds were orally inoculated with 1×10^6 sporulated oocysts using a 1 mL syringe, except for the NNC- group who received 1ml of water. *Eimeria* spp. were found in 236 (96.3%) out of 245 individual houses, representing 180 (92.8%) out of 194 farms. *Eimeria acervulina* was the most prevalent species (35.0%) followed by *Eimeria tenella* (30.9%), *Eimeria maxima* (20.4%), and other *Eimeria spp.* (13.6%). However, mixed species infections were common, with the most prevalent combination being mixtures of *E. acervulina, E. maxima, E. tenella,* and other species in 31.4% of the *Eimeria*-positive samples. PCR analysis identified *E. acervulina, E. maxima, E. tenella, Eimeria necatrix, Eimeria mitis,* and *Eimeria praecox* with variable prevalence across farms and regions. Anticoccidial sensitivity testing of strains of *Eimeria* isolated from 1 region, no treatment difference ($P > 0.05$) was observed in final weight (BW), weight gain (BWG) or feed conversion (FCR). For the global resistance index (**GI**) classified SAL and MET.CLO as good efficacy (85.79 and 85.49, respectively) and DIC as limited efficacy (74.52%). These results demonstrate the ubiquitous nature of *Eimeria* spp. and identifies the current state of sensitivity to commonly used anticoccidials in a region of poultry importance for Colombia.

Key words: broiler chicken, *Eimeria* monitoring, oocyst, litter, anticoccidial sensitivity

2021 Poultry Science 100:101239
https://doi.org/10.1016/j.psj.2021.101239

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180 (92.8%) out of 194 farms. *E. acervulina* was the most prevalent species (35.0%) followed by *E. tenella* (30.9%), *E. maxima* (20.4%), and other *Eimeria spp.* (13.6%). However, mixed species infections were common, with the most prevalent combination being mixtures of *E. acervulina, E. maxima, E. tenella,* and other species in 31.4% of the *Eimeria*-positive samples. PCR analysis identified *E. acervulina, E. maxima, E. tenella, E. necatrix, E. mitis,* and *E. praecox* with variable prevalence across farms and regions. Anticoccidial sensitivity testing of strains of *Eimeria* isolated from one region, no treatment difference ($P > 0.05$) was observed in final weight (BW), weight gain (BWG) or feed conversion (FCR). For the global resistance index (**GI**) classified SAL and MET.CLO as good efficacy (85.79 and 85.49, respectively) and DIC as limited efficacy (74.52%). These results demonstrate the ubiquitous nature of *Eimeria* spp. and identifies the current state of sensitivity to commonly used anticoccidials in a region of poultry importance for Colombia.

Key words: broiler chicken, *Eimeria* monitoring, oocyst, litter, anticoccidial sensitivity

INTRODUCTION

Coccidiosis is the costliest parasitic disease in commercial poultry. Global estimates of the economic loss caused by coccidiosis in chickens range from USD 3 billion in 1995 (Williams, 1999) to over USD 13 billion annually in 2016 (Blake et al., 2020). Such losses include decreased feed consumption and growth rate, increased feed conversion (Williams, 1999; Dalloul and Lillehoj, 2006), and the cost of prophylactic and therapeutic control of the disease (Blake, D.P. and Tomley, F.M., 2014; Blake et al., 2020). Currently, intensive chicken farming relies heavily on anticoccidial drugs and live vaccines to control coccidiosis (Jenkins et al., 2017a). Anticoccidial control programs can be optimized by knowing the severity and timing of challenge as well as the species present and their anticoccidial drug sensitivity profile (Jenkins et al., 2017a). The number of *Eimeria* oocysts per g of litter follows a general pattern throughout the life of a flock and these data can be used to evaluate coccidiosis control programs (Chapman et al., 2002; 2016; Williams, 2002). In addition, *Eimeria* can be identified and speciated by visual evaluation of species-specific intestinal lesions (Johnson and Reid, 1970) and through oocysts morphology as assessed by the microscopic evaluation of intestinal scrapings (Hadipour et al., 2011; Györke et al., 2013).

However, under field conditions, the common occurrence of mixed infections makes it difficult to reach a specific diagnosis (Long and Joyner, 1984; Györke et al., 2013) that is essential for optimal prevention and control of coccidiosis. Molecular techniques such as the polymerase chain reaction (PCR) have proven to be effective tools for *Eimeria* diagnosis in broilers (Györke et al., 2013; Moraes et al., 2015). Molecular techniques reduce possible misdiagnosis between species with similar morphometric characteristics and can identify infections that might be missed through gross evaluation (Kučera, 1990). Nevertheless, PCR is not commonly used in field conditions.

Apart from accurately assessing the presence and severity of coccidia in the field, it can be helpful to understand the sensitivity of field isolates against commonly used anticoccidials (Abbas et al., 2011). Sensitivity testing usually consists of infecting groups of medicated and non-medicated birds with *Eimeria* isolated from the litter of commercial farms (Chapman, 1998). Sensitivity tests can provide valuable information to help producers design effective *Eimeria* control strategies (Abbas et al., 2008).

In Colombia the broiler and layer sectors have an annual growth rate of approximately 7-8%, as reported by the Colombian Agriculture Institute (ICA: Instituto Colombiano Agropecuario in spanish) (ICA, 2019), with a current population of 187.5 million birds, of which 104.8 million are broilers (ICA, 2019). The production of chicken meat in 2019 was 1.69 million tons (Fenavi, 2019). Poultry production is concentrated in four States: Santander (24.4%), Cundinamarca (17.06%), Valle del Cauca (15.98%) and Antioquia (6.25%) -. Each state has different climates, general management conditions and production environments. The ubiquitous nature of coccidia and concentrated poultry production can make disease control a challenge (Carvalho et al., 2011).

In Colombia there is no information on the incidence or prevalence of coccidiosis and only production companies know which anticoccidial drugs are used. In addition, vaccines are not commonly used to control coccidia (personal communications, March to October 2019). Having more information on *Eimeria* prevalence and the sensitivity of field isolates to commonly used anticoccidials can help producers make better treatment and control decisions. Therefore, the objective of this work is to survey the prevalence of *Eimeria* spp. on broiler farms in 4 important broiler-producing regions of Colombia and perform an anticoccidial sensitivity test with strains isolated from one of the surveyed areas.

MATERIALS AND METHODS

Ethics Committee Approval

The work was approved by the animal ethics committee of Universidad de Antioquia (Act No. 122 of February 5, 2019).

Sample Area and Farms

The study was conducted between March and August 2019. Using data from the MAPS-Fenavi - (2018) national database on poultry farms, the sample size was calculated (Grisales, 2011) for studies with known populations assuming an error of 10%. A total of 219 farms were sampled: 62 in Cundinamarca, 61 in Santander, 58 in Valle del Cauca and 38 in Antioquia. In each state, commercial farms that were in the finishing phase of the productive cycle (beyond d 21) were selected. A survey was used to collect additional information from each farm (e.g location, size, number of birds, number of sheds, management practices and biosecurity).

Litter sampling and analysis of Eimeria spp.

Samples were collected from 194 of the 219 proposed farms, corresponding to 88.6% of the initial objective. It was not possible to comply with the entire sampling because confidentiality of the farms. Litter samples were randomly collected from each shed while walking in a zigzag pattern (Goan, 2009). Depending on the size of the shed, between 6 and 12 grab samples of approximately 100g were taken, pooled, and homogenized. A 500g subsample was taken from each composite sample, placed in a hermetically sealed plastic bag, and transported to the laboratory where it was kept refrigerated at 4 °C for 1 to 3 days. Oocysts per g of litter (**OPG**) was calculated by microscopic (Olympus CH30, Olympus Optical Co., Ltd., Tokyo, Japan) enumeration using a McMaster chamber (Conway and McKenzie, 2007). To identify the *Eimeria* species, positive litter samples were washed in tap water, kept at room temperature overnight and filtered through sieves (Endecotts, London, England) of different diameters in a descending manner: 500, 212, 180, 75 and 45 µm. The resulting liquid was diluted in a proportion 1:1 with 5% potassium dichromate ($K_2Cr_2O_7$) [200 mL liquid in 200 mL potassium dichromate in an Erlenmeyer flask], and aerated with an aquarium pump (DG-302A, Aquarium air pump CE, China) at room temperature (approximately 27 °C) for 1 wk. Oocysts were isolated in saturated saline using a flotation technique (López et al.,

2018) and 100 sporulated oocysts were microscopically (100X objective. Olympus Optical Co., Ltd., Tokyo, Japan) speciated using morphometric measurements of length, width and shape index (Castañon et al., 2007; Haug et al., 2008).

Determination of Eimeria species by Polymerase Chain Reaction (PCR)

***Sample Collection*.** Litter samples from each of the 4 geographic regions were processed as described previously except a saturated sugar solution (density 1.18 to 1.20 g/mL- Sheather) was used for oocyst flotation prior to the addition of 2.5% potassium dichromate ($K_2Cr_2O_7$) for storage at 4 °C until analysis. Prior to the DNA extraction, 500 µL of the final solution was washed 5 times with PBS and centrifuged at 847 RCF (3000 rpm) for 5 minutes (Hettich Mikro 120 centrifuge (Tuttlingen, Germany)), to remove the potassium dichromate.

***DNA Extraction*.** DNA extraction was carried out for Antioquia, Santander, Cundinamarca and Valle del Cauca, taking 200 µL of the suspension containing 100 oocysts from each zone and using the commercial NucleoSpin Soil KIT (Macherey -Nagel, Düren, Germany). Following the methodology of López et al. (2018), the lysis time of the sample was modified by adjusting the agitation time in A-NucleoSpin Bead tubes (50 preps., Cat. No. 74078050, Macherey-Nagel GmbH & Co.KG, Düren, Germany) to 20 minutes. The quality of the DNA was verified and quantified in an Epoch-spectrophotometer using a nucleic acid quantification Take 3 plate (BioTek Instruments, Inc., Winooski, USA), with Gen5 2.04 software (BioTek Instruments, Inc., Winooski, USA).

***PCR analysis*.** PCR analysis was performed using a modified method of Moraes et al. (2015). The reaction was performed in a T3-Thermoblock thermocycler (Biometra GmbH, Gottingen, Germany), with initial denaturation at 96 °C for 5 min, followed by 35 cycles of 1 minute at 94 °C and 2 minutes at 55 °C (50 °C for *E. necatrix*, *E. tenella*, and *E. acervulina*), an extension at 72 °C for 30 seconds and a final extension at 72° C for 10 minutes. The PCR products were mixed with DNA-6X loading buffer (Thermo scientific, Waltham, Massachusetts, USA) and separated on a 1% agarose gel with the electrophoresis technique, marking them with GelRed (Bioutium, San Francisco, USA). The specific size fragments were identified, using a molecular weight pattern with a 100 bp ladder under ultraviolet light (UV Transilluminator Model M-20, Upland, California, USA).

The PCR was performed for each *Eimeria* species, preparing a solution with 200 µM of dNTps, 2.5 mM of $MgCl_2$; 5 U of Taq DNA polymerase (Thermo Scientific- Waltham, Massachusetts, USA), 10 pM Primer R, 10 pM Primer F and 10 ng of DNA. The solution for the species *E. necatrix*, *E. maxima*, *E. praecox*, and *E. mitis* was brought to a final volume of 33 µL and for *E. acervulina* and *E. tenella* to

a final volume of 20 µL. The primers used are shown in Table 1. As positive controls, Evant-Hipramunet vaccine (HIPRA-The Reference in Prevention for Animal Health) was used for *E. acervulina*, *E. maxima*, *E. tenella*, *E. praecox*, and *E. mitis* and Livacox Q vaccine (BIOPHARM, Research Institute of Biopharmacy and Veterinary Drugs) was used for *E. necatrix*. *Eimeria brunetti* is not reported since there was no positive control available.

Table 1. Forward (F) and reverse (R) primers in end-point PCR for detection of 6 *Eimeria* species and size of generated amplicon (TA).[1]

Name of Primer	Primer sequence	Size (pb)
ac-A03-F	AGT CAG CCA CAC AAT AAT GGC AAA CAT G	
ac-A03-R	AGT CAG CCA CAG CGA AAG ACG TAT GTG	811
tn-K04-F	CCG CCC AAA CCA GGT GTC ACG	
tn-K04-R	CCG CCC AAA CAT GCA AGA TGG C	539
mt-A03-F	AGT CAG CCA CCA GTA GAG CCA ATA TTT	
mt-A03-R	AGT CAG CCA CAA ACA AAT TCA AAC TCT AC	460
pr-A03-F	AGT CAG CCA CCA CCA AAT AGA ACC TTG G	
pr-A03-R	GCC TGC TTA CTA CAA ACT TGC AAG CCC T	354
mx-A09-F	GGG TAA CGC CAA CTG CCG GGT ATG	
mx-A09-R	AGC AAA CCG TAA AGG CCG AAG TCC TAG A	272
nc-A18-F	TTC ATT TCG CTT AAC AAT ATT TGG CCT CA	
nc-ENec-R	ACA ACG CCT CAT AAC CCC AAG AAA TTT TG	200

[1]*Eimeria* species: *E. acervulina (ac), E. tenella (tn), E. mitis (mt), E. praecox (pr), E. maxima (mx), E. necatrix (nc)*. (Pb): base pair. Adapted from Moraes *et al*., 2015.

In vivo anticoccidial sensitivity test with field strains isolated from Cundinamarca (Colombia)

***Eimeria* strain isolation.** Cundinamarca isolate was chosen for being one of the most important states between the higher poultry production states in Colombian. The 64 samples of litter containing *Eimeria* from Cundinamarca state were used to isolate oocysts for sensitivity testing. Litter were processed as described previously and oocysts were isolated by flotation in a saturated sugar solution (density 1.20 g/mL-Sheather). The saturated solution was removed by 5 serial washes with buffered water and centrifugation at 1211 RCF

(2500 rpm) for 10 minutes (Thermo Scientific IEC Centra GP8R, Needham Heights, MA, USA). The oocysts of the 64 samples were mixed and deposited in 2.5% potassium dichromate, at room temperature and with constant stirring for 4 days to achieve at least 70% sporulation. Oocyst concentration, speciation, and sporulation rate was determined using a hemocytometer (Neubauer Chamber). Subsequently, the potassium dichromate was removed replacing it with buffered water, performing 5 centrifugations at 1211 RCF (2500 rpm) X 10 minutes (Thermo Scientific IEC Centra GP8R, Needham Heights, MA, USA), and the challenge inoculum was adjusted to $1x10^6$ sporulated oocysts/mL (Gerhold, et al., 2011). The mixed inoculum had a similar composition of oocysts of *E. acervulina*, *E. maxima* and *E. tenella*, with approximately 33% of each of these species (333.000 oocyst for each *Eimeria*), differentiated on the basis of oocyst morphology (Stephan, et al., 1997).

The farms poultry in this area that entered the work and from where the Eimeria isolate was obtained, are different owners, all report the use of anticoccidial via feed, but do not information which type anticoccidial it is. In direct communication with technical personnel in the area, they report that the basic anticoccidial plan for the area is the supply of ionophore for the starter period (D. 1 to 21) and a combination of ionophore with chemical for the growth period (D. 21 to 42).

Anticoccidial sensitivity test. The test was carried out following the guidelines for evaluation of anticoccidial efficacy by the World Association for the Advancement of Veterinary Parasitology (Holdsworth et al., 2004) and the methodology used by Stephan et al. (1997) and Thabet et al. (2017). The anticoccidial sensitivity test lasted 21 days and was carried out during October and November 2019, in an experimental farm located in the town of Rionegro (Antioquia, Colombia) at an altitude of 2,125 m, with an average temperature of 17 °C. A total of 160 one-day-old Ross AP males were used to test the sensitivity of the *Eimeria* spp. strain to three commonly used anticoccidial medications. Broiler chickens were sourced from a commercial hatchery, received a standard vaccination program, and were housed together in floor pens previously flamed and sanitized with Farm Fluid S (NEOGEN, Lansing, Michigan) with *ad libitum* access to water and starter feed without anticoccidials or antibiotic growth promoters. Feed was formulated to complying with Ross 308 AP nutrient recommendations (Aviagen, 2019). At 12 d of age, animals were weighed, randomized into 4 replicates of 8 birds each (Table 2) and placed in wire-floor cages previously randomized to treatment, without blocking by cage. The wire-floor cages had been thoroughly cleaned and fumigated with concentrated ammonia solution prior to use. Birds received experimental diets from 12 d of age to trial

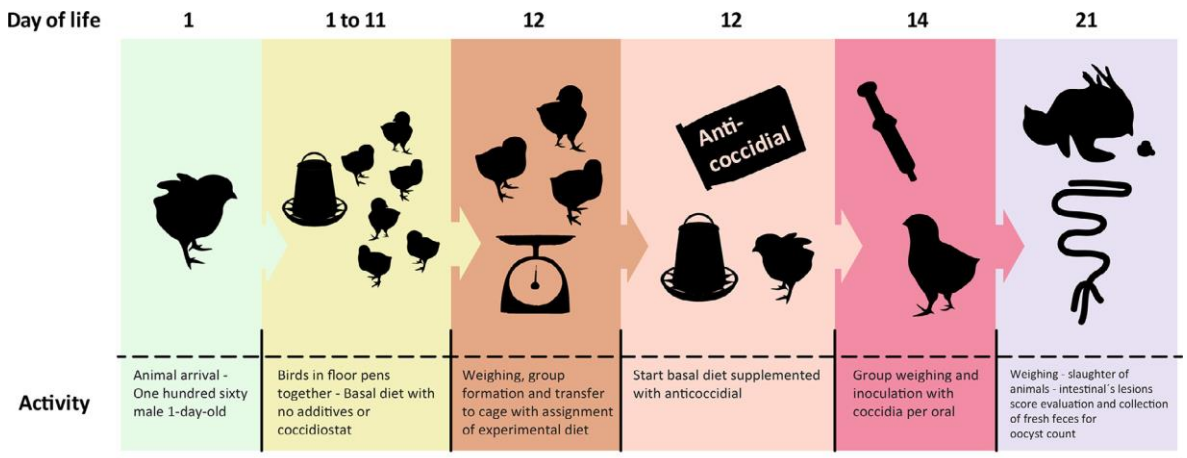
termination at 21 d of age. The following experimental groups were evaluated: Non-infected non-medicated control (**NNC**), Infected non-medicated control (**INC**) and 3 infected medicated treatments including Salinomycin (**SAL, dose: 66 ppm**), Diclazuril (**DIC, dose: 1 ppm**), and Methylbenzocuate Clopidol (**MET.CLO, dose: 100 ppm**).

Table 2. Description of treatments for *in vivo* anticoccidial sensitivity test in Broilers from Colombia.

Treatment[1]	**Number of replicates**	**Dose (ppm)**
NNC	4	
INC	4	
SAL	4	66
DIC	4	1
MET.CLO	4	100

[1] Experimental group of 4 replicates with 8 individuals each. Each challenged bird was gavaged with 1 x 10^6 sporulated oocysts containing equal portions of *E. acervulina*, *E. maxima*, and *E. tenella*, 333.000 oocysts each specie). Non-infected non-medicated control (**NNC**), Infected non-medicated control (**INC**) and 3 infected medicated treatments including Salinomycin (**SAL-66 ppm**), Diclazuril (**DIC-1 ppm**), and Methylbenzocuate Clopidol (**MET.CLO -100 ppm**).

On day 14, each bird was weighed and orally inoculated with 1x10^6 sporulated oocysts using a 1 mL syringe. On day 21 birds were group weighed and 2 individuals per replicate were randomly selected, for a total of 8 individuals per experimental group, to evaluate intestinal lesion scores (Johnson and Reid 1970) and fresh feces were collected directly from the bird at death to perform the count of oocysts per gram of feces (OPG) (Figure 1. Experimental test scheme).



Day of life	Activity
1	Animal arrival - One hundred sixty male 1-day-old
1 to 11	Birds in floor pens together - Basal diet with no additives or coccidiostat
12	Weighing, group formation and transfer to cage with assignment of experimental diet
12	Start basal diet supplemented with anticoccidial
14	Group weighing and inoculation with coccidia per oral
21	Weighing, slaughter of animals for evaluation intestinal´s lesions score evaluation and collection of fresh feces for oocyst count

Figure 1. Experimental test scheme.

Calculation of the global resistance index. The global resistance index (**GI**) for each experimental group was calculated using a modified version of the formula developed by Stephan et al. (1997) where oocyst index (**OI**) was obtained from the OPG count in fresh feces not from mucosa scrapings and used the following 0 to 5 categorical ranking: 0 = no, 1 = 1-10,000, 2 = 10,001-49,999, 3 = 50,000-79,999, 4 = 80,000-99,999, and 5 >99,999 OPG of feces.

$$\mathbf{GI\ (\%) = BWG_{NNC} - [(FCR_M - FCR_{NNC}) \times 10] - (OI_M\text{-}OI_{INC}) - [(LS_M - LS_{INC}) \times 2] - (\%MR/2).}$$

Where, OI = Oocyst index (Score 0-5), BWG_{NNC} = relative weight gain calculated as the percentage (%) gain of the NNC group, LS = Lesion score (score 0-4), FCR = feed conversion, MR = mortality (%), M = medicated, NNC = non-infected, non-medicated control, and INC = infected, non-medicated control.

In addition, the GI for each test group was calculated as a percentage of the GI for the NNC (Table 3).

Table 3. *In vivo* sensitivity rating parameters.

Efficacy	GI_{NNC} (%)
Very good	≥ 90
Good	80-89
Limited	70-79
Partial resistance	50-69
Resistance	<50

The global resistance index (**GI**). Non-infected non-medicated control (**NNC**).

Adapted from Thabet et al. (2017).

***Histopathological Analysis*.** On the final day of the test, 3 birds per experimental group were randomly chosen for intestinal histopathology at approximately 4 cm from the duodenal loop, 5 cm after Meckel's diverticulum (ileum), and from the cecum. Tissue samples were fixed in 10% buffered formalin, routinely processed, embedded in paraffin, sectioned, and stained with hematoxilyn and eosin (H&E). Microscopic evaluation was conducted at 100X and 400X magnification after staining with hematoxylin and eosin and was performed in duplicate by an expert avian pathologist who was blinded to treatments. Histopathological lesions and the presence or absence of *Eimeria* spp were categorized (Table 4) and summed across birds and intestinal locations for each experimental group. Finally, the total score is presented, a higher score indicates a greater degree of damage and a greater amount of intestinal Eimeria.

Table 4. Categorization histopathological of intestinal lesions and presence of *Eimeria* spp.

Score	Intestinal microscopic-lesions	Presence of *Eimeria*
0	Without lesions	No presence
1	Mild lesion	Sparse - scattered
2	Moderate lesion	Moderate - medium
3	Severe lesion	Numerous

Statistical Analysis

Survey data (OPG and *Eimeria* species present) were entered manually into Microsoft Excel (Microsoft Office 2016, Microsoft Corporation, Redmond, WA) to determine frequencies and prevalence of species by state. R version 4.0.2 was used to calculate a Pearson correlation coefficient between flock age and logarithmically transformed OPG. Results of the sensitivity test were analyzed using JMP 15 (SAS Institute Inc., Cary, NC). Growth performance data were analyzed as a randomized block (for high and low weights) design with

cage as the experimental unit. For intestinal lesions and oocyst count the non-parametric Wilcoxon test was used, with bird as the experimental unit. The OPG data were transformed by (Ln + 1) (Oviedo et al., 2006) and evaluated by a mixed model with cage as the random factor. Histopathology results are presented descriptively.

RESULTS

Field survey study

The density of birds in most farms was between 12 to 14 birds/m2, with an average number of 5.5 (± 3.0) sheds per farm, an average flock size of approximately 80,000 birds (range 10,000 to 330,000), and an average slaughter age of 37.5 days (Table 5). The most common building design was traditional open-sided sheds with tarpaulin or curtains with concrete floors using rice hull or wood shaving litter. In regions with warmer temperatures, mechanical fans are used to regulate the climate inside the sheds. Very few farms had controlled environment systems in the sampling areas that coincide with the warmest areas in the departments of Santander and Valle del Cauca. The commercial broiler lines used in the 194 farms studied were: Ross AP 51.2% (83/160), Ross AP x Cobb 500 33.8% (54/160), Cobb 500 13.7% (21/160) and others 1.3 % (2/160) (Table 6). Except for farms in Santander, which milled their own feed, most of the farms used contract feed suppliers that manufacture diets for pre-starter (hatching until day 10, starter from 11 to 21 days, and grower/finisher from 22 to 35-42 days). In Colombia, the use of anticoccidial vaccines is not very frequent and none of the farms included in the study were using coccidial vaccines at the time of sample collection. The use of recycled litter between flocks was only reported as a frequent practice in the region of Cundinamarca, where litter was typically reused for 3-5 cycles; 80% of the farms in the other sampling areas used new litter between each flock. At the end of each flock farms are chemically cleaned and disinfected, with an average down time of 10 to 15 days.

Table 5. Management characteristics of broiler farms in 4 regions of Colombia during 2019.

Department	**Cundinamarca**	**Santander**	**Valle del Cauca**	**Antioquia**
Sampled / registered Farms[1]	37/803	72/648	57/379	28/86
Annual average temperature (°C)	24.2	26.5	24.5	23.2
Altitude (masl)	1,781	1,098	1,150	1,679
Genetic line in number of sampled farms [2]				
Ross AP	14	45	11	13
Ross 308	0	1	0	1
Cobb 500	2	5	5	9
Mix of multiple lines	2	21	26	5
Density, birds per m^2 (min. to max.)	14.0 (13.0 to 15.0)	12.2 (8.8 to 17.0)	12.6 (11.0 to 17.9)	14.1 (13.5 to 14.5)
Litter composition	44.4% WS 55.6% RS	100% RS	56.1% WS 43.9% RS	92.9% WS 7.1% M
Birds placed per farm (min.to max.)	36.591 (10.200 to 162.000)	56.346 (5.700 to 195.000)	126.715 (9.180 to 330.000)	83.672 (20.400 to 147.600)
Sheds per farm (min.to max.)	2.7 (1 to7)	4.4 (1 to 14)	6.4 (1 to 18)	7.6 (3 to14)
Average age of birds at sampling (min.to max.)	29 (21 to38)	30 (21 to39)	31 (25 to43)	32 (22 to 41)

Abbreviations: M, mix of wood shavings with rice hulls; RS, rice hulls; WS, wood shavings.
1Register of farms by department - MAPS-Fenavi.
2160 total surveys.

The overall prevalence of *Eimeria* spp. was 92.8% (180/194) and OPG of litter varied widely between farms and departments (Figure 2), with an average of 1,931 (± 5,543) (Table 6). *Eimeria* species identified in descending order of frequency were: *E. acervulina* (35.0%), *E. tenella* (30.9%), *E. maxima* (20.4%), and other *Eimeria* spp. (13.6%). Infections of mixed species with two, three, and more species were

common and the most frequently found combination was the mix of at least 4 *Eimeria* spp., of which three are *E. acervulina*, *E. tenella*, *E. maxima*, and others, in 74 of 236 positive samples (Table 7).

No association was found between the age of the flock of the litter samples collected and the number of OPG found (R2= 0.00 and p = 0.2227) (Data not shown).

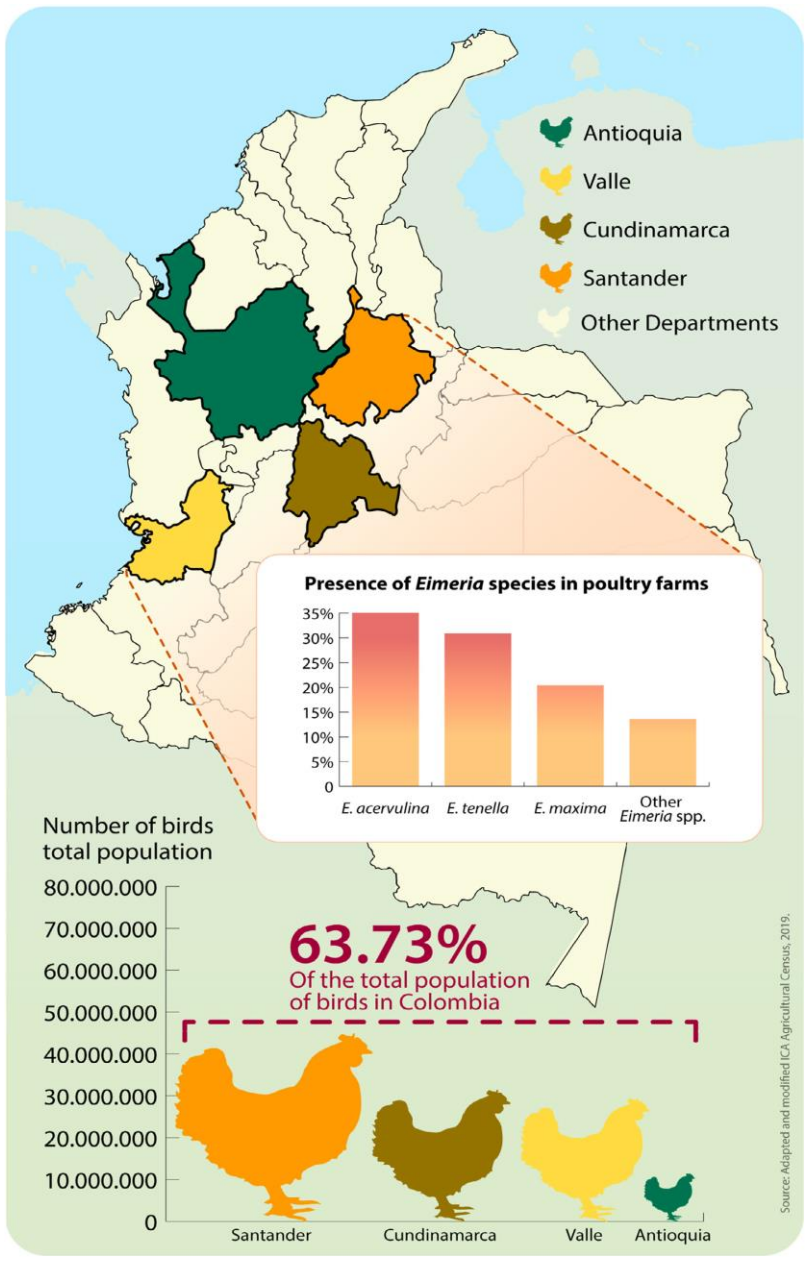


Figure 2. *Eimeria* species distribution in 4 departments of Colombia

Table 6. Frequency of *Eimeria* species and average (± SD) oocysts per gram (OPG) of farm litter in 4 regions of Colombia. Percentage of diversity and distribution of species.

Department	**Cundinamarca**	**Santander**	**Valle del Cauca**	**Antioquia**	**Total**
Number of litter samples analyzed[a]	67	72	62	44	245
Number of farms positive for *Eimeria* spp/total analyzed (%)	29/37 (78.4%)	69/72 (95.8%)	55/57 (96.5%)	27/28 (96.4%)	180/194 (92.8%)
Average OPG values of positive samples	1,592 ± 4,735	1,386 ± 3,185	1,018 ± 3,711	4,728 ± 9,827	1,931 ± 5,543
(min. to max.)	(0 to 32,800)	(0 to 24,300)	(0 to28,044)	(0 to 48,960)	(0 to 48,960)
***Eimeria* spp. (%)**					
E. acervulina	39.3	38.9	37.3	20.2	35.0
E. maxima	13.6	21.4	8.5	35.5	20.4
E. tenella	35.3	22.6	36.1	36.0	31.0
Other spp.	11.8	17.2	18.2	8.3	13.6

Percentage of diversity and distribution of species.

Table 7. Number of litter samples with different combinations of *Eimeria* species for each department sampled

Region	**No. of farms**	**No. of litter**	**No. of Posit.**	**No. of Negat.**	**a+m+t+o**	**a+m+t**	**a+t+o**	**m+t+o**	**a+m**	**a+t**	**a+o**	**m+t**	**m+o**	**t+o**	**a**	**m**	**t**	**Not identified[1]**
Ant	28	46	44	2	9	6	1	1	1	2	1	8	0	1	0	1	2	13
San	72	72	69	3	35	7	1	0	0	0	0	0	0	0	0	0	0	26
Vall	57	62	59	3	15	0	6	0	0	0	0	0	0	0	0	0	0	36
Cun	37	75	64	11	15	3	3	0	0	0	0	0	0	0	0	0	0	43

Abbreviations: a, E. acervulina; Ant, Antioquia; Cun, Cundinamarca; m, E. maxima; o, other spp.; San, Santander, t, E. tenella; Vall, Valle del Cauca.
[1]Not identified: samples with an OPG ≤170 species identification was not possible.

Confirmation of the Presence of *Eimeria* species by the PCR Technique in Four Regions of Poultry Importance in Colombia

The end-point PCR results showed that Antioquia and Santander were positive for the 6 *Eimeria* species analyzed *E. necatrix, E. maxima, E. praecox, E. mitis, E. acervulina,* and *E. tenella*; Cundinamarca was positive for *E. necatrix, E. maxima, E. praecox, E. acervulina,* and *E. tenella* and negative for *E. mitis* while, Valle del Cauca was positive for *E. maxima, E. praecox, E. mitis, E. acervulina,* and *E. tenella* and negative for *E. necatrix* (Figures 3A-1F).

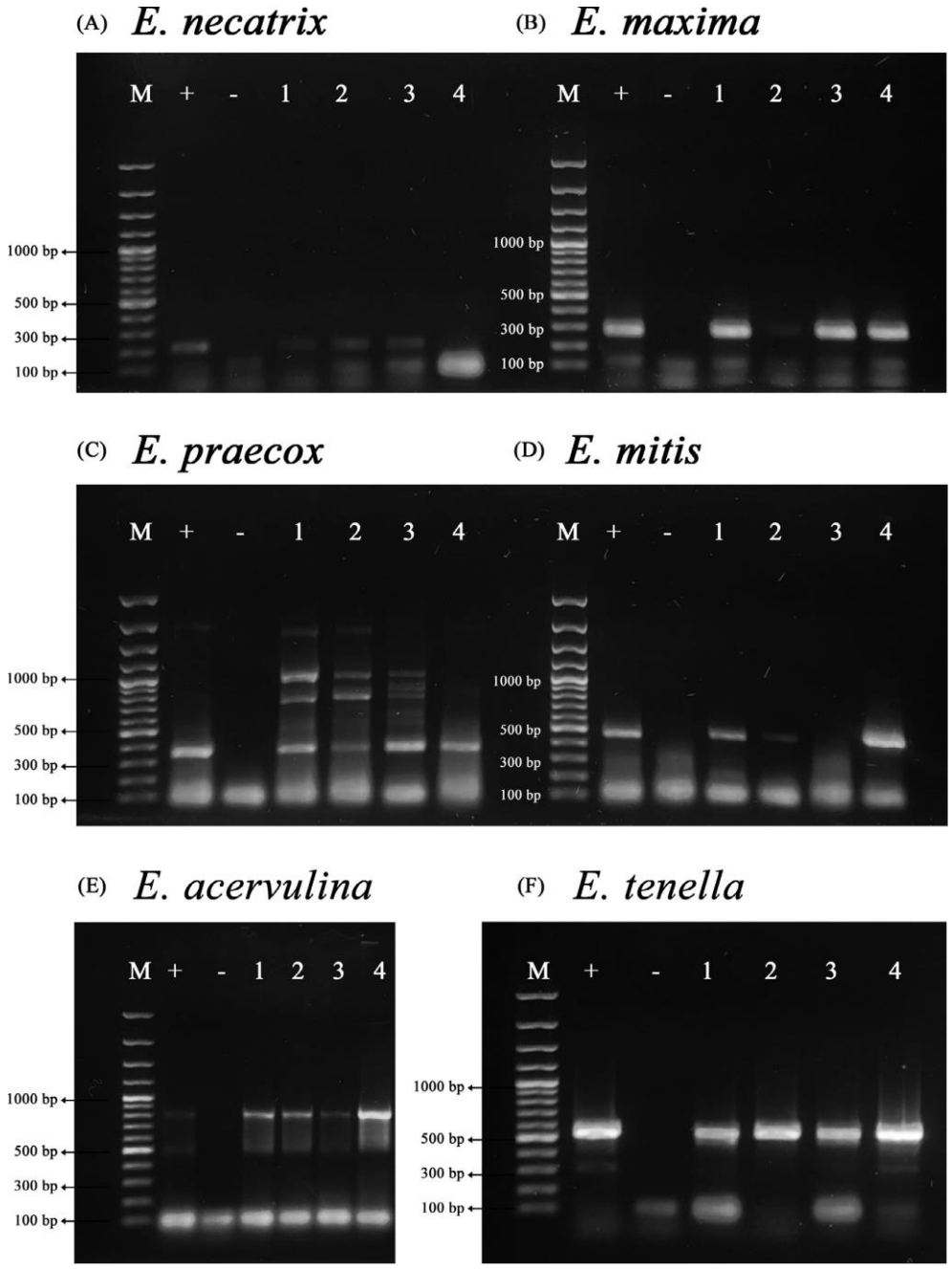


Figure 3. Gel electrophoresis. PCR result: molecular weight (marker 100 base pairs (bp)) (A) *Eimeria necatrix* (amplicon size 200bp) + (positive control) </*; - (negative control); 1 (Antioquia); 2 (Santander); 3 (Cundinamarca); 4 (Valle del Cauca). (B) *Eimeria maxima* (amplicon size 272bp). (C) *Eimeria praecox* (amplicon size 354bp). (D) *Eimeria mitis* (amplicon size 460bp). (E) *Eimeria acervulina* (amplicon size 811bp). (F) *Eimeria tenella* (amplicon size 539bp). <Positive control LIVACOX Q vaccine with *E. necatrix.* *Positive control EVANT-HIPRAMUNET *with E. maximum, E. praecox, E. mitis, E. tenella, and E. acervulina.*

In Vivo *Anticoccidial Resistance Test with Field Strains Isolated from Department of Cundinamarca (Colombia)*

No treatment difference ($P > 0.05$) was observed in final weight (BW), weight gain (BWG) or feed conversion (FCR) (Table 8).

No intestinal lesions, oocyst excretion, or mortality was observed in the NNC group (Table 9). The oocyst index presented a statistical difference between the SAL and MET.CLO groups. The only death recorded was in the DIC group but there were no treatment differences (P > 0.05) in mortality. SAL and MET.CLO treatments received a good efficacy with GI scores of 85.79 and 85.49, respectively. With a GI score of 74.52, the DIC treatment received a limited efficacy rating (Table 10).

Table 8. Summary of the zootechnical results for the *In vivo* Anticoccidial sensibility test.

Group	BW	BWG	FCR
NNC	760.48 ± 36.78	403.62 ± 35.24	1.33 ± 0.110
INC	746.28 ± 36.78	395.59 ± 35.24	1.38 ± 0.110
SAL	750.12 ± 36.78	398.25 ± 35.24	1.39 ± 0.110
DIC	731.60 ± 36.78	356.81 ± 35.24	1.59 ± 0.110
MET.CLO	732.53 ± 37.17	379.49 ± 35.90	1.44 ± 0.121

Measurements taken 7 days after the challenge with 1 X 10^6 sporulated oocysts/mL of field *Eimeria* spp.
Final weight (BW), weight gain (BWG) represented in grams, and feed conversion (g/g) (FCR) (Least squares mean ± standard error). Non-infected non-medicated control (**NNC**), Infected non-medicated control (**INC**) and 3 infected medicated treatments including Salinomycin (**SAL-66 ppm**), Diclazuril (**DIC-1 ppm**), and Methylbenzocuate Clopidol (**MET.CLO-100 ppm**).

Table 9. Gross lesion score (LS) for *E. acervulina*, *E. tenella* and total mean lesion score (TMLS), oocyst index (OI) and mortality rate (MR)

Group	LS - *E. acervulina*	LS - *E. tenella*	TMLS[1]	OI	MR (%)
NNC	0.00^b	0.00^b	0.00^b	0.0^b	0
INC	0.50 ± 0.53^a	1.37 ± 1.19^a	1.88 ± 1.46^a	2.2 ± 0.64^a	0
SAL	0.62 ± 0.52^a	1.37 ± 0.74^a	2.00 ± 1.07^a	2.8 ± 1.19^c	0
DIC	0.50 ± 0.53^a	0.62 ± 0.74^a	1.13 ± 1.55^a	3.4 ± 1.69^a	3.21
MET.CLO	0.37 ± 0.52^a	1.50 ± 1.31^a	1.88 ± 2.07^d	1.2 ± 1.13^d	0

Measurements taken 7 days after the challenge with 1 x10^6 sporulated oocysts of field Eimeria spp.
[a-d] Different letter indicates statistical significance ($P < 0.05$).
[1]TMLS: Sumatory of the average score of Eimeria species intestinal lesions, 8 chickens per treated group. Non-infected non-medicated control (NNC), Infected non-medicated control (INC) and 3 infected medicated treatments including Salinomycin (SAL-66 ppm), Diclazuril (DIC- 1 ppm), and Methylbenzocuate Clopidol (MET.CLO- 100 ppm).

Table 10. Relative weight gain (% BWG_{NNC}), global resistance index (GI) and Global index of NNC (%), estimated average values (± standard deviation) for field *Eimeria* spp.

Group	BWG_{NNC} (%)	GI	Global index of NNC (%)	Status
INC	98.01 ± 3.75			
SAL	98.67 ± 5.09	97.27	85.79	Good efficacy
DIC	88.40 ± 25.79	84.49	**74.52**	**Limited efficacy**
MET.CLO	96.93 ± 9.02	96.93	85.49	Good efficacy

Histopathology Analysis

The NNC group showed a degree of inflammation categorized as enteritis and minimal eosinophilic typhlitis, but no presence of *Eimeria* (Score 18) (Table 11). The INC group showed evidence of intestinal damage, with tissue erosion and severe focal cecal hemorrhage and *E. acervulina*, *E. maxima* and *E. tenella* organisms were observed in each segment (Score 18); coccidial typhlitis was diagnosed. The groups medicated with SAL (Score 25) and with DIC (Score 23) showed mild to moderate hyperplasia of gut-associated lymphoid tissue (**GALT**) in the lamina propria, slight necrosis in some segments (ceca) and *E. acervulina, E. maxima,* and *E. tenella* organisms were identified. *Eimeria necatrix* was only found in one bird receiving DIC. In the DIC group, there were large oocysts that coincide with *E. necatrix*, where severe coccidial typhlitis was diagnosed. The group treated with MET.CLO (Score 33) showed moderate hyperplasia of the GALT in the lamina propria, moderate necrosis of the cecal tissue, and all the sections evaluated had coccidia: *E. acervulina, E. maxima,* and *E. tenella.* Unidentified *Eimeria* species were found in the ileum of all 3 anticoccidial treated groups but not in the infected control group. In the matters of histological score, the group with MET.CLO shows the highest score indicating greater intestinal damage and greater amount of coccidia.

Table 11. Histological description of lesions and presence of *Eimeria* in 3 intestinal segments of individuals challenged with coccidia.

Histopathological Findings[1]	Experimental group				
	NNC	**INC**	**SAL[66 ppm]**	**DIC[1 ppm]**	**MET.CLO[100 ppm]**
Hyperplasia GALT[2]	4	0	2	1	2
Hyperplasia lamina propria	3	0	2	1	2
Eosinophils	9	0	1	0	0
Heterophiles	0	0	0	0	1
Lymphocytes	1	0	0	0	0
Fibrin	0	0	1	1	1
Necrosis	1	0	1	0	2
Erosion	0	1	0	0	0
Hemorrhage	0	1	0	0	4
E. acervuline	**0**	**2**	**4**	**4**	**4**
E. maxima	**0**	**7**	**6**	**6**	**7**
E. tenella	**0**	**7**	**6**	**6**	**8**
E. Necatrix	**0**	**0**	**0**	**1**	**0**
Other Eimerias	**0**	**0**	**2**	**3**	**2**
Total score	**18**	**18**	**25**	**23**	**33**

[1]Sum of 3 individuals and 3 intestinal segments (duodenum, ileum and cecum) per treatment. The table shows the number of individuals that presented different cell types in the different intestinal layers and segments; different type of lesions: necrosis, erosion, hemorrasge; and the presence of different Eimeria spp. stages. [2]GALT: gut-associated lymphoid tissue, NNC: non-infected non-medicated, INC: infected non-medicated, SAL: infected treated with Salinomycin, DIC: infected treated with Diclazuril, MET.CLO: infected treated with Methylbenzocuate Clopidol

DISCUSSION

This is the first study we are aware of that reports the prevalence and anticoccidial sensitivity of *Eimeria* on commercial broiler farms in Colombia. In this study, litter samples were taken since it can be difficult to get fresh feces from commercial farms (Chapman et al., 2016). Chapman et al. (2016) reported that sampling built-up litter allows the evaluation of accumulated oocysts across several grow-outs and indirectly verifies the conditions of the litter to know whether the parasite sporulation process is taking place (Venkateswara et al., 2015). In this work at the time of sampling it was not possible to sacrifice birds to identify scores of intestinal lesions by *Eimerias spp*, as in other reported studies (Gharekhanietal, 2014; Belal, 2017; Gazoni et al., 2020) to be able to complement the OPG found in litter with the intestinal health of the birds. Of the total litter sampled, 92.8% were positive for *Eimeria* spp. This high frequency of *Eimeria* spp. has also been reported in other studies using litter or fecal samples: 90% in Argentina (Mattiello et al., 2000); 92% in Romania (Györke et al., 2013), 79.4% in North India (Prakashbabu et al., 2017), 65.8% in East China (Sun et al., 2009) and 78.7% in South Korea (Lee et al.,

2010); but different from the reports of Gharekhani et al., 2014 in Hamedan (Western Iran) and Kaboudi, et al., 2016 in Sidi Thabet, (Tunisia), where the general prevalence of coccidia was 31.8%. Additionally, 41.1% (74/180) had mixed *Eimeria* (*E. acervulina, E. maxima, E. tenella* and others) infections, although one species was always predominant in each farm. Results that agree with other studies in various regions of the world such as Iran, India, Alegeria, Brazil (Gharekhanietal., 2014; Prakashbabu, et al., 2017; Debbou-Iouknane et al., 2018; Gazoni et al., 2021). This determination of the distribution of coccidial species is important because it depends not only on the level of oocysts ingested, but also on the species involved and the host's immune response (Yun et al., 2000). It is known that *E. acervulina* can develop resistance to anticoccidials at a faster rate, due to its high reproductive potential and short life cycle (Williams, 2001; Chapman, et al., 2010). This could explain the higher frequency of *E. acervulina* (35.0%) in all sampling areas of this study, as well as in the study by Haug et al. (2008) in Norwey with 100%, Györke et al. (2013) in Romania with 91%, and in the work by Gazoni et al. (2015) in Brazil with 13.5%.

Others have reported that there is an increase in the number of oocysts in litter between 3 to 4 weeks after bird placement and OPG can be a good way to monitor the course of infection throughout the life of a flock (Chapman et al., 2016; Jenkins et al., 2017a). In this work, there was no evidence of a relationship between the age of the flock at the time of sampling and the oocyst count, possibly because only one sample was taken per farm. On the contrary, Chapman et al. (2016) evaluated the course of infection at weekly intervals in 6 successive flock showing a bell-shaped curve with a oocyst peak around 21 to 33 d of age. The average OPG in the current study (1.9 x 10^3), equal to that reported in Hamedan (Western Iran) by Gharekhani et al (2014) (1.8 x 10^3), was lower than the 52 x 10^3 (range 35.8 x 10^3 to 73.6 x 10^3) reported by Chapman et al. (2016), but relatively similar considering production difference between the US and Colombia and the fact we only sampled farms once and potentially missed the peak of oocyst production. Of the regions sampled, Antioquia presented the highest oocyst count (average 4.73 x10^3, range 0 to 48.96 x 10^3), this could be speculatively due to particular conditions of the area, such as handling a higher density of birds in the sheds (average 14.1 birds /m^2), where 92.9% wood shavings are used as litter material and the climatic conditions of the area at the time when the sampling was carried out, coinciding with the rainy months of the year between March-May 2019. These results agree with the work of Balel (2017) in Bangladesh, showing a higher occurrence of coccidiosis in the rainy season, with higher relative humidity and temperatures. These conditions could favor humidity,

facilitating the spread of the parasite, reflecting in a higher oocyst count (Bachaya, et al., 2012, Lawal et al., 2016) from this sampling area.

As reported by other authors, coccidia is ubiquitous in poultry farms (Bachaya, et al., 2012; Debbou-Iouknane et al., 2018), but the variation in terms of hygiene conditions, management and geographic location of the farms can be the main causes of the differences (Bachaya et al., 2012; Gharekhani et al., 2014) in the presence of the parasite in the field, as reflected in the results of the OPG counts in the 4 sampling areas. In this work, as a differential management practice, the use of recycled litters was reported more frequently in the Cundinamarca zone, a condition that can directly affect the amount of oocysts in litter (Chapman, 2016), research work such as Garcés et al (2018) in Ecuador, who showed that the use of recycled litter more than twice, improves productive parameters at day 35 of the chickens' life, compared to the use of new litters between each batch. Possibly due to the immunity generated by early exposure to oocysts in the litter, in addition to the effect of earlier colonization with microbiota that can act as a probiotic or direct feed microbial supplement, leading to better chicken performance (Garcés et al., 2018). Another condition that can alter the results of the oocyst counts is the anticoccidial treatment program implemented in each farm at the time of sampling, wich was not possible to obtain due to the confidentiality claimed for the producer (Chapman and Jeffers, 2015; Garcés et al., 2018).

Epidemiological studies on the frequency of *Eimeria* spp. complemented with molecular analyzes are valuable tools for the prevention and control of coccidiosis (Haug et al., 2008; Ogedengbe et al., 2011; Györke et al., 2013; Jenkins, et al., 2019). They can identify in a short time and with precision, for example the viability of oocysts capable of causing infection (Jenkins, et al., 2019) and which species of *Eimeria* are compromising the productive parameters in poultry farms, so an effective control strategy can be developed. This study, like the study by Hamidinejat et al. (2010) and Moraes et al. (2015), used the PCR technique to identify the species of *E. acervulina, E. maxima, E. praecox, E. mitis, E. necatrix* and *E. tenella*. Despite the differences in the sampling locations and methods, both studies showed that it is common to find mixed infections which can be difficult to characterize through clinical signs alone which require more specialized training (Carvalho et al., 2011). Finally, in this work, due to morphological characteristics of the oocysts in order of prevalence, the species of *E. acervulina* (35.0%), *E. tenella* (31.1%) and *E. maxima* (20.4%) were identified, data similar to those presented by Debbou -Iouknane et al (2018) in Algeria, with prevalence of 32.05%, 26.92% and 11.53% and to the work of Gharekhani et

al (2014) in Western Iran, with higher but consistent prevalence in the order of frequency 75.7%, 54.3 % and 20% for *E. acervulina*, *E. tenella* and *E. maxima*, respectively; species that are classified between moderate and high pathogenicity (Kaboudi et al., 2016), additionally with the PCR *E. praecox, E. mitis* and *E. necatrix* were identified, the latter being highly pathogenic (Kaboudi et al., 2016).

These findings are of great importance, since the species with moderate and high pathogenicity are possibly interfering with the productive response of the birds in the sampled farms (Debbou-Iouknane et al., 2018), in addition to this subclinical form (mild infections that do not show symptoms) of coccidia is responsible for causing a reduction in the feed conversion of birds (Gazoni et al., 2021) and taking into account that the feed account for approximately 70% of the cost of broiler chicken production, the economic impact of coccidia on broiler chicken production is very high (Jordan et al., 2002; Molla, B., & Ali, A. 2015 ; Gazoni et al., 2021) only due to the reduction in this productive parameter.

In this work, the PCR technique complements the diagnosis of *Eimeria* species, which due to morphological characteristics of the oocysts was not easy to identify, for this reason the PCR technique is a methodology that in recent years has gained relevance for its rapid and accurate diagnosis (Brown et al., 2018).

In countries such as Norwey (Haug et al., 2008), Romania (Györke et al., 2013), Brazil (Moraes et al., 2015), and India (Brown et al., 2018), the PCR technique is frequently used for the identification and diagnosis of *Eimeria.* Additionally, data from PCR can complement control programs against coccidia, which, due to the reduced use of antibiotic feed additives in the European Union and other regions of the world (Regulation EC N° 1831/2003), have been focused on reinforcing biosecurity and hygiene practices in poultry farms, combined with the prophylactic use of live vaccines (Thabet et al., 2017) and natural feed additives (Peek and Landman, 2010).

Although there is a trend to reduced chemotherapy in poultry production, anticoccidial products are still used frequently (Thabet et al., 2017) and are considered critical for broiler production in many regions of the world (Chapman et al., 2010; Peek, 2010). Widespread use of anticoccidial products has led to the appearance of resistant strains, which is a serious challenge for the poultry industry in general (Quiroz-Castañeda and Dantán-González, 2015). In this study, representatives of 3 classes of anticoccidials was evaluated in a sensitivity test. For synthetic compounds popularly known as “chemicals” (Chapman and Jeffers, 2015), DIC was used. In the category of ionophores that are produced by fermentation (Noack et al., 2019), SAL was evaluated (Chapman and Jeffers, 2015). And MET.CLO was used as an example of a combined product (Peek, 2010; Noack et al., 2019). Strain sensitivity was measured using the global resistance index

methodology, which considers several relevant parameters related to the pathogenic effects of coccidia and the zootechnical performance (Thabet et al., 2017). This methodology considers oocyst excretion, lesion score, growth performance, and mortality, with weight gain and feed conversion weighted heaviest because of their economic impact (Stephan et al., 1997; Arabkhazaeli et al., 2013).

Oocyst excretion is influenced by various factors such as the reproductive potential of the various species, overcrowding, and the host's immune response (Fayer, 1980), therefore, as the only variable to measure anticoccidial efficacy, it can be misleading (Reid, 1975). Several publications have shown that oocyst production has little correlation with weight gain, lesion score and, in highly pathogenic species (*E. tenella* and *E. necatrix*), even with mortality (Fayer, 1980; Stephan et al., 1997). The intestinal lesion score is one of the most common methods for evaluating intestinal damage caused by coccidia, but it involves the evaluator's subjectivity and experience (Conway et al., 1990; Stephan et al., 1997; Li et al., 2004). In field conditions for highly pathogenic species, the mortality variable is the strongest sign of resistance (Bedrnik, 1983; Stephan et al., 1997).

In this work, a modification was made in the IO calculation of the formula by Stephan et al. (1997), since the oocyst count was done from fresh feces and not from intestinal scrapings. However, they were categorized on a scale of 0-5 to be able to include them in the same way in the formula for sensitivity analysis. The DIC group presented the highest IO values (3.4 ± 1.69). However, it was not found to be statistically significant with the other treated groups, and although the DIC group had higher IO, poorest weight gain and feed conversion, it did not present the highest intestinal lesion scores. This is similar to Chapman's (1998) report that high counts oocyst are not always related to greater intestinal damage.

Similarly, in this study, it was not possible to find a statistical difference in the intestinal lesion score for any of the groups evaluated, which could possibly reflect a low pathogenicity of the field strains used (Thabet et al., 2017).

E. maxima is classified as a species with a moderate to high pathogenicity (Chapman, 2014, Quiroz-Castañeda and Dantán-González, 2015) and the infective dose used in this work was high compared to other reports such as Oviedo et al. (2006), (3.33×10^5 vs 100×10^3 oocysts, respectively), but during the experimental period, lesions caused by *E. maxima* could not be observed. Although, in the evaluation of coprological analysis on the final day of the test, it was possible to oocysts count of this species, which, due to their large size, shape, and color, are difficult to confuse. To corroborate these findings, the histology analysis performed on the different intestinal sections was of great help, as it showed the presence of *Eimeria maxima* and other species such as *E. mitis* and *E. necatrix*. Possibly, on

the day of the final evaluation, this variety of *Eimeria maxima*, was just reaching its oocyst excretion peak, which, as reported by Jenkins et al. (2017b), can occur between 130 and 162 hours post inoculation. Another possibility is that this field strain of *E. maxima* was not pathogenic and had little replication capacity (Dalloul and Lillehoj, 2006) to show intestinal lesions. This corroborates results reported by Schwarz et al. (2009), that indicated there are genetic variants with different pathogenic capacities among the same species of *Eimeria maxima.*

The values of the global index of resistance with respect to the NNC group were in the range of good efficacy for the groups medicated with SAL and MET.CLO (85.79% and 85.49% respectively) and the group treated with DIC reported a score of 74.52%, classified as limited efficacy. These data is consistent with the field results evaluated, since this last group was the only one that presented mortality caused by coccidia. According to Stephan et al. (1997), this is the greatest evidence of resistance problems, what generated a decrease in the value of the index of this group, down to the category of limited efficacy. The results in this work are alike with other results under similar evaluation conditions: strains from the field in a mixture of diverse species, where drugs such as DIC, for coccidia control reported limited efficacy to total resistance but differs for SAL and MET.CLO who report resistance (Stephan et al., 1997; Abbas et al., 2008; Arabkhazaeli et al., 2013; Thabet et al., 2017; Lan et al; 2017).

For future work, performing a dose titration to confirm the estimated level of challenge, include others anticoccidial treatments and make a comparison with different methodologies for evaluating anticoccidial sensitivity. For example: Anticoccidial index (**ACI**), Percent of optimum anticoccidial activity (**POAA**), Reduction of lesion scores (**RLS**) and Relative oocyst production (**ROP**) (Arabkhazaeli et al., 2013; Lan et al; 2017), that allow broadening the criteria to determine anticoccidial sensitivity.

This work is the first epidemiological report of coccidia in 4 regions responsible for 63.7% (ICA, 2019) of the broiler production in Colombia. In addition, this study provides information on the identification of the most frequently identified coccidial species and their distribution, along with information on the sensitivity of field strains to some of the most commonly used anticoccidials in the region. More research is needed, but this is a great first step to providing more knowledge about the current state of coccidial challenge in Colombia.

ACKNOWLEDGEMENTS.

This research was funded by CIBAV- the Strategy of Consolidation of Research Groups (CODI 2018-2019), Universidad de Antioquia, (UdeA), Medellín, Colombia and the company Solla S.A. Special thanks to the Veterinarian and Zootechnician **Jorge Hernán Duque** for his advice and support during the development of the proposal, to the poultry companies that voluntarily participated in the project and provided technical staff to take samples, to the technical staff of Solla S.A. and the Avilandia research farm for field support, and to all the staff of the CIBAV and Nutri-Solla. We sincerely acknowledge the contributions of **Dr. Kevin Watkins** who carried out an outstanding review of our manuscript.

DISCLOSURES

The authors declare no conflict of interest

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CONCLUSIONES GENERALES

Revisar el estado actual de la coccidiosis aviar en el mundo permite ampliar y actualizar conocimientos sobre el agente causal de esta enfermedad que sigue presente en la industria avícola ocasionando enormes pérdidas al sector, como se muestra en los resultados de este trabajo donde se evidenció la presencia de *Eimeria* spp. en el 92.8% (180/194) de las granjas muestreadas, confirmando que es un agente que siempre esta presente y contra el cual se deben seguir creando medidas eficaces que puedan disminuir el impacto en la salud del animal y por ende mejorar los parámetros económicos de los sistemas de producción avícolas; para lo cual sigue siendo necesario continuar evaluando la respuesta de la *Eimeria* spp. a los diferentes anticoccidiales que se ofertan en el mercado a través de pruebas de resistencia *in vivo* como una herramienta para establer programas de rotación anticoccidial dirigidos que pueden reducir el riesgo a la perdida de sensibilidad del parásito; además vincular pruebas de diagnostico, molecular como la PCR que puedan identificar con mayor certeza la especie de *Eimeria* presente y que se deba controlar.

PERSPECTIVAS

El mercado mundial de huevo y carne de pollo cada vez es más exigente, las producciones libres de medicamentos que incluyen los productos anticoccidiales son una realidad para el sector avícola, por lo tanto, es necesario seguir trabajando en esta línea de investigación e incluir evaluaciones de productos naturales o alternativos para el control de agentes patógenos como la *Eimeria,* que se puedan llevar con seguridad y eficacia a

una escala comercial de producción y permita mantener la competitividad del sector avícola. Por lo tanto, dar continuidad en este campo de la investigación aviar es muy importante para ampliar los conomientos sobre coccidiosis y su agente causal, permitiendo el desarrollo de programas de control de manera holística incluyendo bioseguridad, rotación de anticoccidiales, sanitización adecuada de camas reutilizadas y programas de sensibilización y capacitación, para el personal operativo y profesional en granjas avícolas. Adicional, reforzar en campo la capacitación del personal en técnicas de diagnóstico para coccidia e incluir como una práctica rutinaria en las granjas avícolas pruebas de sensibilidad anticoccidial, pueden ser una herramienta de gran ayuda para el monitoreo constante de la respuesta del parásito y para evaluar la eficacia de los diferentes programas de control anticoccidial utilizados en granjas.

Con los resultados mostrados en la PCR se confirma la presencia de otras especies de *Eimeria* como E. mitis, *E. preacox y E. necatrix*, que si bien las dos primeras son consideras poco patógenas, están presentes alterando la integridad intestinal afectando los parámetros productivos en las aves, por lo tanto, se hace necesario tenerlas presentes en los programas de control y seguir perfeccionando las técnicas de su detección en campo.

Finalmente se hace necesario poder establecer una estrategia de comunicación entre el sector empresarial y académico a través de entidades como FENAVI o el Instituto Colombiano Agropecuario-ICA sobre la problemática de la coccidiosis en campo, lo que sería de gran ayuda para continuar combatiendo un patógeno que afecta el rendimiento productivo del sector avícola en general.

ANEXOS

Anexo I. Soporte artículo de revisión sometido a revista Journal of Applied Poultry Research

---------- Forwarded message ---------
De: **Journal of Applied Poultry Research** <em@editorialmanager.com>
Date: sáb., 8 ago. 2020 a las 20:42
Subject: Confirming submission to Journal of Applied Poultry Research
To: Jenny Jovana Chaparro-Gutiérrez <jenny.chaparro@udea.edu.co>

This is an automated message.

General concepts about avian coccidiosis: a current problem in global poultry farming.

Dear Dr. Chaparro-Gutiérrez,

We have received the above referenced manuscript you submitted to Journal of Applied Poultry Research.

To track the status of your manuscript, please log in as an author at https://www.editorialmanager.com/JAPR/, and navigate to the "Submissions Being Processed" folder.
Thank you for submitting your work to this journal.

Kind regards,

Journal of Applied Poultry Research

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Anexo II. Reglamentación de la revista Journal of Applied Poultry Research

Se adjunta en formato PDF, la reglamentación de la revista

ELSEVIER

JOURNAL OF APPLIED POULTRY RESEARCH

An official journal of the Poultry Science Association.

AUTHOR INFORMATION PACK

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ISSN: 1056-6171

Anexo III. Reglamentación de la revista Poultry Science

Se adjunta en formato PDF, la reglamentación de la revista

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POULTRY SCIENCE
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Anexo IV. Protocolo de colecta muestras de camas

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Protocolo para la toma de muestras de camas en granjas Avícolas de pollo de engorde

Esta es una guía para la toma de muestras de cama dentro del galpón, en granjas de pollo de engorde, en el marco del proyecto: "Caracterización de las especies de *Eimeria* en granjas de pollo de engorde de algunas regiones de Colombia ".

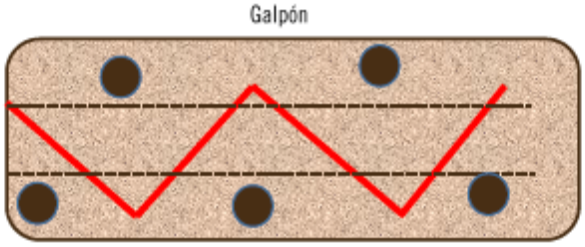
Materiales

- Nevera de icopor pequeña
- Bolsa Ziploc
- Marcador Sharpie
- Recipiente plástico (aprox- 50 ml)
- Hielo/gel refrigerante
- Bolsa de basura grande
- Balde plástico grande (20 litros)
- Guantes plásticos
-

Procedimiento *

Importante: Previo a la toma de la muestra dentro de cada galpón recordar que es muy importante el diligenciamiento de la **encuesta** adjunta a este documento.

1. Hacer un reconocimiento general del galpón
2. Comenzar con el recorrido dentro del galpón para la toma de las muestras, la idea es hacer un recorrido en forma de "W", evitando coger la línea del alimento y del agua.



* Metodología propuesta por: Goan Charles. Poultry Litter Sampling and Testing. The University of Tennessee, Agricultural Extension Service, 2009. disponible en: https://extension.tennessee.edu/publications/Documents/SP563.pdf

5. Una vez realice el muestreo de los galpones, mezcle todas las submuestras colectadas de todos los galpones de la misma edad, puede ser en un balde grande o en plástico, con el fin de hacer una correcta homogenización del material.

6. De la mezcla anterior tome una sub-muestra de aproximadamente 500- gramos (bolsa Ziploc 20*30 cm). Identifique correctamente la muestra: Código de la granja, ubicación (departamento, municipio) fecha de colecta, edad del lote.

Nota: es importante que una vez se empaque la muestra en estas bolsas, se le coloque refrigeración (gel/hielo) lo más pronto posible.

7. Haga entrega de la muestra al personal encargado de Solla en cada seccional (Girón: Feisal Rances, teléfono 3144429431, Valle y Zona Centro: pendiente definir)

Para cualquier información adicional comunicarse con Carolina Mesa al celular 3113343075.

UNIVERSIDAD DE ANTIOQUIA
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Anexo V. Formato de encuesta para la colecta de las muestras en campo.

Encuesta Factores de Riesgo Proyecto Caracterización Especies *Eimeria* en granjas Avícolas de Colombia

Universidad de Antioquia
Laboratorio de parasitología veterinaria

Información general del granja				
Número del cuestionario (consecutivo)				
Fecha (día/mes/año)				
Nombre de la granja				
Nombre del propietario/administrador				
Teléfono(s) de contacto				
E-mail				
Departamento	Antioquia (1)			Cundinamarca (3)
	Santander (2)			Valle del Cauca (4)
Municipio / Vereda				
Altura sobre el nivel del mar (m.s.n.m)		Temperatura promedio (°C)		
mm Lluvia/ año		Clasificación zona de vida		
Tamañode la granja (Número animales encasetados)	_____			
Tipo de granja	(1) Ambiente controlado _____	(2) Manejo Ventiladores____	(3) Manejo de Cortinas _____	(4) Otro____ ¿Cuál?_____
Densidad (animal por m2)	_____			
Número de Galpones	_____			

¿Qué Línea de aves maneja en su granja?	(1) Ross 308 _____	(2) Ross Ap _____	(3) Cobb _____	(4) Otra_____ ¿Cuál?
	(5) Mixto____, ¿Cuál?_____			
Fecha de encasetamiento		Edad del lote al momento del muestreo		
Género	(1) Machos_____	(2) Hembras_____	(3) Mixto_____	
Edad de Sacrificio (días)	(1) Machos_____	(2) Hembras_____		
Peso Sacrificio (kg)	(1) Machos_____	(2) Hembras_____		
Tipo de alimento concentrado utilizado	(1) Comercial _____	(2) Propio_____	(3) Otro_____ ¿Cuál?	
Fases de alimentación utilizadas	(1) Preinicio: Si___ No___	(2) Inicio: Si___ No___	(3) Engorde: Si___ No___	(4) Finalizador: Si___ No___
Tipo de bebederos Uitlizados	(1) Campana_ _____	(2) Niple _____	(3) Otro_____ ¿Cuál?	
Tipo de Comederos Utilizados	(1) Tolvas_____	(2) Lineales____	(3) Otro_____ ¿Cuál?	

Prácticas de manejo de la granja					
¿El predio esta certificado como granja Biosegura?	(0) No	(1) Si			
¿La granja tiene acceso al público en general?	(0) No	(1) Si			
¿Qué tipo de practicas de desinfección utiliza?	(1) Arco de desinfección para vehiculos_ ____	(2) Duchas para el personal ____	(3) Desinfección de ropa____	(4) Desinfección de Botas/zapatos____	(5) otro_____, ¿Cuál?___ ____

Cuando sale el lote que tipo de aseo/desinfección utiliza para el galpón	(0) ninguno_____	(1) Lavado general agua y jabón_____	(2) Aplicación desinfectaten adicional _____, ¿Cuál?_____	(3) Utilizan flameo? Si_____ No____ ¿Qué estructura flamea? Paredes__ Pisos___ ambas___	(4) Otro Método ¿Cuál?_____
Tipo de cama utilizada en el galpón	(1) piso en tierra_____	(2) Cascarilla de Arroz _____	(3) Viruta_____	(4) Otro_____ ¿Cuál?_____	
Reutiliza cama	(0) No ____	(1) Si _____	(1) Cuantas veces____		
	Que tipo de santización le realiza a la cama _____				
Utiliza anticoccidiales	(0) No____ (1) Si _____		¿Cuál es la vía?	(1) alimento _____ (2) agua _____ (3) ambas_____	
Realizan control de Moscas	(0) No		(1) Si		
	Por favor especifique _____				
Realizan control de Roedores	(0) No		(1) Si		
	Por favor especifique _____				
Historial de la granja-Coccidia					
¿Ha tenido animales sintomáticos compatibles con coccidiosis en el galpón (diarrea, deshidratación, pérdida de peso, bajo consumo alimento)?	(1) Actualmente				
	(2) En el último año, pero no en el presente				
	(3) Nunca		Observaciones:		
¿ Han ocurrido muertes en su lote debido a la coccidia?	(1) Recientemente _____	(2) En los último año__	(3) Nunca _____		
Condiciones ambientales					

¿Durante la epoca de lluvias presenta zonas de inundación en el galpón?	(0) No	(1) Si	
Fuente de agua	(1) Acueducto veredal_____ _____	(2) Fuente propia (quebrada _____ nacimiento _____)	
	En caso de ser propia, cuenta con planta de tratamiento de aguas (0)No (1) si		
Epoca en la que se encuentra	(1) Lluvia	(2) Seca	Hace cuanto comenzó _____
Manejo de Residuos en granja (mortalidas, camas)	(0) ninguno_____	(1) Compostaje_____	