UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS DE LA PRODUCCIÓN Y DE LA SALUD ANIMAL

EFECTO DE MICROPARTÍCULAS CON CURCUMINA (*Curcuma longa*) SOBRE ALGUNOS PATÓGENOS QUE AFECTAN LA PRODUCCIÓN AVÍCOLA, COMO: *Eimeria* sp, REOVIRUS Y *Mycoplasma* sp.

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RESUMEN

Esta tesis tuvo como objetivos evaluar el papel de la curcumina en el estrés oxidativo y su efecto sobre la *Eimeria maxima*, el *Mycoplasma synoviae* (MS) y el *Mycoplasma gallisepticum* (MG). Además, se evaluó el efecto de la cepa S1133 de reovirus sobre la integridad intestinal y la producción de pollo en engorda. Para lograr el primer objetivo se evaluó y determinó la concentración de prostaglandina $GF_{2\alpha}$ ($PGF_{2\alpha}$) e isoprostano 8-iso-$PGF_{2\alpha}$ en plasma e intestino de pollos Leghorn libres de patógenos específicos (SPF) desafiados con *Eimeria maxima*, con o sin suplementos dietéticos de curcumina mediante microextracción en fase sólida y cromatografía líquida de ultra rendimiento / espectrometría de masas en tándem. Niveles entéricos de isoprostano 8-iso-$PGF_{2\alpha}$ y $PGF_{2\alpha}$ a los 7 días y a los 9 días posteriores a la inoculación con *Eimeria maxima* aumentaron significativamente en comparación con los pollos de control sin exposición. En contraste, los niveles entéricos de isoprostano 8-iso-$PGF_{2\alpha}$ y $PGF_{2\alpha}$ a los 7 días posteriores a la exposición se redujeron significativamente en pollos alimentados con curcumina, en comparación con el desafío de pollos de control con *E. maxima*.

Los resultados de la investigación arrojaron que las propiedades antioxidantes antiinflamatorias de la curcumina redujeron el daño oxidativo y la consiguiente sobreproducción de productos de oxidación de lípidos en la mucosa intestinal. Para evaluar el efecto de curcumina sobre el MS y MG, estos se aislaron (39.5°C), se identificaron por RAPD y se les calculó las concentraciones mínimas inhibitorias (MIC) de los aislados de gallinas reproductoras y de postura de huevo para plato vacunadas con las cepas MS-H termosensible positivo (ts+) de MS y TS11 ts+ de MG de los principales estados avícolas de México. Los resultados observados en el experimento nos permitieron proponer que MS es el más importante de los micoplasmas en la producción avícola mexicana. Las razones de esto son que el MS es el *Mycoplasma* más frecuentemente aislado en México, ya que se encontró mayor prevalencia de MS, mientras que MG solo en la mitad de las regiones avícolas estudiadas. Además, MG resultó más sensible para los antimicoplásmicos más usados como tilosina y tiamulina. Estos hallazgos sirven como base para el control y posible erradicación de la micoplasmosis en México; así como, las dosificaciones de fármacos antimicoplásmicos según la especie de *Mycoplasma*. La cepa vacuna MS-H se pudo de aislar a 39.5°C; lo cual es evidencia de la reversión de la termosensibilidad positiva (ts+), de pasar de ts+ a

ts-. Los micoplasmas no fueron sensibles a la curcumina, utilizando los mismas MIC de los antibióticos antimicoplásmicos como indicadores de la sensibilidad. Para evaluar el efecto de la cepa S1133 de reovirus sobre la integridad intestinal y la producción de pollo en engorda, se consideró que la artritis viral (ARV) es una enfermedad de importancia productiva avícola producida por reovirus. La vacunación con la cepa S1133 de las gallinas reproductoras controla la artritis viral y transmite anticuerpos maternos a la progenie. La vacunación de pollos de engorde con la cepa ARV S1133 se utiliza para prevenir la artritis viral. Sin embargo, los efectos entéricos posteriores a la vacunación no se han caracterizado bien. El propósito en este experimento fue evaluar el efecto de la vacunación con la cepa S1133 sobre el aumento de peso y conversión alimenticia de pollos de engorde, así como caracterizar las lesiones gástricas, entéricas y pancreáticas que podrían ser inducidas por la cepa. Un total de 672.000 pollos se dividieron cada uno en dos grupos: un grupo vacunado con la cepa S1133 de ARV (S1133ARV) y un grupo de control (no vacunado). Tras el análisis histológico, el grupo de la vacuna mostró menos tejido glandular proventricular y atrofia del páncreas y vellosidades duodenales, además de tener una ganancia diaria promedio más baja. La conclusión basada en los resultados de esta investigación es que la vacunación neonatal con S1133ARV causa atrofia de los acinos pancreáticos, glándulas proventriculares y vellosidades intestinales, lo que lleva a un aumento del diámetro de la luz glandular y atrofia de las vellosidades entéricas, así como a pérdida de peso, en pollos de engorda

Palabras clave: Curcumina, *Eimeria maxima*, *Mycoplasma synoviae*, *Mycoplasma gallisepticum*, reovirus, isoprostano 8-iso-$PGF_{2\alpha}$, prostaglandina, $PGF_{2\alpha}$, MS-H, S1133

ABSTRACT

Solid-phase and ultra-performance liquid chromatography/tandem mass spectrometry. This thesis aimed to evaluate the role of curcumin in oxidative stress and its effect on *Eimeria maxima*, *Mycoplasma synoviae* (MS) and *Mycoplasma gallisepticum* (MG). In addition, the effect of reovirus strain S1133 on intestinal integrity and broiler production was evaluated. To achieve the first objective, the concentration of prostaglandin $GF_{2\alpha}$ ($PGF_{2\alpha}$) and isoprostane 8 iso $PGF_{2\alpha}$ was evaluated and determined in the plasma and intestine of Leghorn chickens free of specific pathogens (SPF) challenged with *Eimeria maxima*, with or without dietary supplements of curcumin by microextraction. Enteric levels of isoprostane 8 iso $PGF_{2\alpha}$ and $PGF_{2\alpha}$ at seven days and nine days after inoculation with Eimeria maxima increased significantly compared to control chickens without challenge. Research results showed that curcumin's anti-inflammatory, antioxidant properties reduced oxidative damage and the consequent overproduction of lipid oxidation products in the intestinal mucosa. In contrast, enteric levels of isoprostane 8-iso-$PGF_{2\alpha}$ and $PGF_{2\alpha}$ at seven days post-challenge were significantly reduced in chickens fed curcumin, compared to the challenge of control chickens with *E. maxima*.

To evaluate the effect of curcumin on MS and MG, these were isolated (39.5 °C), identified by RAPD, and the minimum inhibitory concentrations (MIC) of the isolates from breeding hens and egg-laying hens for vaccinated dishes were calculated. With the MS-H thermosensitive positive (ts+) strains of MS and TS11 ts+ of MG from the leading poultry states of Mexico. The results observed in the experiment allowed us to propose that MS is the most important of the mycoplasmas in Mexican poultry production. The results are because MS is the *Mycoplasma* most frequently isolated in Mexico since a higher prevalence of MS was found, while MG was only found in half of the poultry regions studied. Furthermore, MG was more sensitive to the most used anti-mycoplasmic drugs such as tylosin and tiamulin. These findings serve as the basis for the control and possible eradication of mycoplasmosis in Mexico and the dosages of anti-mycoplasmic drugs according to the species of *Mycoplasma*. The MS H vaccine strain could be isolated at 39.5 ° C, which is evidence of the reversal of the positive thermosensitivity (ts+), from going from ts+ to ts-. Mycoplasmas were not sensitive to curcumin, using the same MICs of anti-mycoplasmic antibiotics as indicators of sensitivity.

To evaluate the effect of the reovirus strain S1133 on the intestinal integrity and the production of broilers, viral arthritis (ARV) was considered a disease of poultry production importance caused by reovirus. Vaccination with strain S1133 of breeder hens controls viral arthritis and transmits maternal antibodies to progeny. Vaccination of broilers with the ARV S1133 strain is used to prevent viral arthritis. However, post-vaccination enteric effects have not been well characterized. The purpose of this experiment was to evaluate the effect of vaccination with the S1133 strain on the weight gain and feed conversion of broilers and characterize the gastric, enteric and pancreatic lesions that the strain could induce. A total of 672,000 chickens were divided into two groups: a group vaccinated with the ARV strain S1133 (S1133ARV) and a control group (not vaccinated). After histological analysis, the vaccine group showed less proventricular glandular tissue and atrophy of the pancreas and duodenal villi and a lower average daily gain.

Based on this investigation, the conclusion is that neonatal vaccination with S1133ARV causes atrophy of the pancreatic acini, proventricular glands, and intestinal villi, leading to increased glandular lumen diameter and enteric villus atrophy, as well as to weight loss, in broilers.

Key words:
Curcumina, *Eimeria maxima*, *Mycoplasma synoviae*, *Mycoplasma gallisepticum*, reovirus, isoprostane 8-iso-$PGF_{2\alpha}$, prostaglandin, $PGF_{2\alpha}$, MS-H, S1133

CONTENIDO

LISTA DE CUADROS

1. INTRODUCCIÓN

La tesis contiene los requisitos indispensables para la graduación doctoral con base en publicaciones científicas en revistas indexadas. Por lo que se estructuró con introducción, discusión y conclusiones; además, se anexaron cuatro publicaciones científicas que incluyen la evaluación de la curcumina *in vivo* en pollos tipo ligeros libres de patógenos específicos desafiados con *Eimeria maxima* y la evaluación *in vitro* de la curcumina y algunos antibióticos contra *Mycoplasma synoviae* (MS) y *Mycoplasma gallisepticum* (MG). Además, se evaluó la reversión de la patogenicidad de la cepa vacunal MS-H de MS y el efecto en la integridad intestinal y la producción de pollo de engorda de la cepa vacunal S1133 de reovirus aviar.

1.1. CURCUMINA

La *Curcuma longa*, familia de las Zingiberáceas, es originaria de la india y en la actualidad se cultiva en países tropicales. Es usada comúnmente como especia en la cultura asiática, donde se considera planta mágica dadas sus características organolépticas y sus indudables propiedades terapéuticas y protectoras, sobre todo a nivel hepático y cutáneo (1). El principal componente es la curcumina, el cual es extraído de la raíz de la *Curcuma longa*; es un polvo naranja-amarillo cristalino, uno de los ingredientes activos responsables de su actividad biológica (2).

La farmacología y el posible valor anticancerígeno de la curcumina han sido objeto de artículos de revisión desde 1991 (3). Sus principales efectos contra patógenos que afectan la productividad animal son: actividad antibacteriana *in vitro* contra bacterias Gram positivas (4), antiinflamatorio (5), propiedades antioxidantes (6), y los efectos antiprotozoarios de la curcumina han sido descritos para *Plasmodium falciparum* (7), *Leishmania* spp. (8), *Trypanosoma* spp. (9), trofozoitos de *Giardia lamblia* (2) y esporozoitos de *Eimeria tenella* (10).

Se han realizado grandes esfuerzos para desarrollar fármacos más potentes, eficaces y bien tolerados, pero sobre todo que sean seguros para ambiente y para los humanos. Por lo tanto, la modificación de moléculas vegetales ha sido prominente en el diseño de nuevos compuestos con mayor actividad antibacteriana y menos efectos tóxicos como la curcumina y sus derivados. La curcumina (1, $C_{21}H_{20}O_6$) o diferuloilmetano es un polifenol identificado por primera vez en 1815, es

el principal polifenol curcuminoide responsable de las propiedades medicinales y farmacológicas de la cúrcuma (*Curcuma longa* L, Zingiberaceae). La *C. longa* tiene un amplio espectro de acciones biológicas (11,12), que incluyen actividades antioxidantes, analgésicas, antiinflamatorias, antisépticas, anticancerosas, antivíricas, antibacterianas, antifúngicas y antiplaquetarias. La curcumina se ha utilizado ampliamente en la medicina ayurvédica durante siglos, sin informes de toxicidad (13,14). Desde 1956, se conoce la actividad antimicrobiana *in vitro* de la Curcuma, ya que el *Staphylococcus aureus* (*Micrococcus pyogenes*) fue inhibido por dilución de una parte curcuminato de sodio en un millón de partes del solvente; posteriormente en la década del 1970, se encontró actividad contra cepas de los géneros *Sarcinia*, *Gaffkya* (*Aerococcus*), *Corynebacterium*, *Streptococcus* y *Bacillus* (15,16) . Ya en esta década, en aves de corral y camarones, el efecto *in vitro* antibacterial de la curcumina se ha demostrado por medio de halos de inhibición y concentración mínima inhibitoria (CMI) contra: *Vibrio harveyi*, *V. alginolyticus*, *V. vulnificus*, *V. parahaemolyticus*, *V. cholerae*, *Bacillus subtilis*, *B. cereus*, *Aeromonas hydrophila*, *Streptococcus agalactiae*, *Staphylococcus aureus*, *S. intermedius*, *S. epidermidis*, y *Edwardsiella tarda*; sin embargo, la curcumina no tuvo efecto sobre *Salmonella* Enteritidis*, S.* Typhi, *S.* Typhimurium, *E. coli*, *Proteus vulgaris*, *P. mirabilis*, *Shigella sonnei*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Erwinia carotovora*, y *Citrobacter frundii* (17). Otras investigaciones encontraron que la curcumina tiene efecto en bacterias cariogénicas, como *Streptococcus mutans* y *Streptococcus mitis*; efecto moderado contra *S. sanguinis*, *Lactobacillus casei*, y *Streptococcus salivarius* (18). También se encontró efecto de la curcumina contra *Mycobacterium abscessus* (19). Trabajos realizados por Changtam *et al.* (20) demostraron que una molécula derivada de la curcumina, llamada diacetilcurcumin, fue eficaz contra *Mycobacterium tuberculosis*. También el extracto y fracciones de *C. longa* han demostrado actividad contra *S. aureus* cepas resistentes a meticilina (MRSA). En análisis isobolográficos, la curcumina redujo entre 2 y 128 veces las concentraciones mínimas inhibitorias de los antibióticos comúnmente usados contra el MRSA, como la oxacilina, la ampicilina, la ciprofloxacina y la norfloxacina. El extracto y las fracciones de *C. longa* tienen actividad antibacteriana contra bacterias patógenas, incluido *S. aureus* (21,22). La CMI de la curcumina oscila entre 125 y 250 $\mu g \cdot mL^{-1}$ contra diez cepas de *S. aureus* cepas resistentes a meticilina (MRSA) y susceptibles a la meticilina (MSSA). Además, al combinar la curcumina con antibióticos comúnmente usados contra el RMSA, como la oxacilina, la ampicilina, la ciprofloxacina y la norfloxacina se redujo de 2 a 128 veces las CMI de estor antibióticos, lo que demuestra la potencial eficacia clínica de la

curcumina y sus derivados para tratar Infecciones por MRSA (21). Muchos otros autores también han estudiado la acción de la curcumina sobre patógenos fúngicos (23,24) .

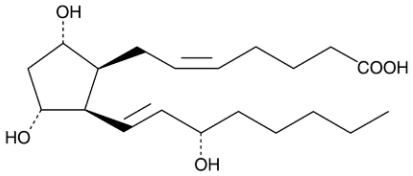
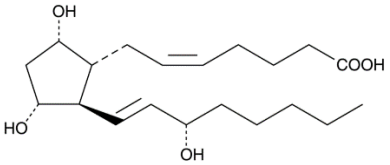
1.2. INDICADORES DEL ESTRÉS OXIDATIVO E INFLAMACIÓN AGUDA ENTÉRICA

Morrow en 1990 (25,26) demostró la producción de una serie de compuestos similares a las prostaglandinas F_2 (F_2-isoprostanos, F_2-IsoPs), que se forman *in vitro* (26) e *in vivo* (25) por radicales libres, peroxidación catalizada del ácido araquidónico unido a fosfolípidos, una vía que es independiente de la vía de la ciclooxigenasa. Estos compuestos son conocidos como isoprostanos, se forman *in situ* en la membrana citoplasmática, originados por la oxidación directa de los fosfolípidos, y después son liberados a la circulación. Los isoprostanos son menos reactivos que otros productos de la lipoperoxidación como aldehídos y lipoperóxidos, se pueden encontrar con mayor facilidad en plasma y orina. Por lo tanto, los isoprostanos pueden ser hoy en día considerados como los indicadores más fiables de estrés oxidativo y se pueden utilizar para evaluar el estado oxidativo en una serie de patologías (27) . La cuantificación de isoprostanos como un biomarcador provee una oportunidad para la investigación de lipoperoxidación en diversas enfermedades, también provee un importante biomarcador del uso racional en dosis y selección de antioxidantes en enfermedades (28).

La oxidación no enzimática del ácido araquidónico produce una gran variedad de compuestos, algunos de estos se ciclan formando anillos de ciclopentano, estos compuestos son similares a las prostaglandinas es decir son isómeros de estas y se conocen con el nombre de isoprostanos (29) . La diferencia entre las prostaglandinas y los isoprostanos es la vía de oxidación del ácido araquidónico. Las prostaglandinas se forman vía oxidación enzimática (COX_1, COX_2) y se clasifican en seis diferentes series D, E, F, G, H e I dependiendo de la oxidación del anillo ciclopentano; en cambio los isoprostanos se forman cuando la oxidación es vía no enzimática en presencia de radicales libres que catalizan la peroxidación, son formados *in situ* en los fosfolípidos de las membranas celulares y después son transportados extracelularmente por fosfolipasas, circulan en sangre y después son excretados en orina (30) .

En Cuadro 1 se presentan las principales características de la prostaglandina PGF2α y el isoprostano 8-iso-PGF2α (iPF2α-III, 15-F2t-IsoP, 15-F2t-isoprostano, 8-iso-15(S)-Prostaglandina F2α), se observa que las principal diferencias entre la prostaglandina y el isoprostano además de su vía de formación es la conformación de su estructura, en la prostaglandina las cadenas laterales al ciclopentano están orientadas en forma *cis*, en cambio en el isoprostano las cadenas están orientadas en forma *trans* (30).

Cuadro 1. Principales características de F2-Isoprostanos (8-iso-PGF2α) y de prostaglandinas (PGF2α) (30)

	8-iso-PGF2α	**PGF2α**
Estructura		
No. CAS	27415-26-5	38562-01-5
Fórmula molecular	$C_{20}H_{35}O_5$	$C_{20}H_{35}O_5 \cdot C_4H_{11}NO_3$
Peso molecular	254.5	254.5
Solubilidad	100 mg·mL^{-1} de etanol, DMSO, dimetil formamida	Agua, metanol
Formación	Formación *In situ*, en los fosfolípidos de la membrana citoplasmática (productos no enzimáticos de la lipoperoxidación)	Vía enzimática

El descubrimiento de los isoprostanos como productos no enzimáticos de la lipoperoxidación abrió una nueva era de investigación con respecto al rol de los agentes oxidantes en las patologías humanas, la cuantificación de isoprostanos como marcador exacto del estatus de estrés oxidativo *in vivo* (31). La cuantificación de isoprostanos en fluidos biológicos es representativa de su producción endógena y por lo tanto da una alta precisión del grado de estrés oxidativo *in vivo* (32). Se considera la medición de isoprostanos como el estándar de oro para la evaluación del daño oxidativo *in vivo* (33) . Además, la evaluación de los isoprostanos puede ser aplicada al estudio de la eficacia de los alimentos, fármacos y antioxidantes (34). El 8-iso-PGF2α se ha cuantificado en diversas especies, y sus concentraciones difieren dependiendo su estado de salud (Cuadro 2).

Cuadro 2. Concentración de F2 Isoprostanos (8-iso-PGF2α) en fluidos biológicos de diversas especies según su estado fisiológico (actualizado y adaptado de Rodriguez-Patiño (30))

Muestra	**Concentración**
Plasma mujer embarazada (35)	195 [164, 551] pg·mL^{-1} Testigo 40-170 pg·mL^{-1}
Plasma ratón (36)	DOX 1.94 ng·mL^{-1} TBHP 2.02 ng·mL^{-1} Testigo 1.49 ng·mL^{-1}
Plasma humano. Distrofia muscular (37)	DMD 240 pg·mL^{-1} BMD 220 pg·mL^{-1} Testigo 50 pg·mL^{-1}
Orina (MG creatinina) bovina (38)	Enfermo 5.62 ng·mg^{-1} creatinina Testigo 12.5 ng·mg^{-1} creatinina
Orina (MG creatinina) canina (38)	Enfermos 38.32 ng·mg^{-1} creatinina Testigo 2.62 ng·mg^{-1} creatinina
Orina (MG creatinina) equina (38)	Enfermos 4.94 ng·mg^{-1} creatinina Testigo 0.83 ng·mg^{-1} creatinina
Orina (MG creatinina) Felina (38)	Enfermos 0.80 ng·mg^{-1} creatinina Testigo 0.36 ng·mg^{-1} creatinina
Plasma humano (39)	55-348 ng·g^{-1} 3-5 ng·L^{-1}, creatinina/orina
Plasma humano. Enfermedad renal terminal (40)	Después de hemodiálisis 5.42 ng·mL^{-1} Antes de hemodiálisis 1.13 ng·mL^{-1} Testigo 0.96 ng·mL^{-1}
Plasma ratón (41)	Deficientes de Zinc 210 pg·mL^{-1} Suplementados Zinc 180 pg·mL^{-1}
Plasma humano (42)	Diabetes Mellitus tipo 2, 310 pg·mL^{-1} Testigo 237 pg·mL^{-1}
Líquido amniótico humano (43)	Subsecuente preclamsia 123.1±57.6 pg·mL^{-1}
Plasma seminal humano (44)	Normozoospérmicos 6.95±2.10 ng·mL^{-1} Astenozoospérmicos 14.66±4.10 ng·mL^{-1} Astenoteratozoospérmicos 16.71±5.58 ng·mL^{-1} Oligoastenoteratozoospérmicos 3.42±8.36 ng·mL^{-1}
Condensado de aliento humano (45)	Trabajadores en minas 5 pg·mL^{-1} Testigo 3 pg·mL^{-1}
Plasma humano (46)	Niños con autismo 103.27 ± 12.56 pg·mL^{-1} Testigo 24.03 ± 2.65 pg·mL^{-1}
Plasma ovino (47)	Patología pulmonar 1113.3 ± 6 45.2 pg·mL^{-1} Testigo 155.3 ± 67.2 pg·mL^{-1}
Plasma ratón (48)	CC14, 4520 pg·mL^{-1} Testigo 130 pg·mL^{-1}
Plasma canina (49)	CHF (Falla cardiaca congestiva) 44.55 pg·mL^{-1} CVD (Enfermedad valvular crónica) 43.51 pg·mL^{-1} DCM (Cardiomiopatía dilatada) 59.36 pg·mL^{-1} Testigo 25.29 pg·mL^{-1}
Plasma halcones polluelos (50)	Peregrinos urbanos 118.4±7.0 pg·mL^{-1} Peregrinos rurales 126.9±5.0 pg·mL^{-1}
Plasma ovino (51)	Hembra 130.9±41.4 pg·mL^{-1} Macho 122.9±49.1 pg·mL^{-1}
Plasma caprino (51)	Hembra gestante 214.7±79.5 pg·mL^{-1} Macho 140.5 ± 70.5 pg·mL^{-1}

1.3. PATÓGENOS Y SU EFECTO EN LA PRODUCCIÓN AVÍCOLA

La producción avícola es basada en la conversión de alimento a proteína para consumo humano. Esta conversión incluye varios factores, de los cuales las más importantes son: la calidad del alimento, genética y manejo del ave, así factores que afectan el consumo y la absorción entérica de nutrientes. En esta investigación incluirá el consumo y absorción entérica de nutrientes, al estudiar las patologías primordiales que influyen en la salud general y entérica avícola. El primer signo de aves con enfermedad sistémica es el rechazo al alimento; la principal enfermedad sistémica bacteriana que produce este rechazo, además de causar grandes mortalidades y pérdidas productivas, es la enfermedad respiratoria crónica (ERC). Mientras que ente las principales patologías que dañan la integridad intestinal que se ocasionan mala absorción de nutrientes son coccidiosis y reovirosis. Estas patologías causan principalmente inflamación y necrosis de la mucosa digestiva; lo que origina mala absorción de nutrientes. Entre los indicadores inflamación y necrosis, están los isoprostanos son reflejo del estrés oxidativo producido por inflamación y necrosis. Ambos eventos son indicadores la inflamación aguda y necrosis.

1.3.1. EIMERIOSIS

La eimeriosis o coccidiosis es enfermedad entérica causada por parásitos protozoarios del género *Eimeria* afectando el intestino delgado y los sacos ciegos. Los animales mayormente infectados son pollos de engorda y pollas reproductoras jóvenes, la infección es por vía oral (52). Esta enfermedad tiene impacto sanitario, pero sobre todo económico; ya la mortalidad causada por *Eimeria tenella*, causa menos pérdidas económicas que *Eimeria acervulina* y *Eimeria maxima*; ya que la primera aumenta la conversión alimenticia y disminuye la ganancia de peso, mientras que la infección por *E. maxima* disminuye la absorción de pigmentos cutáneos, que son condición primordial para la venta del pollo en el mercado mexicano. La disminución de peso y de pigmento cutáneo causan pérdidas económicas; ya que el déficit del 10% en la ganancia de peso representa un 10% en la producción total de carne de pollo y una mala pigmentación provoca rechazo del pollo por consumidor, disminuyendo significativamente su valor (53). El principal inconveniente al cual se enfrentan los avicultores es la resistencia de la *Eimeria* spp que se tiene a los fármacos anticoccidiales, la cual se han reportado principalmente en pollos de engorda (54).

1.3.2. MICOPLASMOSIS

La bacteria que produce más mortalidad como patología primaria es el *Mycoplasma* spp, etiología determinante de la enfermedad respiratoria crónica (ERC), y su complicación con otras bacterias principalmente *Escherichia coli* para conformar la ERC complicada (ERCC) (55). MS y MG son las especies de *Mycoplasma* más relevantes para aves comerciales desde el punto de vista clínico y económico. La importancia de MG fue reconocida hace décadas, como etiología de enfermedad respiratoria crónica (ERC) (56). Mientras que para MS en el pasado solo se informaron infecciones subclínicas, enfermedades respiratorias (ERC), articulares y anormalidades de ápice del cascarón del huevo (55,57). Las infecciones por MS y MG causan en reproductoras disminución en la producción de huevo fértil; pero sobre todo transmite el *Mycoplasma* spp al embrión donde produce muerte tardía y nacimientos de pollitos infectados que desarrollaran la ERC. La ERC disminuye la inmunidad innata local respiratoria y predispone a la infección por *E. coli* produciendo la ERC complicada (ERCC) (58). La ERCC produce pérdidas económicas importantes, al causar poliserositis, septicemia, muerte y decomisos en planta de procesamiento (55,56) .

En la actualidad el control de la micoplasmosis en México se realiza por medio de vacunas y antibióticos. Las vacunas registradas en México son contra MS es la MS-H (Vaxsafe®MS, Laboratorio Avimex, Ciudad de México). La cepa de vacuna MS-H ts+, desarrollada por la mutagénesis química de cepa de campo australiana (86079/7NS). Las vacunas contra MG son la cepa F (F Vax-MG®, Merck Sharp & Dohme Animal Health, Madison, NJ), y dos cepas mutadas para tornarlas sensibles a la temperatura de 37°C, la cepa 6-85 (Nobilis® MG 6/85, Merck Sharp & Dohme Animal Health, Madison, NJ) y la cepa TS-11 (TS-11®, Boehringer Ingelheim Vetmedica, Guadalajara, México). La cepa vacunal también positiva a ser termosensible (ts+). Las cepas ts+ tienen la ventaja diagnóstica de que al aislamiento no se reproducen a temperatura de 37°C, por lo que es fácil diferenciarlas de cepas de campo termosensibles negativas (ts-) (59,60).

Para el uso de antibióticos antimicoplásmicos es importante tener el origen genético y estructura del *Mycoplasma* spp. El *Mycoplasma* spp es de la clase de los Mollicutes que son las formas de vida libre y autorreplicantes más pequeñas y sencillas que se conocen. Son bacterias de origen Gram positivo, como lo indica su ARNr 16S. Pero en lugar de ser primitivos, se separaron hace unos 65 millones de años, por evolución regresiva y reducción del genoma y pérdida de pared celular, de

ancestros más complejos en la rama del árbol filogenético bacteriano que contiene los lactobacilos, bacilos, clostridios y estreptococos (61) . Por lo que son sensibles a los antibióticos dirigidos contra los clostridios y estreptococos, exceptuando a los antimicrobianos que tienen como diana a la pared celular, tal es el caso de las penicilinas, betalactámicos, glucopéptidos, polimixinas, rifampicina y sulfonamidas. Los antibióticos más utilizados como metafilaxia o tratamiento de presentaciones clínicas de la ERC, son los macrólidos, pleuromutilinas, lincosamidas y fluoroquinolonas; otros antibióticos con pobre sensibilidad contra la micoplasmosis; sin embargo, se utilizan principalmente en combinación para tratar la ERCC, son anfenicoles y tetraciclinas (62) . Debido a que la principal transmisión del *Mycoplasma* spp es de la gallina a la progenie, la vacunación y los tratamientos tienen como su principal objetivo la gallina reproductora.

La utilización de antibióticos en animales de consumo se debe evitar; sin embargo, cuando el uso de antibióticos por justificación clínica no se puede restringir, se deben considerar la sensibilidad bacteriana al antibiótico y la dosis correcta para disminuir el riesgo de resistencia del *Mycoplasma* spp a los fármacos; así como la eficacia de un polifenol contra esta bacteria. Por lo que se importante tener información actualizada de la sensibilidad al antibiótico por los micoplasmas de importancia clínica, así como distribución de los micoplasmas en las zonas avícolas más importantes del país. Por tanto, un objetivo de este trabajo fue determinar la actividad antimicrobiana *in vitro* de aislados mexicanos de MS y MG frente a seis agentes antimicrobianos y un polifenol como la curcumina. No se encontraron informes científicos que prueben la eficacia de la curcumina contra el *Mycoplasma* spp, por lo que en esta investigación se evaluó el efecto *in vitro* de la curcumina.

1.3.3. REOVIROSIS

El reovirus es un virus ARN con doble cadena desnudo; el nombre reovirus se forma con las iniciales de Respiratorio, Entérico y *Orphănus* (huérfano en latín) aislado por primera vez en humanos; sin embargo, se encuentra en aves de engorda y de postura. En los pollos de engorda, los reovirus patógenos causan pérdidas económicas importantes debido a la artritis y la tenosinovitis en los tendones del gastrocnemio (63). La artritis viral afecta principalmente a los pollos de carne, pero también se ha diagnosticado en ponedoras comerciales (64). Las parvadas de reproductoras que desarrollan artritis viral durante la producción de huevos pueden caracterizarse por cojera y aumento de la mortalidad, disminución de la producción de huevos, proporción

incubabilidad/fertilidad subóptima y transmisión vertical del reovirus a la progenie (65). La eliminación vertical del reovirus patógeno por parvada reproductora puede infectar a la progenie y causar graves pérdidas. Además, dado que el reovirus aviar se replica en el tracto gastrointestinal, también se asocia con otras patologías como el síndrome de malabsorción con retraso del crecimiento, hepatitis, gastroenteritis, miocarditis y enfermedades respiratorias (66-68). Los reovirus aviares poseen antígenos específicos de grupo y serotipo y los anticuerpos neutralizantes pueden detectarse 7 a 10 días después de la infección.

La vacunación contra reovirus en reproductoras de pollos de engorda se realiza con vacunas apatógenas activas (cepa 2177) y vacunas modificadas (cepa S1133) e inactivadas producidas con reovirus patógenos (cepas S1133, 2408, SS412 y 1733), incluidos los virus homólogos del área geográfica avícola. La vacuna viva apatógena se administra por vía subcutánea, las vacunas vivas modificadas a través del agua de bebida en la granja o por aspersión en incubadora, mientras que las vacunas inactivadas se administran por vía subcutánea. La vacunación en reproductoras de pollos de engorda es fundamental para protegerse; sobre todo, para proteger a su progenie contra la artritis viral. Los altos niveles de anticuerpos antireovirales producidos por la vacunación de reproductoras reducen considerablemente la presentación clínica de artritis viral en la progenie (69). Se espera que estos anticuerpos prevengan la infección de la parvada reproductora que estar seguida de la transmisión vertical del virus a la descendencia a través del saco vitelino (70) y que proporcionen anticuerpos derivados de la madre a la descendencia (71). Los pollos recién nacidos son muy susceptibles a la infección por reovirus patógenos (72,73). Por lo tanto, la vacunación adecuada de las reproductoras de pollos de engorda es fundamental (74). La vacunación se puede realizar mediante vacunas vivas o inactivadas modificadas basadas en la cepa de reovirus aviar S1133 (75). Los anticuerpos maternos pueden brindar protección a los pollitos de 1 día contra infecciones naturales y experimentales, pero el nivel de protección conferido por los anticuerpos está relacionado con la similitud del serotipo, la virulencia del virus, la edad del hospedador y el título de anticuerpos (66). La recuperación de la infección por reovirus implica la actividad de las células B y T, pero la protección está mediada predominantemente por células B (anticuerpos). Por lo tanto, la inmunidad materna es esencial para la protección contra la artritis viral (76). La supresión experimental de la inmunidad mediada por células T dio como resultado un aumento de la mortalidad en aves infectadas con reovirus, pero la gravedad relativa de las lesiones de diez

pacientes no se vio afectada (77). Las células T CD8 + pueden desempeñar un papel importante en la patogénesis y / o la eliminación de reovirus en el intestino delgado. En este proceso, la inmunidad materna no juega un papel importante (78).

La artritis viral se controla mediante la vacunación con virus activos modificados o inactivados. La cepa de reovirus aviar S1133 (S1133ARV) es la más utilizada para la vacunación y se ha demostrado que es eficaz contra la artritis viral en la mayor parte del mundo (70). Sin embargo, no se ha informado de ninguna evidencia de la expectativa clínica sobre el uso de la cepa S1133 del reovirus aviar modificado vivo en pollos de engorda recién nacidos en condiciones comerciales. Por lo tanto, el propósito de este estudio fue evaluar el efecto de S1133ARV sobre el aumento de peso y la conversión alimenticia de pollos de engorda después de la vacunación con esta cepa, además de caracterizar las lesiones gástricas, entéricas y pancreáticas inducidas en respuesta en un día. pollos de engorda viejos en una prueba de campo comercial a gran escala en México.

1.4. OBJETIVOS

- Evaluar *in vivo* el efecto de la curcumina sobre el estrés oxidativo y la *Eimeria maxima*, en pollos ligeros libres de patógenos específicos; por medio de la cuantificación plasmáticas y entérica de isoprostano 8-iso-$PGF_{2\alpha}$ y prostaglandina $PGF_{2\alpha}$.
- Evaluar *in vitro* el efecto de la curcumina y los principales antibióticos utilizados en México contra MS y MG.
- Evaluar la termoestabilidad y trasmisión de la cepa vacunal termosensible MS-H de MS de las reproductoras pesados y ligeras a la progenie.
- Evaluar el efecto de la cepa S1133 de reovirus sobre la integridad intestinal y la producción de pollo en engorda.

2. PUBLICACIONES GENERADAS

Se elaboraron tres experimentos. Los dos primeros fueron para probar el efecto de las micropartículas de curcumina sobre patologías que disminuyen la productividad de las granjas avícolas y el tercero para evaluar el efecto del reovirus sobre la producción avícola. Como resultado de los experimentos se publicaros en revistas científicas con reconocimiento internacional tres artículos y uno más se encuentra en revisión.

2.1. TRES EXPERIMENTOS REALIZADOS Y CUATRO ARTÍCULOS CIENTÍFICOS

2.1.1. EXPERIMENTO 1. EFECTO DE LA CURCUMINA SOBRE LA INTEGRIDAD INTESTINAL E INFLAMACIÓN AGUDA EN POLLOS SPF INOCULADOS CON *Eimeria maxima*

2.1.1.1. Publicación 1: Curcumin reduces enteric isoprostane 8-iso-PGF2α and prostaglandin GF2α in specific pathogen-free Leghorn chickens challenged with *Eimeria maxima* (79) (Anexo 1)

Scientific Reports 2021; 11:11609

Factor de impacto: 7.1

Editorial: ***Nature***

DOI: https://doi.org/10.1038/s41598-021-90679-5

2.1.2. EXPERIMENTO 2. EFECTO IN VITRO DE LA CURCUMINA Y DE LA CEPA MS-H SOBRE *Mycoplasma synoviae* Y *Mycoplasma gallisepticum*

2.1.2.1. Publicación 2: Isolation and antimicrobial sensitivity of *Mycoplasma synoviae* and *Mycoplasma gallisepticum* from vaccinated hens in Mexico (80) (Anexo 2A)

Revista: Pathogens 2020; 9(11): 924

Factor de impacto: 4.4

Editorial: ***MDPI***

DOI: https://doi.org/10.3390/pathogens9110924

2.1.2.2. Publicación 3: Vertical transmission and reverse thermosensitivity of the MS–H vaccine strain of *Mycoplasma synoviae* in commercial laying hens (81) (Anexo 2B)

Heliyon 2021, preimpresión en revisión

Factor de impacto: 2.9

Editorial: ***Cell Press* (ELSEVIER)**

DOI: https://dx.doi.org/10.2139/ssrn.3882378

2.1.3. EXPERIMENTO 3. EFECTO DE LA VACUNA CONTRA REOVIROSIS SOBRE EL GROSOR DE LA MUCOSA INTESTINAL E INDICADORES PRODUCTIVOS EN GRANJAS DE POLLOS DE ENGORDA

2.1.3.1. Publicación 4: Evaluation of avian reovirus S1133 strain in neonatal broiler chickens in gastrointestinal integrity and performance in a large-scale commercial field trial (82) (Anexo 3)

Vaccines 2021; 9(8):817

Factor de impacto: 3.5

Editorial: ***MDPI***

DOI: https://doi.org/10.3390/vaccines9080817

3. DISCUSIÓN

3.1.1. EXPERIMENTO 1. EFECTO DE LA CURCUMINA SOBRE LA INTEGRIDAD INTESTINAL E INFLAMACIÓN AGUDA EN POLLOS SPF INOCULADOS CON *Eimeria maxima*

Publicación 1

Curcumin reduces enteric isoprostane 8-iso-$PGF_{2\alpha}$ and prostaglandin $GF_{2\alpha}$ in specific pathogen-free Leghorn chickens challenged with *Eimeria maxima* (79)

La coccidiosis sigue siendo una de las enfermedades más críticas en la industria avícola. Debido a las regulaciones internacionales y las presiones de los consumidores, existe la necesidad de desarrollar alternativas para los antibióticos promotores del crecimiento en la alimentación animal y avícola. Los fitogénicos parecen ser candidatos de interés como alternativas a los antibióticos promotores del crecimiento porque se ha demostrado que controlan las infecciones por *Eimeria* debido a la asociación de la infección por coccidios con la peroxidación lipídica de la mucosa intestinal (83). Otros estudios han confirmado los beneficios de los fitogénicos para reducir las infecciones gastrointestinales y aumentar el rendimiento (84-86). Además, varios estudios han confirmado la reducción de la gravedad de la infección por *E. maxima* en pollos de engorde debido a las propiedades antioxidantes de la curcumina (87-89). Además del trabajo crítico de absorber agua y nutrientes, los enterocitos también juegan un papel esencial en la respuesta inmune de la mucosa, manteniendo la tolerancia a la microbiota benéfica e identificando patógenos luminales. La invasión de Eimeria spp. en las células epiteliales intestinales es un proceso complejo que incluye varios eventos, comenzando con la liberación de los esporozoítos tras la ingestión oral de los ooquistes (90,91).

Como parásitos intracelulares, la adhesión e invasión de los esporozoitos a la célula huésped es reconocida por los receptores tipo Toll 4 y Toll 15, involucrados en el reconocimiento y activación de patógenos del inflamasoma mucoso del eje IL-1/IL-18, que es responsable del reclutamiento y activación de heterófilos, células asesinas naturales, mastocitos, macrófagos y aumento de la producción del factor de transcripción NF-κB (92-95). Sin embargo, los esporozoitos han desarrollado un sistema molecular único que alimenta la motilidad y la invasión de las células epiteliales a través de la motilidad deslizante, lo que les permite invadir rápidamente las células

huésped y formar una vacuola parasitófora intracelular que las protege del entorno hostil intracelular (96-99). Dentro de esta vacuola, estos parásitos Apicomplexa ganan un tiempo precioso para continuar con su ciclo de vida multifacético. Cada fase de las etapas sexual, asexual, intracelular o extracelular de este parásito prehistórico y notable se asocia con inflamación local grave, autofagia, apoptosis, muerte celular, hemorragias y necrosis en la mucosa intestinal (96,98,99,100-102). Por tanto, las infecciones por coccidios se caracterizan por un daño tisular excesivo causado por la infección del parásito y la inflamación crónica de la respuesta inmune del huésped provocada contra los invasores.

En los pollos, los macrófagos son las fuentes primarias de óxido nítrico, superóxido y peróxido de hidrógeno; además, los macrófagos aumentan durante las infecciones por coccidios (103-106). En el presente estudio, los pollos desafiados con *E. maxima* presentaron un aumento significativo ($P<0.01$) en $PGF_{2\alpha}$ entérica a los 7 y 9 días después del desafío en comparación con los pollos no desafiados. Sin embargo, los niveles séricos de $PGF_{2\alpha}$ permanecieron similares en ambos grupos. Curiosamente, los pollos desafiados con *E. maxima* y suplementados con curcumina mostraron una reducción significativa de los niveles de $PGF_{2\alpha}$ a los 7 días después del desafío en comparación con los pollos de control de *E. maxima*. Las PG se producen a partir de la liberación de ácido araquidónico de los fosfolípidos en la membrana celular por las ciclooxigenasas (COX). Son fundamentales para generar respuestas inflamatorias frente a patógenos (107,108). Si bien tienen una respuesta rápida durante las fases agudas de la respuesta inflamatoria, existe una interferencia con las citocinas para activar sinérgicamente el factor NF-κB e inducir la expresión génica de citocinas proinflamatorias y más COX, mediando ciclos de retroalimentación positiva y, en consecuencia, inflamación crónica (109,110). Dado que los componentes celulares que sufren un daño inmediato son los lípidos y proteínas de la membrana celular y la membrana mitocondrial por peroxidación lipídica, la fisiología de la célula completa se ve comprometida. Uno de los mecanismos celulares para revertir el estrés oxidativo es la producción de varias proteínas de choque térmico que reparan las proteínas dañadas y regulan la apoptosis (111-113).

Un resultado notable observado en este estudio fue el aumento significativo de isoprostano 8-iso-$PGF_{2\alpha}$ en el yeyuno de pollos desafiados con *E. maxima* a los 7 y 9 días posteriores al desafío en comparación con los pollos de control sin desafío. Además, los pollos del grupo suplementado

con curcumina mostraron una reducción significativa en el isoprostano 8-iso-$PGF_{2\alpha}$ en el yeyuno de los pollos desafiados con *E. maxima* en los dos días de evaluación después del desafío en comparación con los pollos de control de desafío con *E. maxima*. La generación excesiva de especies reactivas de oxígeno se ha relacionado con una variedad de eventos patológicos. Sin embargo, la peroxidación lipídica es el principal marcador de estrés oxidativo en muchas condiciones patológicas, por lo que los isoprostanos son biomarcadores de evaluación fiables valorar (114,115). En contraste, los F2-isoprostanos (8-Iso-$PGF_{2\alpha}$) tienen bioactividades dañinas y potentes, que incluyen vasoconstricción, agregación plaquetaria e hipertrofia cardíaca (116-119). Hasta donde sabemos, este es el primer informe de detección de 8-Iso-$PGF_{2\alpha}$ después de un desafío de *E. maxima* en el yeyuno, además de demostrar las propiedades antioxidantes protectoras de la curcumina reduciendo los niveles entéricos de 8-Iso-$PGF_{2\alpha}$, a pesar de que los niveles plasmáticos de 8-Iso-$PGF_{2\alpha}$ permanecieron similares en todos los grupos, independientemente del desafío con *E. maxima* . Se sabe que, en humanos, la vida media plasmática de 8-Iso-$PGF_{2\alpha}$ es de un minuto en la etapa de distribución y la vida media de la etapa de eliminación es de cuatro minutos (120). Por lo tanto, la vida media en el plasma de pollo también puede ser corta, razón por la cual no pudimos detectarla. Sin embargo, se requieren estudios farmacocinéticos y metabólicos que evalúen puntos anteriores, así como el recuento diario de ooquistos, para confirmar y ampliar estos resultados.

3.1.2. EXPERIMENTO 2. EFECTO IN VITRO DE LA CURCUMINA Y LA CEPA MS-H SOBRE *Mycoplasma synoviae* Y *Mycoplasma gallisepticum*

Publicaciones 2 y 3

Isolation and antimicrobial sensitivity of *Mycoplasma synoviae* and *Mycoplasma gallisepticum* from vaccinated hens in Mexico (80)

Vertical transmission and reverse thermosensitivity of the MS–H vaccine strain of *Mycoplasma synoviae* in commercial laying hens (81)

En este trabajo se encontró mayor tasa de aislados de MS (reproductoras: 25.57%; ponedoras: 5.58%; total*:* 19.49*%*) comparada con la tasa de los aislados de MG *(reproductoras: 5.88%; ponedoras: 0%; total: 3.64%), lo que* concuerda con investigaciones realizadas en Europa y América. Aunque, en África y Asia existen investigaciones que concuerdan con este trabajo, también informes que indican mayor prevalencia de MG sobre MS (Cuadros 3, 4 y 5).

Cuadro 3. Porcentaje de gallinas ponederas y reproductoras ligeras positivas a *Mycoplasma synoviae* (MS) y *Mycoplasma gallisepticum* (MG) en países que tienen la misma proporción de MS y de MG que esta investigación.

Países que tienen mayor porcentaje de MS que de MG al igual que esta investigación			
País	**MS (%)**	**MG (%)**	**Referencias**
Holanda	73.0	10.5	121
Bélgica	2.7	0.9	122
Serbia	20.74	7.99	123
Estados Unidos	89.0	73.0	124
Chile	71.1	52.8	125
Brasil	19.88	0.19	126
Irán	45	10	127
Irán	100	25 Reproductoras	128
Países que tienen mayor porcentaje de MG que de MS a diferencia que esta investigación			
Paquistán	42.6	44.9	129
Argelia	22.2	51.9	130
	10	23.3	131
	66.33	69.90	132

Cuadro 4. Porcentaje de pollos de engorda y reproductoras pesadas positivas a *Mycoplasma synoviae* (MS) y *Mycoplasma gallisepticum* (MG) en países que tienen la misma proporción de MS y de MG que esta investigación.

Países que tienen mayor porcentaje de MS que de MG al igual que esta investigación			
País	**MS (%)**	**MG (%)**	**Referencias**
Serbia	22.86	14.29	123
	22.91	12.50 Reproductoras	123
Turquía	4	2.54	124
Jordania	31	23	133
Paquistán	10	7.14	129
Libia	6.4	3.4	134
Marruecos	66.67	26.67 Reproductoras	135
Estados Unidos	89.4	84.0	136
Países que tienen mayor porcentaje de MG que de MS a diferencia que esta investigación			
Myanmar	8.8	10.5	137
India	10.56	28.87	138
Irán	0.0	0.0	128
Egipto	1.9	27.5	139
Paquistán	16.67	35.03	140
	50.13	59.6 Reproductoras	129

Cuadro 5. Porcentaje de aves de traspatio y de ornato positivas a *Mycoplasma synoviae* (MS) y *Mycoplasma gallisepticum* (MG) en países que tienen la misma proporción de MS y de MG que esta investigación.

Países que tienen mayor porcentaje de MS que de MG al igual que esta investigación			
País	**MS (%)**	**MG (%)**	**Referencias**
Bélgica	96.4	73.2 Aves de ornato	141
Argentina	100	55.1	142
Mozambique	84.5	48.8	143
Países que tienen mayor porcentaje de MG que de MS a diferencia que esta investigación			
Irán	2	85	144
Paquistán	47	68	140

La prevalencia más alta de MS en comparación con MG se puede deber al desarrollo de la industria avícola. Otros factores que influye en la mayor prevalencia de MS son los programas de erradicación de MG implantados en algunos países o regiones, como el programa de control y erradicación de MG con base en la Directiva 2009/158/CE del Consejo de la Unión Europea (UE, 2009) y la Decisión 2011/214 / UE de la Comisión Europea (UE, 2011), que establece que no permite el comercio de la progenie de parvadas positivas a MG en la Unión Europea (121). La prevalencia también está influida por la capacidad de ambas micoplasmas de interactuar con otros patógenos como los virus de enfermedad de Newcastle, influenza aviar y virus de la bronquitis infecciosa (58,145,146) . La disminución de MG ha estado inducida por eficacia de los programas antimicoplásmicos, ya sea vacunas o fármacos.

3.1.3. EXPERIMENTO 3. EFECTO DE LA VACUNA CONTRA REOVIROSIS SOBRE EL GROSOR DE LA MUCOSA INTESTINAL E INDICADORES PRODUCTIVOS EN GRANJAS DE POLLOS DE ENGORDA

Publicación 4

Evaluation of avian reovirus S1133 strain in neonatal broiler chickens in gastrointestinal integrity and performance in a large-scale commercial field trial (82)

Los resultados de este estudio indican que la vacunación de pollos de engorde de un día con la cepa de reovirus aviar S1133 empeora la ganancia diaria promedio, FCR y factor de eficiencia de producción. La reducción en el rendimiento se asoció con cambios histopatológicos y morfométricos en la glándula proventricular, el duodeno y el páncreas de pollos de engorde hembras que se

procesaron comercialmente a los 38 días de vida. Estos hallazgos coinciden con los de investigadores anteriores que informaron que el reovirus aviar aislado del contenido intestinal de pollos de engorde con síndrome de malabsorción producía una depresión transitoria, pero significativa, en el aumento de peso corporal cuando se inoculaba por vía oral en pollitos de un día (147). Además, Jones y Georgiou (65) encontraron que la resistencia al reovirus está relacionada con la edad porque, aunque el reovirus puede infectar a las aves más viejas, la enfermedad resultante es generalmente menos grave y el período de incubación es más largo.

La disminución en el rendimiento de los pollos de engorde vacunados puede explicarse por los hallazgos histológicos. El diámetro luminal de la glándula proventricular fue significativamente mayor en el grupo vacunado, y este aumento de la luz se debe a una reducción del tejido glandular, lo que hizo que aumentara la relación entre la luz y el radio total del diámetro glandular. Como se sabe, los tejidos glandulares producen ácido clorhídrico y pepsinógeno, que son esenciales para la digestión de proteínas (148).

En las vellosidades duodenales del grupo vacunado, hubo una disminución de las vellosidades altas y un aumento de la lámina propia de tal manera que aumentó la relación entre el espesor de la lámina propia y el espesor total del radio de la mucosa. La apoptosis es el proceso por el cual los reovirus causan atrofia epitelial de las glándulas proventriculares y del epitelio en la punta de las vellosidades (149,150). Este fenómeno puede explicar la ausencia de un proceso inflamatorio evidente en los tejidos observado en este trabajo (151). Estos cambios duodenales inducen una disminución de los nutrientes, especialmente de las proteínas. La mayor absorción de proteínas en el duodeno se produce en su parte proximal, que es la más afectada por la vacuna. Si bien el efecto del virus de la vacuna sobre las vellosidades entéricas se perdió en el yeyuno distal, la absorción de nutrientes fue menor (148,152).

El páncreas de los pollos de engorde vacunados mostró una fibrosis moderada, pero esta fibrosis fue mayor en los pollos vacunados en comparación con los controles no vacunados. Un aumento en la cantidad y densidad de los tejidos conectivos intersticiales con atrofia por compresión de los acinos caracteriza las fases crónicas de la inflamación en el páncreas (153-155). Normalmente, las bandas de tejido fibroso maduro separan pequeños lóbulos de los tejidos acinares (156). La fibrosis

pancreática se atribuye a la deficiencia de selenio en pollos de engorde con retraso del crecimiento. Una de las limitaciones del presente estudio es que no se midió la concentración de selenio. Sin embargo, la reducción de la absorción de nutrientes está asociada con la deficiencia de selenio (157). Se sugiere que las actividades normalmente bajas de la glutatión peroxidasa dependiente de selenio (SeGSHpx) en el páncreas pueden predisponer a ese órgano a la atrofia debido al estrés oxidativo en condiciones de deficiencia de selenio nutricional, lo que da como resultado un mayor agotamiento de SeGSHpx (148). La atrofia pancreática parece depender de la concentración de selenio. En el presente estudio, la atrofia pancreática fue moderada, mientras que en los trabajos de Whitacre *et al*. (158) y Xu *et al*. (154), la deficiencia y la atrofia fueron graves.

Los resultados del ELISA revelaron un alto título de anticuerpos maternos, lo que concuerda con el vigoroso programa de vacunación de las reproductoras de pollos de engorde contra el reovirus. Sin embargo, los anticuerpos maternos no evitaron la infección de la cepa S1133 de reovirus aviar modificado vivo en el epitelio gastroentérico. Por lo tanto, la replicación de la cepa S1133 causó daño a las glándulas proventriculares y a los enterocitos. En el presente estudio, los anticuerpos maternos disminuyeron a las 3 semanas de edad en ambos grupos. Curiosamente, se observó un aumento en los títulos de anticuerpos en ambos grupos, presumiblemente, debido a un desafío de reovirus salvaje que no fue controlado por vacunación como se informó previamente por Zhong *et al*. (74). Los reovirus pueden aislarse de aves sanas y los anticuerpos séricos se encuentran a menudo tanto en aves afectadas como sanas (66). Otra limitación del presente estudio de prueba de campo es la falta de aislamiento y caracterización de las cepas silvestres responsables de inducir una respuesta inmune en pollos de control no vacunados. Sin embargo, en el estudio actual, los parámetros de rendimiento de los pollos de control no vacunados no se vieron afectados, a pesar de que mostraron títulos de anticuerpos contra el reovirus aviar. El kit comercial ELISA utilizado para evaluar los títulos de anticuerpos (Reo ELISA CK100®, BioCheck®) detecta anticuerpos séricos contra todos los serotipos de reovirus aviar en parvadas vacunadas y no vacunadas, por lo que también se utiliza para la detección de infecciones de campo. como seguimiento del éxito de la vacunación en aves de corral.

4. CONCLUSIONES

En el presente estudio, los pollos SPF Leghorn desafiados con *E. maxima* mostraron una respuesta inflamatoria asociada con un aumento significativo a los 7 días y 9 días después del desafío en $PGF_{2\alpha}$ entérica. Estos cambios se relacionaron con un aumento significativo de la excreción entérica de 8-Iso-$PGF_{2\alpha}$ y de ooquistos en los dos días de evaluación, lo que sugiere que la fase activa de la enfermedad estuvo acompañada de inflamación y estrés oxidativo dentro de la capa intestinal. Sin embargo, la suplementación dietética de curcumina redujo los niveles de $PGF_{2\alpha}$ y 8-Iso-$PGF_{2\alpha}$ a los 7 días después del desafío, y de 8-Iso-$PGF_{2\alpha}$ a los 9 días después del desafío en comparación con los pollos de control desafiados con *E. maxima*. Dado que los ácidos grasos poliinsaturados y el colesterol son los principales objetivos del estrés oxidativo, los productos finales de la peroxidación lipídica, como el 8-Iso-PGF2α, también forman parte de la patogenia de los cambios relacionados con la inflamación causados por *E. maxima*, lo que confirma el papel de 8-Iso-$PGF_{2\alpha}$ como biomarcador sensible al estrés oxidativo en pollos. Los resultados de este estudio piloto sugieren que las propiedades antioxidantes y antiinflamatorias de la curcumina pueden reducir el daño oxidativo y, posteriormente, la sobreproducción de productos de oxidación de lípidos en la mucosa intestinal. Se requieren más estudios para confirmar y ampliar estos resultados en pollos de engorde.

Con respecto los experimentos realizados en micoplasmosis aviar se encontró que hay mayor prevalencia de MS en los principales estados de México, mientras que MG solo cuatro de las ocho regiones avícolas estudiadas. Además, *M. gallisepticum* resultó ser más sensible para los antimicoplásmicos más usados como tilosina y tiamulina. Estos hallazgos sirven como base para el control y posible erradicación de la micoplasmosis en México; así como, las dosificaciones de los fármacos antimicoplásmicos según la especie de *Mycoplasma* a tratar. La cepa vacuna MS-H se pudo de aislar a 39.5°C de las muestras de gallina; lo cual es evidencia de la reversión de TS+ a TS-. La reversión de la TS concuerda con otros autores que también que indican la reversión de la patogenicidad de las vacunas termosensibles contra la micoplasmosis. Además, se encontró evidencia molecular de la transmisión y por lo tanto reversión de la patogenicidad de la cepa MS-H. Los micoplasmas no fueron sensibles a la curcumina, utilizando los mismas CMI de los antibióticos antimicoplásmicos como indicadores de la sensibilidad.

Con respecto el efecto de la cepa vacunal S1133 en pollos de engorda que los programas de vacunación de reproductoras de pollos de engorde con la cepa de reovirus aviar S1133 están diseñados para prevenir la artritis viral en pollos de engorde a través de la inmunidad pasiva. Sin embargo, los resultados del presente estudio sugieren que la vacunación neonatal con la cepa del reovirus aviar S1133 causa reacciones posvacunal caracterizadas por atrofia del acino pancreático, glándulas proventriculares y vellosidades intestinales, lo que lleva a un aumento del diámetro de la lumen glandular y atrofia de las vellosidades entéricas, así como pérdida de peso, en pollos de engorde. Por lo tanto, el costo-beneficio de la vacunación con reovirus aviar para prevenir la mala absorción en bandadas sin signos clínicos de artritis viral no es rentable. Debido a que este trabajo se llevó a cabo en instalaciones comerciales, se deben realizar más investigaciones para confirmar las conclusiones de este trabajo en otras instalaciones, entornos y con diferentes niveles de anticuerpos maternos.

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ANEXO 1. Artículo científico publicado

PUBLICACIÓN 1. CURCUMIN REDUCES ENTERIC ISOPROSTANE 8-ISO-PGF$_{2\alpha}$ AND PROSTAGLANDIN GF$_{2\alpha}$ IN SPECIFIC PATHOGEN-FREE LEGHORN CHICKENS CHALLENGED WITH *Eimeria maxima* (79)

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Curcumin reduces enteric isoprostane 8-iso-PGF2α and prostaglandin GF2α in specific pathogen-free Leghorn chickens challenged with *Eimeria maxima*

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The purpose of this pilot study was to evaluate and determine the concentration of prostaglandin GF2α (PGF2α) and isoprostane 8-iso-PGF2α in plasma and intestine of specific pathogen-free (SPF) Leghorn chickens challenged with *Eimeria maxima*, with or without dietary supplementation of curcumin using solid-phase microextraction and ultra-performance liquid chromatography/tandem mass spectrometry. Eighty 1-day-old male SPF chickens were randomly allocated to one of four groups with four replicates (n = 5 chickens/replicate). Groups consisted of: (1) Control (no challenge), (2) Curcumin (no challenge), (3) *Eimeria maxima* (challenge), and (4) *Eimeria maxima* (challenge) + curcumin. At day 28 of age, all chickens in the challenge groups were orally gavaged with 40,000 sporulated *E. maxima* oocysts. No significant differences ($P > 0.05$) were observed in the groups regardless of the treatment or challenge with *E. maxima*. Enteric levels of both isoprostane 8-iso-PGF2α and PGF2α at 7 days and 9 days post-challenge were significantly increased ($P < 0.01$) compared to the non-challenge control chickens. Interestingly, the enteric levels of both isoprostane 8-iso-PGF2α and PGF2α at 7 days post-challenge were significantly reduced in chickens fed curcumin, compared to control chickens challenge with *E. maxima*. At 9 days post-challenge, only levels of isoprostane 8-iso-PGF2α in the enteric samples were significantly reduced in chickens challenged with *E. maxima* supplemented with curcumin, compared with *E. maxima* challenge chickens. No differences of isoprostane 8-iso-PGF2α or PGF2α were observed in plasma at both days of evaluation. Similarly, no significant differences were observed between the challenge control or chickens challenge with *E. maxima* and supplemented with curcumin at both times of evaluation. The results of this pilot study suggests that the antioxidant anti-inflammatory properties of curcumin reduced the oxidative damage and subsequent intestinal mucosal over-production of lipid oxidation products. Further studies to confirm and extend these results in broiler chickens are required.

Coccidiosis is a parasitic enteric disease of animals caused by coccidian protozoa from the Apicomplexa phylum. In a recent study, the global cost of coccidiosis in broiler chickens was estimated at ~ £10.36 billion[1]. In

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Group	Body weight 21 days	Body weight 28 days	Body weight gain	Daily body weight gain
Control non-challenge	258.25 ± 9.28	275.55 ± 8.53	17.30 ± 2.11	2.47 ± 0.30
Curcumin non-challenge	254.95 ± 8.22	273.80 ± 8.14	18.85 ± 1.63	2.69 ± 0.23
E. maxima challenge	248.95 ± 6.91	266.30 ± 6.32	17.35 ± 2.07	2.48 ± 032
E. maxima + curcumin	251.40 ± 7.79	267.90 ± 8.51	16.50 ± 2.30	2.36 ± 0.29

Table 1. Evaluation of body weight and body weight gain (in grams) of specific pathogen-free Leghorn chickens with or without *Eimeria maxima* challenge (7 days post-challenge). Data expressed as mean ± standard error. $P > 0.05$.

commercial poultry, coccidiosis has been controlled effectively with anticoccidial products, however, the extensive use of anticoccidial drugs has led to development of resistance against all these drugs[2]. To reduce the occurrence of resistance, the rotation of various anticoccidial drugs in single and shuttle programs is used[3]. Unfortunately, this has not solved the anticoccidial resistance problem. Live anticoccidial vaccines have been incorporated into rotation programs, resulting in an increased incidence of anticoccidial drug-sensitive *Eimeria* spp. field isolates, which may improve the efficacy of anticoccidial drugs[4]. Nevertheless, possible upcoming bans restricting the use of anticoccidials as feed additives, consumer concerns on residues, and increasing regulations have prompted the quest for alternative coccidiosis control strategies[5,6]. Although management and biosecurity measures could halt the introduction of *Eimeria* spp. to a farm, in practice, they do not suffice to prevent coccidiosis outbreaks.

Several phytogenics have been evaluated as feed additives in the poultry industry to protect feed from degradation and deterioration during storage, as well as for nutritional purposes[7]. However, it has been reported that these additives play an essential role in the prevention of several diseases in poultry due to their antioxidant, anti-inflammatory, antibacterial, antiviral, antifungal, and immunomodulatory properties[8–10]. Hence, in recent years, our laboratory has been evaluating curcumin as a feed additive to control *Salmonella* Enteritidis and necrotic enteritis in broiler chickens[11–13]. Curcumin is a bright yellow chemical and the principal curcuminoid of turmeric (*Curcuma longa*), a member of the ginger family (Zingiberaceae). For centuries, curcumin has been used as spice and food-coloring agent. In the poultry industry, curcumin has been used as anticoccidial, anti-inflammatory, immunomodulatory, antimicrobial, antioxidant and to promote growth performance[14–16]. Diets supplemented with of 1% of curcumin reduced intestinal lesion scores, oocyst per gram excretion (OPG) and improved weight gains during *E. maxima* infections and this anticoccidial activity was suggested to result from its antioxidant properties[17]. Other studies have been shown that curcumin inhibits induction of nitric oxide synthase in macrophages stimulated with endotoxin, as well as serum nitrogen dioxide and nitrate in *E. maxima*-infected chickens fed curcumin[18,19].

Eimeria spp. have a remarkable and complex life cycle, including sexual and asexual reproduction with intracellular and extracellular phases[20–22]. Hence, during the disease, the gut-associated lymphoid tissues respond with a series of innate and acquired immune reactions against the parasite[23,24]. Several investigators have extensively studied and documented the immunopathology of cellular responses involving the secretion of pro-inflammatory cytokines to *Eimeria* infections in chickens[25–30]. However, little is known about the role of prostaglandins (PG) and isoprostanes (F_2-Ips) as part of the innate response during clinical coccidiosis. Prostaglandins are a group of lipid compounds from the eicosanoid family implicated in inflammation, allergy, fever, and other immune responses that are generated from arachidonic acid by the action of cyclooxygenases (COXs) isoenzymes. Conversely, F_2-Ips are PG-like complexes formed from free radical catalyzed oxidation of arachidonic acid, without the action of COXs. The measurement of F_2-Ips, especially 8-epi-$PGF_{2\alpha}$, is recognized as a consistent biomarker of lipid peroxidation and is currently used as a sensitive index of oxidative stress in vivo.

The purpose of this pilot study was to evaluate and determine the concentration of prostaglandin GF2α (PGF2α) and isoprostane 8-iso-PGF2α in plasma and intestine of specific pathogen-free (SPF) Leghorn chickens challenged with *Eimeria maxima*, with or without dietary supplementation of curcumin, using solid-phase microextraction and ultra-performance liquid chromatography/tandem mass spectrometry.

Results

The evaluation of body weight and body weight gain (in grams) of specific pathogen-free Leghorn chickens without or with *Eimeria maxima* challenge (7 days post-challenge) are summarized in Table 1. In the present study, challenge with 40,000 sporulated oocysts of *E. maxima* did not affect the body weight or body weight gain of SPF Leghorn chickens. No significant differences ($P > 0.05$) were observed in the groups regardless of the treatment or challenge with *E. maxima* (Table 1).

Table 2 presents the results of the evaluation of isoprostane 8-iso-PGF2α and PGF2α from jejunum and plasma in SPF chickens challenged with *E. maxima* at 7- and 9-days post-challenge. Enteric levels of both isoprostane 8-iso-PGF2α and PGF2α at 7 days and 9 days post-challenge were significantly increased ($P < 0.01$) compared to the non-challenge control chickens (Table 2; Fig. 1). Interestingly, the enteric levels of both isoprostane 8-iso-PGF2α and PGF2α at 7 days post-challenge were significantly reduced in chickens fed with curcumin compared to control chickens challenge with *E. maxima*. At 9 days post-challenge, only levels of isoprostane 8-iso-PGF2α in the enteric samples were significantly reduced in chickens challenged with *E. maxima* supplemented with curcumin, as compared with *E. maxima* challenge chickens. No differences of isoprostane 8-iso-PGF2α or PGF2α were observed in the plasma at both days of evaluation (Table 2; Fig. 1).

Group	Prostaglandin GF2α		Isoprostane 8-iso-PGF2α	
	Enteric (pg/g)	Plasma (pg/mL)	Enteric (pg/g)	Plasma (pg/mL)
7 days post-challenge				
Control non-challenge	6934.47 ± 572.87[b]	107.92 ± 11.64	760.10 ± 75.56[b]	97.17 ± 8.96
Curcumin non-challenge	5843.27 ± 631.55[b]	117.29 ± 22.34	582.97 ± 70.68[b]	95.58 ± 15.27
E. maxima challenge	12,076.52 ± 770.55[a]	151.50 ± 17.87	1272.80 ± 81.97[a]	101.99 ± 17.87
E. maxima + curcumin	8,088.87 ± 698.27[b]	118.54 ± 9.89	864.93 ± 55.21[b]	97.97 ± 17.16
9 days post-challenge				
Control non-challenge	8984.66 ± 603.25[b]	162.05 ± 15.73	669.16 ± 81.47[b]	110.55 ± 9.22
Curcumin non-challenge	7606.78 ± 721.99[b]	121.19 ± 14.62	602.21 ± 79.93[b]	106.39 ± 19.53
E. maxima challenge	14,191.48 ± 750.61[a]	124.38 ± 11.82	1363.84 ± 89.12[a]	105.33 ± 8.83
E. maxima + curcumin	10,884.00 ± 740.07[a,b]	135.66 ± 22.59	834.82 ± 125.43[b]	104.83 ± 15.92

Table 2. Evaluation of isoprostane 8-iso-PGF2α and prostaglandin GF2α from enteric (jejunum) and plasma of specific pathogen-free Leghorn chickens at 7- and 9-days post-challenge. Data expressed as mean ± standard error. [a,b]Different superscripts within columns and days indicate a significant difference at $P < 0.01$.

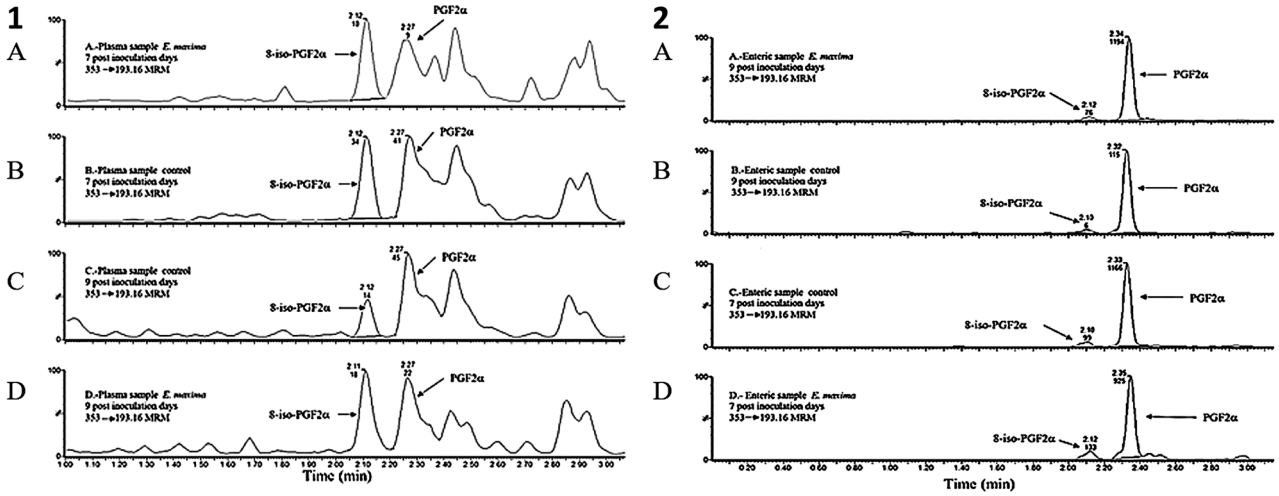

Figure 1. Chromatograms of 8-iso-PGF2α and PGF2α. (**1**) Obtained from plasma samples: A—sample of *E. maxima* 7 days post-inoculation chickens, B—sample of control of 7 days post-inoculation chickens, C—sample of control of 9 days post-inoculation chickens, and D—sample of *E. maxima* 9 days post-inoculation chickens. (**2**) Obtained from enteric (jejunum) samples: A—sample of *E. maxima* 9 days post-inoculation chickens, B—sample of control of 9 days post-inoculation chickens, C—sample of control of 7 days post-inoculation chickens, and D—sample of *E. maxima* 7 days post-inoculation chickens.

Group	7 days post-challenge	9 days post-challenge
Non-challenge control	0 (0)[b]	0 (0)[b]
Non-challenge Curcumin	0 (0)[b]	0 (0)[b]
E. maxima challenge	24,240 (20,200)[a]	2750 (1700)[a]
E. maxima + Curcumin	22,593 (28,962.5)[a]	3023 (1650)[a]

Table 3. *Eimeria maxima* oocyst per gram in the feces of specific pathogen-free Leghorn chickens at 7- and 9-days post-challenge. Each value represents the mean (median). [a,b]Values within groups columns with different superscripts differ significantly at $P < 0.05$.

The results of the evaluation of *E. maxima* oocyst per gram in the feces of specific pathogen-free Leghorn chickens at 7- and 9-days post-challenge are summarized on Table 3. No significant differences were observed between the challenge control or chickens challenged with *E. maxima* and supplemented with curcumin at both times of evaluation (Table 3).

Discussion

Coccidiosis remains one of the most critical diseases in the poultry industry. Due to international regulations and consumer pressures, there is a need to develop alternatives for antibiotic growth promoters in animal and poultry feed. Phytogenics seem to be candidates of interest as alternatives to antibiotic growth promoters because they have been shown to control of *Eimeria* infections due to the association of coccidial infection with lipid peroxidation of the intestinal mucosa[31]. Other studies have confirmed the benefits of phytogenics in reducing gastrointestinal infections and increasing performance[32–34]. Moreover, several studies have confirmed the reduction *E. maxima* infection severity in broiler chickens due to curcumin's antioxidant properties[17–19].

In addition to the critical job of absorbing water and nutrients, enterocytes also play an essential role in the mucosal immune response, maintaining tolerance to beneficial microbiota, and identifying luminal pathogens. The invasion of *Eimeria* spp. in intestinal epithelial cells is a complex process that includes several events, beginning with the excystation of sporozoites after oral ingestion of the oocysts[35,36]. As intracellular parasites, attachment and invasion of the sporozoites to the host cell is recognized by Toll-like receptors 4 and 15, involved in pathogen recognition and activation of the mucosal inflammasome IL-1/IL-18 axis, which is responsible for recruiting and activating heterophils, natural killer cells, mast cells, macrophages, and increased production of transcription factor NF-κB[37–40]. Nevertheless, sporozoites have evolved a unique molecular system fueling motility and invasion of epithelial cells through gliding motility, allowing them to rapidly invade host cells and form an intracellular parasitophorous vacuole that protects them from the intracellular hostile environment[41–44]. Within this vacuole, these Apicomplexa parasites gain precious time to continue with their multifaceted life cycle. Each phase of the sexual, asexual, intracellular, or extracellular stages of this prehistoric and remarkable parasite are associated with severe local inflammation, autophagy, apoptosis, cellular death, hemorrhages, and necrosis in the intestinal mucosa[42–47]. Hence, coccidia infections are characterized by excessive tissue damage caused by the parasite infection and chronic inflammation of the host immune response elicited against the invaders. In chickens, macrophages are the primary sources of nitric oxide, superoxide, and hydrogen peroxide as essential mediators of both innate and acquired immunity, thus increasing during coccidia infections[48–52]. In the present study, chickens challenged with *E. maxima* presented with a significant increase ($P < 0.01$) in enteric PGF2α at 7- and 9-days post-challenge when compared with non-challenged chickens. However, the serum levels of PGF2α remained similar in both groups. Interestingly, chickens challenged with *E. maxima* and supplemented with curcumin showed a significant reduction of PGF2α levels at 7 days post challenge when compared with *E. maxima* control chickens. PGs are produced from arachidonic acid release from phospholipids in the cellular membrane by cyclooxygenases (COXs). They are fundamental in generating inflammatory responses against pathogens[53,54]. While they have a rapid response during the acute phases of the inflammatory response, there is crosstalk with cytokines to synergistically activate NF-κB factor and induce gene expression of pro-inflammatory cytokines and more COXs, mediating positive feedback loops and consequently, chronic inflammation[55,56].

Since the cellular components that suffer immediate damage are the lipids and proteins of the cell membrane and mitochondrial membrane by lipid peroxidation, the whole-cell physiology is then compromised. One of the cellular mechanisms to revert oxidative stress is the production of several heat shock proteins that repair damage proteins and regulate apoptosis[57–59]. A noteworthy result observed in this study was the significant increase in isoprostane 8-iso-PGF2α in the jejunum of chickens challenged with *E. maxima* at 7- and 9-days post-challenge compared to the non-challenge control chickens. Furthermore, chickens in the group supplemented with curcumin showed a significant reduction in isoprostane 8-iso-PGF2α in the jejunum of chickens challenged with *E. maxima* at both days of evaluation post-challenge compared to the *E. maxima* challenge control chickens. Excessive generation of reactive oxygen species has been implicated in a variety of pathological events. However, lipid peroxidation is the primary marker of oxidative stress in many pathological conditions, so isoprostanes are reliable evaluation biomarkers evaluate[60,61]. In contrast, F2-isoprostanes (8-Iso-PGF2α) have harmful and potent bioactivities, including vasoconstriction, platelet aggregation, and cardiac hypertrophy[62–65]. As far as we know, this is the first report of detection of 8-Iso-PGF2α following a challenge of *E. maxima* in the jejunum, as well as demonstrating the protective antioxidant properties of curcumin reducing the enteric levels of 8-Iso-PGF2α, despite plasma levels of 8-Iso-PGF2α remaining similar in all groups, regardless of the challenge with *E. maxima*. It is known that in humans, the plasma half-life of 8-Iso-PGF2α is one minute at the distribution stage and the removal stage half-life is four minutes[66]. Hence, the half-life in chicken plasma may also be short, which may be why we were not able to detect it. However, pharmacokinetic and metabolic studies evaluating earlier points as well as daily oocyst count are required to confirm and extend these results.

In summary, in the present study, SPF Leghorn chickens challenged with *E. maxima* showed an inflammatory response associated with a significant increase at 7 days and 9 days post challenge in enteric PGF2α. These changes were related to a significant increase of enteric 8-Iso-PGF2α and oocyst excretion at both days of evaluation, suggesting that the active disease phase was accompanied by inflammation and oxidative stress within the intestinal layer. Nevertheless, dietary supplementation of curcumin reduced the levels of PGF2α and 8-Iso-PGF2α at 7 days post challenge, and 8-Iso-PGF2α at 9 days post challenge compared with *E. maxima* challenged control chickens. Since polyunsaturated fatty acids and cholesterol are the principal targets of oxidative stress, lipid peroxidation end products, such as 8-Iso-PGF2α, are also a part of the pathogenesis of inflammation-related changes caused by *E. maxima*, confirming the role of 8-Iso-PGF2α as a sensitive biomarker of oxidative stress in chickens. The results of this pilot study suggest that the antioxidant and anti-inflammatory properties of curcumin are able to reduce oxidative damage and subsequently intestinal mucosal over-production of lipid oxidation products. Further studies to confirm and extend these results in broiler chickens are required.

Ingredients	Pre-starter (0–3 weeks)
Yellow corn 7.1%	622.62
Soybean meal 46.5%	323
Limestone 38% Ca	18
Phosphate 21/27%	12
Vegetable oil	10
NaCl (refined salt)	4
Vitamin premix[a]	1.4
Mineral premix[b]	1.1
DL-Methionine 99%[c]	3.700
Liquid L-lysine 50%[d]	3.500
L-Threonine[e]	0.640
6-Phytase[f]	0.040
Nutrients[70]	
Weight	1.0
Dry matter (%)	88.300
Crude protein (%)	20.000
Metabolizable energy (Mcal kg^{-1})	3.087
Choline (mg kg^{-1})	2.000
Arginine (%)	1.210
Linoleic acid (%)	1.200
Total lysine (%)	1.150
Total calcium (%)	1.050
Methionine + cystine (%)	0.830
Valine (%)	0.830
Threonine total (%)	0.820
Isoleucine (%)	0.790
Methionine total (%)	0.510
Phosphorus available (%)	0.480
Phosphorus digestible (%)	0.440
Total tryptophan (%)	0.210
Total chlorine (%)	0.180
Total sodium (%)	0.180

Table 4. Ingredient composition (kg) and nutrient content of feed supplied to the experimental SPF chickens. [a]Vitamin premix supplied per kg of diet: Retinol, 6 mg; cholecalciferol, 150 µg; dl-α-tocopherol, 67.5 mg; menadione, 9 mg; thiamine, 3 mg; riboflavin, 12 mg; pantothenic acid, 18 mg; niacin, 60 mg; pyridoxine, 5 mg; folic acid, 2 mg; biotin, 0.3 mg; cyanocobalamin, 0.4 mg. [b]Mineral premix supplied per kg of diet: Mn, 120 mg; Zn, 100 mg; Fe, 120 mg; copper, 10 to 15 mg; iodine, 0.7 mg; selenium, 0.2 mg; and cobalt, 0.2 mg. [c]MetAMINO® (Evonik, Essen, Germany). [d]Liquid L-lysine 50% (ADM, Chicago, IL, USA). [e]ThreAMINO® (Evonik, Essen, Germany). [f]Axtra PHY TPT 10,000® (Dupont Industrial Biosciences, Marlborough, UK).

Methods

Challenge strain. *Eimeria maxima* M6 oocysts were provided by Dr. John. R. Barta, University of Guelph, Canada. The methods for detecting and recovering oocysts from challenged chickens, oocyst sporulation, and the preparation of infective doses were conducted as described previously[67,68].

Starter diet. A control basal non-supplemented diet and a basal diet supplemented with 2% curcumin were used in this experiment (Table 4). Starter feed used in this experiment was formulated to approximate the nutritional requirements for Leghorn chickens as recommended by the National Research Council[69] and adjusted to Hy-Line Management Guide, W36 Commercial Layers recommendations[70]. No antibiotics, coccidiostats, or enzymes were added to the feed.

Animal source and experimental design. Eighty one-day-old male specific pathogens-free (SPF) Leghorn chickens (ALPES® Tehuacan, Puebla, Mexico) were randomly allocated to one of four groups with four replicates per group (n = 5 chickens/replicate). Chickens were placed in battery cages with a controlled age-appropriate environment at the diagnostic laboratory of the Avian Medicine Department of the Faculty of Veterinary Medicine and Zootechnics (FMVZ) at the National Autonomous University of Mexico (UNAM). Groups consisted of: (1) Control (no challenge), (2) Curcumin (no challenge), (3) *Eimeria maxima* (challenge), and (4) *Eimeria maxima* (challenge) + curcumin. Chickens were provided with ad libitum access to water. At day

28 of age, all chickens in the challenge groups were orally gavaged with 40,000 sporulated *E. maxima* oocysts in a volume of 1 mL of sterile phosphate-buffered saline solution (PBS). The dose used in the present study did not cause clinical coccidiosis in SPF Leghorn chickens. The dose was selected based on a previous trial conducted to determine a challenge dose causing sub-clinical coccidiosis as described previously[13]. Negative control chickens were sham inoculated with 1 mL of PBS. Seven days after challenge, all chickens were bled, and half of them were euthanized to collect the second half of the jejunum to determine plasma and enteric concentrations of isoprostane 8-iso-PGF2α and PGF2α. At 9 days post-challenge, remaining chickens from all groups were bled and jejunum was collected to perform the evaluations. Oocysts per gram (OPG) of feces were evaluated at 7- and 9-days post-challenge.

The standards for 8-iso-PGF2α and 8-iso-PGF2α-d4. The standards for 8-iso-PGF2α and 8-iso-PGF2α-d4 (internal standard) were purchased from Cayman Chemicals (Ann Arbor, MI), while the standard for PGF2α was obtained from Sigma-Aldrich (St Louis, MO). Acetonitrile and methanol (HPLC grade) were purchased from JT Baker. Milli-Q water (Millipore system) was used throughout the experiments. Formic acid (FA: 95%, reactive grade) and isopropanol (LC/MS grade) were purchased from Sigma-Aldrich (St Louis, MO). Ammonium hydroxide (NH_4OH, reactive grade, 29.60%) and potassium hydroxide (KOH) were purchased from JT Baker. For solid-phase microextraction (micro-SPE), 96-well Oasis® MAX µElution cartridges containing a water-wettable reversed-phase strong ammonium exchange mixed-mode polymer, which is selective for acids and stable in organic eluents, were used. A Positive Pressure-96 processor purchased from Waters was also used. Figure 1 shows the chromatograms of standards.

Procedure for the extraction of 8-iso-PGF2α and PGF2α in chicken plasma. Extraction of 8-iso-PGF2α and PGF2α were determined as previously described[71]. An aliquot of 500 µL chicken plasma was transferred to 2 mL vials, followed by the addition of 100 µL of 4 ng/mL 8-iso-PGF2α-d4 as an internal standard and 500 µL of hydrolysis solution (KOH, 15%) to release 8-iso-PGF2α-esterified. The vials were mixed and incubated in an ultrasonic bath for 30 min at 40 °C. Subsequently, the vials were cooled to room temperature and 225 µL of 6 M formic acid (FA) was added, mixed, and centrifuged at 15,000 rpm for 10 min at 4 °C. Solid-phase microextraction using a 96-well Oasis® MAX µElution plate conditioned with 500 µL of methanol and 500 µL of 20 mM FA was used. Finally, the cartridges were loaded with 350 µL of plasma and washed with 350 µL of 2% NH_4OH. Samples were then eluted with 50 µL of a mixture of 5% FA in acetonitrile and isopropanol (40:60) and diluted with 150 µL of Milli-Q water. Samples were analyzed (30 µL) using ultra-performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS).

Procedure for the extraction of 8-iso-PGF2α and PGF2α in chicken intestine. For the extraction of 8-iso-PGF2α and PGF2α, 0.1 g of homogenized second half of the jejunum (Meckel's diverticulum to cecal tonsils) were transferred to 2 mL vials, followed by the addition of 100 µL of 4 ng/mL 8-iso-PGF2α-d4 as the internal standard and 1.5 mL of chloroform: methanol (80:20) mixture. The vials were mixed 30 s by vortex and 15 min in an ultrasonic bath. Samples were then centrifuged at 15,000 rpm for 20 min. The supernatant was evaporated and 500 µL of methanol and 500 µL of hydrolysis solution (KOH 15%) were added, mixed, and incubated in an ultrasonic bath for 30 min at 40 °C. Subsequently, the vials were cooled to room temperature and 225 µL of 6 M formic acid (FA) and 50 µL of 88% FA were added, mixed, and centrifuged at 15,000 rpm for 10 min at 4 °C. Solid-phase microextraction and analysis of samples were performed in the same way as for the determination of 8-iso-PGF2α and PGF2α in chicken plasma using a 96-well Oasis® MAX µElution plate conditioned with 500 µL of methanol and 500 µL of 20 mM FA. Finally, the cartridges were loaded with 350 µL of jejunum sample and washed with 350 µL of 2% NH_4OH. Samples were then eluted with 50 µL of a mixture of 5% FA in acetonitrile and isopropanol (40:60) and diluted with 150 µL of Milli-Q water. The sample (30 µL) was injected into a UPLC-MS/MS system for analysis, under the chromatographic and mass spectrometric conditions described previously by Rodriguez Patiño et al.[71].

Ethics. This study was carried out in accordance with the guidelines for the management of chickens as recommended by the Internal Committee for Care and Use of Experimental Animals (CICUAE, from its abbreviation in Spanish) of the National Autonomous University of Mexico (UNAM), Ethical approval code CICUAE: C20_06, and the study is in compliance with the ARRIVE guidelines where animals are involved.

Quantification of oocysts. The quantification of OPG from feces was performed at 7- and 9-days post-challenge by using the McMaster technique as previously described[67].

Data and statistical analysis. PGF2α and 8-iso-PGF2α data are presented as means with standard deviation (S.D.). The number of samples per variable group was 20, implying a normal distribution (Shapiro–Wilk test), and the homoscedasticity was verified (Levene's test). Accordingly, the parametric test of analysis of variance (ANOVA) was performed, and the differences between the means were evaluated using Tukey's honestly significant difference (HSD) test, and the P value was established with an alpha level of $P < 0.01$. OPG data are presented as means with median. The number of samples per variable group was 20; however, the hypotheses of normal distribution (Shapiro–Wilk test) and homoscedasticity (Levene's test) were not confirmed. Consequently, non-parametric tests of non-parametric tests of the two-tailed Kruskal–Wallis was applied and subsequently the Mann–Whitney's U test to compare between pairs of groups was applied with an alpha level $P < 0.05$[72].

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Author contributions

Conceptualization: V.M.P.-G., R.L.-A., and G.T.-I. Investigation and Methodology: V.M.P.-G., G.R.P., M.A.C.R., D.H.-P., B.S.-C., and X.H.-V. Formal analysis and Software: F.A.-H., C.N.V., I.C.-H. Supervision: V.M.P.-G., and G.T.-I. Validation and Visualization: D.H.-P. and B.S.-C. Writing-original draft: V.M.P.-G. and G.T.-I. Writing-review and editing: X.H.-V. and G.T.-I. All the authors reviewed, edited, and approved the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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ANEXO 2A. Artículo científico publicado

PUBLICACIÓN 2. ISOLATION AND ANTIMICROBIAL SENSITIVITY OF *Mycoplasma synoviae* AND *Mycoplasma gallisepticum* FROM VACCINATED HENS IN MEXICO (80)

pathogens

MDPI

Article

Isolation and Antimicrobial Sensitivity of *Mycoplasma synoviae* and *Mycoplasma gallisepticum* from Vaccinated Hens in Mexico

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Abstract: *Mycoplasma synoviae* (MS) and *Mycoplasma gallisepticum* (MG) strains were isolated at 39.5 °C to rule out temperature-sensitive strains (ts+) and identified using random amplification of polymorphic DNA. Then, their minimum inhibitory concentrations (MIC_{100}) were calculated in isolated strains from broiler breeders and laying hens vaccinated with ts+ MS-H and ts+ MG TS-11 vaccines in Mexico. We sampled 631 lots of hens. A total of 28 of the 123 MS isolates and 12 of the 23 MG isolates were analyzed using random amplification of polymorphic DNA, of which 24 and 3 matched the DNA banding patterns of the MS-H and MG-F strains, respectively. The isolated MS and MG strains were sensitive to tiamulin and tylosin and showed intermediate sensitivity or resistance to lincomycin, florfenicol, erythromycin, enrofloxacin, and curcumin. Although both the MS and MG strains were sensitive to the same antibiotics (MIC_{100} lower than 1 mg mL^{-1}), the MG strains were 5 to 10 times more sensitive than the MS strains. MS is the most frequently isolated mycoplasma in Mexican poultry production. The MS vaccine used (ts+ MS-H) could reverse its thermosensitivity and therefore could regain its virulence. MS was less sensitive to tiamulin and tylosin compared to MG.

Keywords: *Mycoplasma synoviae*; *curcumin*; thermosensitivity; pathogenicity; reversal; MS-H vaccine; tiamulin; tylosin

1. Introduction

Mycoplasma synoviae (MS) and *Mycoplasma gallisepticum* (MG) are the most important *Mycoplasma* species for commercial poultry in Mexico. For decades, these species have been recognized as the cause of chronic respiratory disease (CRD) [1–3]. In addition, they decrease the fertile egg production in broiler breeders, cause late-stage embryonic death, or result in the births of infected chicks that later develop CRD. This disease suppresses the innate immune responses of the respiratory system and predisposes the bird to infection with *Escherichia coli*, producing complicated CRD (CCRD) [4]. CCRD is responsible for significant economic losses as it causes polyserositis, septicemia, and death in poultry farms as well as seizures at slaughterhouses [1,2].

In Mexico, mycoplasmosis is currently controlled by vaccination and antibiotic metaphylaxis. The only vaccine used in Mexico against MS is the MS-H strain. The MS-H strain is temperature sensitive (ts+) and was developed through chemical mutagenesis of an Australian field isolate (strain 86079/7NS) [5]. The ts+ MG vaccine strain used in Mexico is TS-11, which was also developed through chemical mutagenesis of an Australian field isolate (strain 80083) [6]. The most widely used groups of antibiotics against *Mycoplasma* spp. are macrolides, pleuromutilins, lincosamides, and fluoroquinolones. Other antibiotics with poor sensitivity, such as amphenicols and tetracyclines, are mainly used in combination to treat CCRD [7,8]. Because *Mycoplasma* spp. are primarily vertically transmitted from the hen to the chick, vaccination and treatments mainly focus on broiler breeders.

Considerable efforts have been made to develop more powerful, effective, well-tolerated, and, above all, safe medicines for humans. One of these medicines is curcumin (1, C21H20O6) or diferuloylmethane, which is extracted from the turmeric tuber (*Curcuma longa* L. Zingiberaceae) [9]. Studies on the in vitro antibacterial effect of curcumin on *Staphylococcus aureus*, *Staphylococcus intermedius*, *Staphylococcus epidermidis*, and *Edwardsiella tarda* [10] have demonstrated that the minimum inhibitory concentration (MIC) of curcumin solubilized in dimethyl sulfoxide against *Mycoplasma* spp. isolated from mammals ranges from 50 to 100 μg mL^{-1} [11], although its effect on mycoplasmas isolated from birds has not been demonstrated.

Random amplification of the polymorphic DNA (RAPD) identification method of MS and MG strains has proven efficient and particularly useful for epidemiological studies as well as the identification and differentiation of vaccine strains and field isolates [12–14]. A previous work [15] showed that RAPD had a discriminatory index for MS superior to 0.95; consequently, this molecular method was chosen for this study.

Eye or spray vaccination with ts+ MS and MG strains aim to colonize the upper respiratory tract and stimulate the local immune system without causing systemic colonization or transovarian transmission to the progeny. Reversing ts+ to ts− facilitates systemic colonization and transovarian transmission, predisposing the bird to CCRD [16]. Based on the above, the objective of the present study was to isolate and identify ts− vaccine strains from poultry vaccinated with ts+ strains using the RAPD method and evaluate the strains' sensitivity to antimycoplasmic antibiotics.

2. Results

In the present study, 631 lots of hens were sampled from 14 poultry companies (Table 1). *Mycoplasma* spp. were isolated in 23.14% (146/631) of samples from 100% (14/14) of the poultry companies. Of the positive isolates, MS accounted for 84.25% (123/146) of the isolates and was significantly ($p < 0.001$) more frequently isolated than MG, which accounted for 15.75% (23/146) of isolates. Of the 123 MS isolates, 28 were analyzed using RAPD, 24 presented DNA banding patterns matching those of the MS-H strain, and four presented different DNA banding patterns. Of the 23 MG isolates, 12 were analyzed using RAPD, three presented DNA banding patterns matching those of the MG-F strain, and nine were untyped strains with DNA banding patterns different from those of the vaccine control strains (Table 2).

Table 1. *Mycoplasma synoviae* (MS) and *Mycoplasma gallisepticum* (MG) isolates in lots of broiler breeders and laying hens in different states of Mexico.

	Broiler Breeders							Laying Hens			Total Hens
	Ags	**Chis**	**SoM**	**Jal**	**Qro**	**Ver**	**Total**	**Jal**	**Pue**	**Total**	
Sampled lots	6	52	102	90	84	57	391	37	203	240	631
M. synoviae	2	15	16	26	9	32	100	7	16	23	123
M. gallisepticum	2	0	14	0	6	1	23	0	0	0	23

Ags = Aguascalientes; Chis = Chiapas; SoM = State of Mexico, Jal = Jalisco, Qro = Querétaro, Ver = Veracruz, and Pue = Puebla.

Table 2. The RAPD identification of *Mycoplasma synoviae* and *Mycoplasma gallisepticum* vaccine strains isolated at 39.5 °C in broiler breeders and laying hens in different states of Mexico.

	Ags	Chi	SoM	Jal	Pue	Qro	Ver	Total
MS-H strain	2	5	–	5	6	1	5	24
Untyped *M. synoviae*	0	0	–	3	1	0	0	4
MG-F strain	2	–	0	–	–	–	1	3
Untyped *M. gallisepticum*	0	–	9	–	–	–	9	9

RAPD = Random amplification of polymorphic DNA. Ags = Aguascalientes; Chi = Chiapas; SoM = State of Mexico, Jal = Jalisco, Pue = Puebla, Qro = Querétaro, and Ver = Veracruz.

MS isolates were susceptible to tiamulin and tylosin but resistant to curcumin, erythromycin, and florfenicol. The MG isolates were susceptible to erythromycin, tiamulin, and tylosin (Table 3).

Table 3. The mean ± standard deviation in μg mL^{-1} of the minimum inhibitory concentration (MIC_{100}) of *Mycoplasma synoviae* and *M. gallisepticum* isolates from broiler breeders and laying hens in Mexico.

	Curcumin *	Enrofloxacin	Erythromycin	Florfenicol	Lincomycin	Tiamulin	Tylosin
M. synoviae	≥2.50 ± 0.00 ^{a}(R)	1.64 ± 0.74 ^{a}(I)	≥2.50 ± 0.00 ^{b}(R)	2.23 ± 0.52 ^{a}(R)	1.81 ± 0.66 ^{a}(I)	0.43 ± 0.30 ^{b}(S)	0.92 ± 0.41 ^{b}(I)
M. gallisepticum	≥2.50 ± 0.00 ^{a}(R)	1.90 ± 1.09 ^{a}(I)	0.48 ± 0.78 ^{a}(S)	≥2.50 ± 0.00 ^{b}(R)	≥2.50 ± 0.00 ^{b}(R)	0.03 ± 0.02 ^{a}(S)	0.07 ± 0.05 ^{a}(S)

* Five isolates of each *Mycoplasma* species were used for curcumin. Different letters between the same antibiotic indicate significant differences ($p < 0.05$). R = resistant isolates, I = isolates with intermediate sensitivity, and S = sensitive isolates.

The isolates with DNA banding patterns matching those of the MS-H strain, the isolates that presented different patterns, and the ts+ MS-H vaccine strain (control) were all susceptible to tiamulin and tylosin and were resistant to curcumin, florfenicol, and erythromycin (Table 4). The isolates with DNA banding patterns matching those of the MG-F strain and MG-F vaccine strain (control) were susceptible to enrofloxacin, tiamulin, and tylosin but resistant to curcumin, florfenicol, and lincomycin. The isolates with DNA banding patterns matching those of untyped MG strains and the ts+ TS-11 vaccine strain (control) were sensitive to erythromycin, tiamulin, and tylosin but resistant to curcumin, enrofloxacin, florfenicol, and lincomycin (Table 5).

Table 4. The means ± standard deviation in μg mL^{-1} of the minimum inhibitory concentration (MIC_{100}) of *Mycoplasma synoviae* isolates by DNA banding pattern in hens in Mexico.

	Curcumin	Enrofloxacin	Erythromycin	Florfenicol	Lincomycin	Tiamulin	Tylosin
ts- MS-H	≥2.50 ± 0.00 ^{a}(R)	1.72 ± 0.70 ^{b}(I)	≥2.50 ± 0.00 ^{a}(R)	2.19 ± 0.55 ^{a}(R)	1.80 ± 0.67 ^{b}(I)	0.45 ± 0.31 ^{a}(S)	0.95 ± 0.41 ^{b}(S)
Untyped *M. synoviae*	≥2.50 ± 0.00 ^{a}(R)	1.17 ± 0.97 ^{b}(I)	≥2.50 ± 0.00 ^{a}(R)	≥2.50 ± 0.00 ^{a}(R)	1.88 ± 0.72 ^{b}(I)	0.31 ± 0.22 ^{a}(S)	0.70 ± 0.39 ^{b}(S)
ts+ MS-H	≥2.50 ± 0.00 ^{a}(R)	0.31 ± 0.00 ^{a}(S)	≥2.50 ± 0.00 ^{a}(R)	≥2.50 ± 0.00 ^{a}(R)	0.63 ± 0.00 ^{a}(S)	0.08 ± 0.00 ^{a}(S)	0.02 ± 0.00 ^{a}(S)

Five isolates of each *Mycoplasma* species were used for curcumin. Different letters between the same antibiotic indicate significant differences ($p < 0.05$). R= resistant isolates, I = isolates with intermediate sensitivity, and S = sensitive isolates.

Table 5. The means ± standard deviation in μg mL^{-1} of the minimum inhibitory concentration (MIC_{100}) of *Mycoplasma gallisepticum* isolates by DNA banding pattern in hens in Mexico.

	Curcumin	Enrofloxacin	Erythromycin	Florfenicol	Lincomycin	Tiamulin	Tylosin
MG-F strain	≥2.50 ± 0.00 [a] (R)	0.08 ± 0.00 [a](S)	1.67 ± 0.72 [a](I)	≥2.50 ± 0.00 [b] (R)	≥2.50 ± 0.00 [a] (R)	0.05 ± 0.02 [a](S)	0.04 ± 0.00 [a](S)
Untyped *M. gallisepticum*	≥2.50 ± 0.00 [a] (R)	≥2.50 ± 0.00 [c](R)	0.08 ± 0.05 [b](S)	≥2.50 ± 0.00 [b] (R)	≥2.50 ± 0.00 [a] (R)	0.02 ± 0.01 [a](S)	0.08 ± 0.05 [a](S)
MG-F strain	≥2.50 ± 0.00 [a](R)	0.08 ± 0.00 [a](S)	0.01 ± 0.00 [c](S)	≥2.50 ± 0.00 [b] (R)	≥2.50 ± 0.00 [a] (R)	0.01 ± 0.00 [a](S)	0.01 ± 0.00 [a](S)
ts+ MG TS-11	≥2.50 ± 0.00 [a](R)	0.39 ± 0.00 [b](S)	0.01 ± 0.00 [c](S)	0.62 ± 0.00 [a](S)	≥2.50 ± 0.00 [a](R)	0.01 ± 0.00 [a](S)	0.01 ± 0.00 [a](S)

Five isolates of each *Mycoplasma* species were used for curcumin. Different letters between the same antibiotic indicate significant differences ($p < 0.05$). R= resistant isolates, I = isolates with intermediate sensitivity, and S = sensitive isolates.

3. Discussion

CRD and CCRD are two diseases that commonly affect poultry production in Mexico and many other parts of the world. One of the main forms of control is vaccination with live strains of MS and MG that do not grow at 39.5 °C or higher temperatures because vaccinating with strains unable to reproduce at body temperature prevents vaccine strain mycoplasmas from causing sepsis or transmitting to the egg. In this study, from laying hens previously vaccinated with the ts+ MS-H strain, we isolated MS at 39.5 °C with DNA banding patterns identical to those of the ts+ MS-H vaccine strain; therefore, in some cases, the ts+ strains were able to revert their 39.5 °C thermosensitivity and reproduce in hens.

We found a higher rate of MS than MG isolates in both types of hens (broiler breeders and laying hens), most likely owing to the highly technical poultry industry in Mexico, as was also found in highly developed poultry farming countries in Europe and in the Americas [17–22]. In Europe, state-led MG eradication programs have been implemented [21]. In the Americas, the USA has a voluntary testing and certification program for flocks free of both mycoplasmas, while in the rest of the continent, control programs aimed at biosecurity with mycoplasma-free birds or mycoplasmosis control with antibiotic metaphylaxis are voluntary for private poultry farmers [1,23]. Additionally, in this study, we observed that MG was 5 to 10 times more sensitive than MS to two of the world's most widely used antibiotics in the poultry industry (tiamulin and tylosin); therefore, with the use of the same dose of antibiotic treatment, the likelihood of eliminating MG is higher than that of eliminating MS.

A ts+ (39.5 °C) vaccine strain that reverses its sensitivity may reproduce in birds and cause CRD as well as transmit to the progeny. Thermosensitivity reversal in ts+ MS-H strains has been demonstrated by isolating strains typed as MS-H at 39.5 °C using restriction fragment length polymorphism analysis in broiler breeders and laying hens [5]. Under laboratory conditions, without performing molecular tests, it was shown that this thermosensitivity reversal was not a complete reversion to virulence when the ts- MS-H were isolated from lesions in specific-pathogen-free pullets (SPAFAS) [16]. In our study, using RAPD, we isolated and identified MS-H strains that grew at 39.5 °C exclusively from tracheal samples of broiler breeder and laying hens.

The samples were obtained from egg-producing hens vaccinated with the ts+ MS-H strain between two and four weeks of age, suggesting that the MS-H strain reversed thermosensitivity after 16 to 25 weeks of application. Therefore, isolated ts- MS-H strains can cause MS contamination in eggs, reducing egg production and causing economic loss. In broiler breeders, MS transmission to their progeny could cause CRD and mortality in broilers. Due to these disorders, it is essential to consider the possible risk of a reversal of pathogenicity when applying the ts+ MS-H strain or other live ts+ vaccines against *Mycoplasma* spp. In specific-pathogen-free hens vaccinated under laboratory conditions, Armour and Ferguson-Noel [24] and El Gazzar et al. [25] demonstrated that the ts+ MG TS-11 strain could revert its thermosensitivity and pathogenicity, cause septicemia, and invade the ovary, thus infecting the eggs.

In our study, of the 12 isolated and characterized MG strains, no strain showed DNA banding patterns matching those of the ts+ MG TS-11 strain; as a result, we were unable to demonstrate that they reversed their thermosensitivity. In our study, we found three MG strains whose DNA banding patterns matched those of the F strain, which was originally a vaccine strain; currently, the F strain can be considered a vaccine or field strain, with nine strains corresponding to untyped wild-type field strains. In the study by Armour and Ferguson-Noel [24], vaccine mycoplasmas failed to colonize bird tissues; however, under field conditions, as in our study, wild strains can compete for receptors of target cells with the vaccine strain TS-11. Most likely, this competition is one of the reasons why this strain was not isolated successfully.

In Mexico, and in many other countries, CRD is controlled with vaccines and antibiotic metaphylaxis; accordingly, the sensitivity to different antibiotics must be periodically assessed by region and by country. Our findings showed that our *Mycoplasma* isolates, in general, were of intermediate sensitivity or resistant to lincomycin, florfenicol, erythromycin, enrofloxacin, and curcumin. The *Mycoplasma* isolates, both untyped wild type and vaccine strains, were only sensitive to tiamulin and tylosin. The majority of studies on antimycoplasmic effects were performed with MG [7]; however, the MIC_{100} for MG is lower than that for MS. Thus, effective control of both *Mycoplasma* species requires using a dose 3 to 5 times higher than that needed to control MS. Regarding erythromycin, we agree with the work of Gautier-Bouchardon [26], which states that MS is inherently resistant to the antibiotic while MG is not.

In the search for new drugs that are effective against bacteria and safe for the environment as well as for humans, well-characterized products of plant origin, including polyphenols, such as curcumin, have been evaluated. Curcumin previously exhibited an antimycoplasmic effect against *Mycoplasma hominis*, *Mycoplasma capricolum*, *Mycoplasma mycoides*, *Mycoplasma genitalium*, and *Mycoplasma pneumoniae* at concentrations ranging from 50 to 100 μg mL^{-1} [11]. We considered the strains that grew at a dilution greater than 2 μg mL^{-1} as resistant to *Mycoplasma*. In this study, all MS and MG strains grew at 2.5 μg mL^{-1}, which was the maximum concentration, showing that curcumin was ineffective against MS and MG at the concentrations economically profitable for poultry production.

4. Materials and Methods

4.1. Sampled Farms

The present study was conducted from January 2010 to December 2019. Samples were collected from 14 poultry companies with a medical history of CRD in their hens or chicks (eight broiler breeders and four laying hens). Samples of broiler breeders were collected from the following Mexican states: Jalisco, Querétaro, Aguascalientes, State of Mexico, Chiapas, and Veracruz. Samples of laying hens were collected from the following Mexican states: Jalisco and Puebla.

Farm Selection Criteria

Broiler breeder farms whose progeny had a history of serosal-fibrinous airsacculitis or fibrinous arthritis were selected for this study, whereas the selected laying hen farms had a history of hens with airsacculitis or peritonitis and low egg production ranging from 2% to 10%. The hens of the selected farms were previously vaccinated against MS using live strain ts+ MS-H (Vaxsafe® MS, Laboratorio Avimex, Mexico City, Mexico) and against MG with one of the two live strains ts+ TS-11 (TS-11®, Boehringer Ingelheim Vetmedica, Guadalajara, Mexico) or strain F (F VAX-MG®, MSD Animal Health, Mexico City, Mexico).

4.2. Bacterial Isolation

In total, 10 hens were randomly selected from each farm. Tracheal scrapping samples were collected using a swab and transported in FREY liquid medium. The samples were incubated at 39.5 °C for 20 days [27,28]. Cultures were reviewed daily, and subcultures of samples showing changes in the

pH were prepared according to the method described by Poveda [29]. The cultures that showed no change in pH were considered negative. MS and MG were identified in biochemical tests (glucose fermentation, tetrazolium reduction, arginine hydrolysis, and digitonin sensitivity), and the results were confirmed with direct immunofluorescence testing. Cloning was completed by aspirating single colonies with a Pasteur pipette and inoculating broth medium to eliminate clumped cells and then re-plating on agar. The cultures were cloned three times to ensure purity. After sufficient growth in broth, the medium was filtered through a 0.45 μM filter to eliminate clumped cells and re-plated on agar, as described by Poveda [29] and Ferguson-Noel and Kleven [30]. To serve as controls, original strains of the MS-H and TS-11 vaccines were incubated at 33 °C, and the F strain was incubated at 37 °C.

4.3. Molecular Identification of Mycoplasma spp. Vaccine Strains

The DNA of isolated and cloned MS and MG strains was identified by RAPD using the technique and primers designed by Geary et al. [31]. The DNA banding patterns of the isolated strains were compared to the DNA banding patterns of the MS-H, MG-F, and MG TS-11 reference strains.

4.4. In Vitro Sensitivity to Antibiotics and Curcumin

MIC_{100} of six antibiotics and curcumin (1, $C_{21}H_{20}O_6$) was calculated with the isolated *Mycoplasma* strains. The antibiotics evaluated in this study were selected based on widespread usage in poultry in Mexico for mycoplasma control and classification as critically important for both veterinary and human use by the World Health Organization [32]. The following antibiotics were obtained from Merck (Kenilworth, NJ, USA): lincomycin hydrochloride, florfenicol, erythromycin thiocyanate, enrofloxacin, tiamulin hydrogen fumarate, and tylosin tartrate. Polyphenol 4.5% curcumin (Laboratorios Mixim, Public Limited Company, Naucalpan, Mexico) was chemically dispersed into polyvinyl pyrollidone (Plastone K-29/32, Ashland™, Mexico City, Mexico). The dilutions were calculated in relation to the final concentration of the base molecule. MIC_{100} values were defined as the lowest concentration of the antibiotic at which 100% of the isolates were inhibited.

All isolated strains were assayed for antibiotic MIC_{100}, whereas only five strains from each *Mycoplasma* were evaluated against curcumin. MIC_{100} was calculated according to the method described by Hannan [33] using FREY culture medium [27,29]. Following the recommendation of Hannan [33], the culture concentration of each isolated *Mycoplasma* spp. was adjusted to 10^4 color changing units mL^{-1}, with phenol red as the indicator. Two-fold dilutions of the antibiotic were prepared, ranging from 2.5 to 0.01 μg mL^{-1}, although there are no official cut-off points for the interpretation of MIC_{100} for avian mycoplasma [26,33]. In this investigation, isolates were considered susceptible to antibiotics when the MIC_{100} was 0.5 g mL^{-1}. Isolates with MIC_{100} 1 g mL^{-1} were classified as intermediate to antibiotics and those with MIC_{100} 2 g mL^{-1} were classified as resistant. These criteria were adapted from the research of Lysnyansky et al. [34].

4.5. Statistical Analysis

The proportion of the number of isolates was compared using the chi-squared test. The mean MIC_{100} of each antibiotic was compared between MS and MG isolates using Student's *t*-test. An analysis of variance (ANOVA) was performed on the MIC_{100} results of the same antibiotic from typified strains (based on DNA banding pattern), and the differences between the means were evaluated using Tukey's honestly significant difference (HSD) test. Statistical significance was set at $p < 0.05$.

4.6. Ethical Compliance

This study was conducted in accordance with the recommendations of the Institutional Animal Care and Use Committee (IACUC) at the University of Arkansas (Fayetteville, AR, USA) under approved protocol #17073.

5. Conclusions

Based on the findings of this study, we propose that MS is the most important *Mycoplasma* for Mexican poultry production for the following reasons: MS was the *Mycoplasma* species most frequently isolated, the vaccine used (ts+ MS-H) was able to revert its temperature sensitivity and recover virulence, and MS was 5 to 10 times less sensitive than MG to the most widely used antibiotics (tiamulin and tylosin). Further studies evaluating the virulence of these thermosensitive revertant strains on egg production and on the progeny of hens vaccinated with MS-H are necessary to determine the economic and health impact of this reversal.

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ANEXO 2B. Artículo científico (preimpresión en revisión)

PUBLICACIÓN 3. VERTICAL TRANSMISSION AND REVERSE THERMOSENSITIVITY OF THE MS−H VACCINE STRAIN OF *Mycoplasma synoviae* IN COMMERCIAL LAYING HENS (81)

Short communication

Reverse thermosensitivity and vertical transmission of the MS−H vaccine strain of *Mycoplasma synoviae* in commercial laying hens

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Abstract

The purpose of this research was to isolate and identify non-temperature sensitive (ts-) (≥39.5 °C insulation), vaccine *Mycoplasma synoviae* (MS) strains from laying breeder hens vaccinated with temperature sensitive (ts+) strains (<39.5 °C insulation), as well as to confirm the transmission of isolated strain from the hens to their progeny. The minimum inhibitory concentrations (MIC_{100}) were calculated. Isolates of MS from 40 ts+ MS−H-vaccinated Hy−Line® breeder hens and 20 unvaccinated female progenies with and without Infectious synovitis (IS) were produced at 37 and 39.5 °C. Random amplification of polymorphic DNA was performed on the isolates at 39.5 °C, and the same ts- MS−H strains were identified in the breeder hens and their progeny. The isolates from breeder hens and their progeny showed non-significant differences in five antimycoplasmic MIC_{100} values ($P > 0.05$). This investigation demonstrated the reversal of the thermosensitivity, pathogenicity, and vertical transmission of the MS−H strain.

Keywords

RAPD, reversal pathogenicity, MS-H, Vaxsafe®, enrofloxacin

1. Introduction

Mycoplasma synoviae (MS) is the main *Mycoplasma* species in Mexican poultry production [1]. MS infection produces a subclinical respiratory infection and subsequent gonadal infection. The gonadal infection produces vertical transmission to the embryo, which becomes a septicemic chick that results in infectious synovitis (IS). IS produces an acute-to-chronic infectious disease in chickens which primarily involves the synovial membranes of joints, triggering exudative synovitis. *Mycoplasma* spp. are transmitted mainly from hen to chick; vaccination and treatments principally focus on leghorn breeder hens. Vaccination and antibiotic metaphylaxis have been used to try to prevent the vertical transmission of *Mycoplasma* spp. from breeder hens to their progeny [2].

The MS−H strain is temperature-sensitive (ts+) (<39.5 °C insulation), which is produced via chemical mutagenesis of the Australian field strain 86079/7NS [3]. Vaccination with the ts+ MS strain has the purpose of colonizing the extrapulmonary respiratory tract without causing systemic infection and transovarial transmission to the progeny. The reversion from ts+ to ts- (≥39.5 °C insulation) produces infection of the air sacs and gonads [4]. The gonadal infection triggers transovarial transmission, inducing laying chicks to IS. The vaccine has had wide use in Australia, Mexico, and several countries; however, its registration in the United States has not been granted [2].

Another alternative for the control of mycoplasmosis is antibiotic metaphylaxis. The antibiotics most widely used against *Mycoplasma* spp. are doxycycline, enrofloxacin, erythromycin, lincomycin, tiamulin, and tylosin [5][6].

The random amplification of polymorphic DNA (RAPD) recognition technique has been demonstrated to be efficient, as well as especially effective for the epidemiological research of MS strains and for the classification and distinction of vaccine strains and field isolates [7] [8]. Marois et al. demonstrated that RAPD has a discriminatory indicator for MS which is more significant than 0.95; therefore, RAPD was selected for the present work [9].

Our recently published study showed the inverse thermosensitivity of the ts+ MS−H strain [1]. However, there exists limited evidence on the reverse pathogenicity of the ts+ MS−H strain in poultry production, and there are no reports that have demonstrated transmission to the progeny of hens vaccinated with ts+ MS−H. Therefore, the objective of this work is to demonstrate the vertical transmission —that is, from vaccinated hens to their progeny— of ts+ MS−H strains with reverted thermosensitivity. Furthermore, minimum inhibitory concentrations (MIC_{100}) of the isolates (at 39.5 °C) were calculated.

2. Material and methods

2.1 Animal Sources

The birds selected for this work were Hy-line® breeder hens vaccinated with the live strain ts+ MS−H (Vax-safe® MS, Laboratorio Avimex, Mexico City, Mexico), as well as their unvaccinated female progeny without and with IS from the same farm. The progeny received two daily treatments of 20 mg mL^{-1} enrofloxacin in drinking water for 5 days, in order to control the IS [5]. The laying breeders and their progeny were obtained from two farms located in distant counties in the Mexican state of Jalisco.

2.1.1. Research group design and collection of samples

Three research groups were formed: 1) The breeder hen group, 2) the non-IS progeny group, and 3) the IS progeny group. The samples were taken from 24-, 39-, 48-, and 70-week-old breeder hens in the first group, and 9-week-old female chickens in the two progeny groups. Tracheal swab samples were collected from the

breeder hen group and the non-IS progeny group. The IS progeny group was sampled from the elbow joints with serofibrinous arthritis.

2.1.2. Ethical compliance

This study was carried out in accordance with the recommendations for the management of chickens provided by the Internal Committee for Care and Use of Experimental Animals (CICUAE) of the National Autonomous University of Mexico (UNAM). Ethical approval code: CICUAE-FESC C20_06.

2.2. Bacterial isolation

The samples were incubated at 39.5 °C for 21 days [10]. The cultures were carried out according to the methodologies described by Petrone-Garcia et al. [1]. To serve as controls, original strains of the MS−H vaccine were incubated at 33 °C, while the ts+ TS−11 MG strain (TS−11®, Boehringer Ingelheim Vetmedica, Guadalajara, Mexico) was incubated at 33 °C. The ts- F MG strain (F VAX MG®, MSD Animal Health, Mexico City, Mexico) was incubated at 37 and 39.5 °C.

2.3. RAPD identification of Mycoplasma synoviae *isolations*

The DNA of cloned MS strains was characterized by RAPD, utilizing the method and primers proposed by Geary et al. [11]. The DNAbp of the isolated strains were matched to the DNAbp of the MS−H (33 °C), F (37 and 39.5 °C), and TS−11 (33 °C) reference strains.

2.4. Minimum inhibitory concentrations

MIC_{100} values were described as the lowest concentration of the antibiotic at which 100% of the isolates were inhibited. The antimycoplasmics used were doxycycline, enrofloxacin, erythromycin thiocyanate, lincomycin hydrochloride, tiamulin hydrogen fumarate, and tylosin tartrate (Merck, Kenilworth, NJ, USA). The MIC_{100} values were estimated in correspondence to the final dilution of the base molecule. The MIC_{100} was analyzed according to the technique published by Hannan [12] and Gautier-Bouchardon [13] and

appropriated by Petrone-Garcia et al. [1]. Two-fold dilutions of the antibiotic were made, fluctuating from 5 to 0.01 μg mL^{-1}.

2.4.1. Statistical Analysis

For MIC_{100}, the data are expressed as the mean and median. The minimum number of samples per group of variables was ten, and the total was 40. The hypothesis of normal distribution was verified with the Shapiro–Wilk test and homoscedasticity with Levene's test; however, the two statistical conditions were not established. Subsequently, the Kruskal–Wallis non-parametric tests were applied and, afterward, the Mann–Whitney *U* test was carried out, in order to compare between pairs of groups. The statistical significance was set at $p < 0.05$.

3. Results

3.1 Bacterial isolation

At the two temperatures, MS isolation was not obtained from the 24- and 39-week-old breeder hens; however, MS isolation was achieved from scrapings from 9 (90%) and 10 (100%) 48- and 70-week-old breeder hens, respectively. Additionally, MS was isolated from 10 (100%) non-IS progeny and from 9 (90%) IS progeny.

3.2. Molecular identification (RAPD) of Mycoplasma synoviae

All the isolates exhibited DNA banding patterns (DNAbp) corresponding to those of the MS−H strain (Figure 1). It is worth mentioning that the isolates from the two groups of progenies, in addition to being identical to the MS−H strain, were the same as the isolates from the group of breeding hens.

3.3. Minimum Inhibitory Concentrations

No MIC100 differences ($p > 0.05$) were observed between breeders and their progeny for doxycycline, erythromycin, lincomycin, or tylosin; however, different MIC100 values ($p < 0.05$) were observed between breeders and their progeny for enrofloxacin, and between breeders and progeny with clinical signs or progeny with non-clinical signs (Table 1).

4. Discussion

When ts- live strains of MS are grown at 39.5 °C or above, the MS from the vaccine can regain the ability to cause sepsis and be transmitted to the embryo. In this study, we identified MS−H strains from tracheal samples of laying breeder hens vaccinated with the ts+ MS−H strain that grew at 37 and 39.5 °C; the ts- MS−H strains isolated at 39.5 °C showed DNAbp equal to those of the ts+ MS−H vaccine strain. The ts- MS−H strain was also isolated in progeny chickens, several of which even presented SE. Therefore, the ts+ strains could revert their 39.5 °C thermosensitivities, replicate in laying breeder hens, and transfer to progeny chickens. Noormohammadi et al. proved the partial reversion to virulence of the ts- MS−H strain in three-week-old leghorn chickens. The chickens were MS−H vaccinated and had air sac lesions that were not different in severity from those caused by ts- MS strains. The two strains were isolated at 37 °C and identified by restriction fragment length polymorphism (RFLP) [4]. Furthermore, RFLP analysis was utilized to demonstrate the change to ts- of isolates at 39.5 °C in the ts+ MS−H strain from laying hens [3]. In field conditions, reversion of the temperature sensitivity of the MS−H strain has been demonstrated in hens [1].

The laying breeder hens in the present study were positive for the MS−H strain, both by isolation and PCR, until 48 and 70 weeks of age, indicating that the ts+ MS−H strain was slow to revert thermosensitivity; however, when the strain was transmitted to progeny, it produced SE before nine weeks of age. Another report has indicated that the reversal of thermosensitivity occurred between 20 and 29 weeks of age in breeder hens [1]. There also exists a ts+ vaccine (TS−11 strain) against *Mycoplasma gallisepticum* (MG),

which is also relevant in poultry production. The ts+ MG TS−11 strain has been reported to reverse its thermosensitivity and pathogenicity, cause airsacculitis, invade the ovary, and infect eggs [14] [15]. In this study, isolated ts- MS−H strains from laying breeder hens produced MS contamination in embryos, where MS transmission to the progeny caused IS. To avoid the transmission of MS to progeny and the consequent IS, it is critical to contemplate the possibility of a reversal of pathogenicity and vertical transmission to the progeny when using the ts+ MS−H strain.

In this work, no difference was observed in MIC_{100}s between laying breeder hens and progeny, except for the increased MIC_{100} of enrofloxacin in the progeny. This increase could have been due to the use of enrofloxacin to treat IS in the progeny. This difference in MIC_{100} was also confirmed in the report of Le Carrou et al., who found a significant increase in the resistance level to enrofloxacin of five re-isolated *Mycoplasma* clones, which was observed after the second treatment [16]. Furthermore, we found that the antimycoplasmic agent with the highest sensitivity for MS was tylosin, in agreement with the results of Petrone-Garcia et al. [1]

5. Conclusions

Considering the results of this research, the ts+ vaccine strain MS−H can revert its temperature sensitivity to perform vertical transmission to the progeny and recover virulence, thus yielding IS. Therefore, it is crucial to contemplate the danger, in terms of the reversal of pathogenicity and vertical transmission to progeny, when using the MS−H vaccine strain or other live ts+ vaccines versus *Mycoplasma* spp.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Contributions

Conceptualization, V.M.P-G. and F.G.-R.; methodology, V.M.P.-G.; software, V.M.P.-G.; validation, G.T.-I., R.L.-A., and F.A.-H.; formal analysis, G.T.-I.; investigation, V.M.P.-G., F.G.-R., and J.A.C.-R ; resources, V.M.P.-G., F.G.-R., and J.A.C.-R.; data curation, I.C.-H. and X.H.-V.; writing—original draft preparation, V.M.P-G.; writing—review and editing, V.M.P-G, G.T.-I., R.L.-A., and F.A.-H; visualization, V.M.P.-G; supervision, S.E.-A.; project administration, G.T.-I., R.L.-A., and F.A.-H; funding acquisition, G.T.-I., R.L.-A., and V.M.P.-G. All authors have read and agreed to the published version of the manuscript.

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ANEXO 3. Artículo científico publicado

PUBLICACIÓN 4. EVALUATION OF AVIAN REOVIRUS S1133 STRAIN IN NEONATAL BROILER CHICKENS IN GASTROINTESTINAL INTEGRITY AND PERFORMANCE IN A LARGE-SCALE COMMERCIAL FIELD TRIAL (82)

vaccines

MDPI

Article

Evaluation of Avian Reovirus S1133 Vaccine Strain in Neonatal Broiler Chickens in Gastrointestinal Integrity and Performance in a Large-Scale Commercial Field Trial

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Abstract: Avian reovirus (ARV) is the principal cause of several diseases. The vaccination of breeders allows for the control of viral arthritis and delivery of maternal-derived antibodies to the progeny. The vaccination of broiler chickens with ARV strain S1133 is used to prevent viral arthritis. However, the post-vaccination enteric effects have not been well-characterized. The purpose of this study was to evaluate the effect of vaccination with the S1133 strain on the weight gain and feed conversion of broiler chickens and to characterize the gastric, enteric, and pancreatic lesions that the strain could induce. A total of 672,000 chickens were divided into two groups: a group vaccinated with ARV strain S1133 (S1133ARV) and a control group (not vaccinated). Upon histological analysis, the vaccine group showed less proventricular glandular tissue and atrophy of the pancreas and duodenal villi, as well as having a lower average daily profit. The conclusion based on the results of this investigation is that neonatal vaccination with S1133ARV causes atrophy of the pancreatic acini, proventricular glands, and intestinal villi, leading to an increased diameter of the glandular lumen and atrophy of the enteric villous, as well as weight loss, in broiler chickens.

Keywords: reovirus S1133 vaccine; pancreatic and enteric histology; ELISA

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1. Introduction

Avian reoviruses are part of the *Reoviridae* family in the genus *Orthoreovirus*. They are a nonenveloped virus composed of two concentric icosahedral capsids with an external diameter of 80–85 nm [1].

Member viruses have a broad host range, including metazoans, plants, protists, and fungi [2]. As in other species, the virus is abundant in poultry, and most reoviruses are innocuous [1–3]. The term "reovirus" is an acronym for "respiratory, enteric, orphan virus" since it was first isolated from the lungs and intestines in humans with no clinical signs [3]. In commercial poultry, pathogenic viruses cause significant economic losses due to arthritis and tenosynovitis in the gastrocnemius tendons [4]. Viral arthritis mainly

affects meat-type chickens but has also been diagnosed in commercial layers [5]. Breeder flocks that develop viral arthritis during egg production may be characterized by lameness, increased mortality, decreased egg production, suboptimal hatchability/fertility, and vertical transmission of the virus to progeny [6]. Shedding of virulent reovirus vertically by a breeder flock may affect progeny and cause severe losses. Moreover, since avian reoviruses replicate in the gastrointestinal tract, they are also associated with other pathologies such as stunting malabsorption syndrome, hepatitis, gastroenteritis, myocarditis, and respiratory diseases [3,7,8]. Avian reoviruses possess group- and serotype-specific antigens, and neutralizing antibodies can be detected 7–10 days following infection.

Vaccination against reoviruses in broiler breeders is conducted with live apathogenic vaccines (strain 2177), modified vaccines (strain S1133), and inactivated vaccines produced with pathogenic reoviruses (strains S1133, 2408, SS412, and 1733). In some countries, homologous viruses from the poultry geographic area are also used [1,3,4]. The apathogenic live vaccine and inactivated vaccines are administered subcutaneously, while modified live vaccines are used in drinking water. Vaccination in broiler breeders is essential to protect them against viral arthritis. However, strong vaccination programs in breeders are also used to transfer passive immunity to protect their progeny against viral arthritis [1,9,10]. Neonate chickens are highly susceptible to pathogenic reovirus infection [11,12]. Hence, proper vaccination of broiler breeders is crucial [13,14]. Maternal antibodies can afford protection to 1-day-old chicks against natural and experimental infections, but the level of protection conferred by antibodies is related to serotype similarity, virus virulence, host age, and antibody titer [3]. Recovery from reovirus infection involves both B- and T-cell activity, but protection is predominantly B-cell-mediated (antibodies). Therefore, maternal immunity is essential for protection against viral arthritis [15]. The experimental suppression of T-cell-mediated immunity resulted in increased mortality in reovirus-infected birds, but the relative severity of tendon lesions was unaffected [16]. $CD8^+$ T cells may play a major role in pathogenesis and/or reovirus clearance in the small intestine. In this process, maternal immunity does not play an important role [17].

The S1133 avian reovirus strain (S1133ARV) is the most widely used for vaccination, and it has been effective against viral arthritis in most parts of the world [1]. However, as far as we are aware, no evidence of the clinical expectation has been reported on using the live modified S1133ARV strain in neonate broiler chickens under commercial conditions. Hence, the purpose of this study was to evaluate the effect of S1133ARV on the weight gain and feed conversion of broiler chickens following vaccination with this strain, in addition to characterizing the gastric, enteric, and pancreatic lesions induced in response 1-day-old broiler chickens in a large-scale commercial field trial in Mexico.

2. Materials and Methods

2.1. Application of the Avian Reovirus S1133 Strain Vaccine in Broiler Chickens under Commercial Conditions

2.1.1. Location and Facilities of the Large-Scale Commercial Field Trial

This study was conducted at the regional complex for one slaughterhouse from an integrated poultry producer located in Aguascalientes, Mexico, with a clinical history of previous flocks of diarrhea with undigested feed with orange mucus, twisted pancreas, and a deficit of 4 $g \cdot day^{-1}$ of average daily gain, without arthritis or tenosynovitis. Twenty-four chicken houses with a capacity of 28,000 female broiler chickens were selected (n = 672,000 total chickens). Twelve houses were randomly selected, and chickens were vaccinated at 1-day-old using a spray cabinet with the avian reovirus S1133 strain, whereas the other twelve houses served as the nonvaccinated control group. Chickens were raised under normal production conditions and fed a four-phase commercial basal diet [18] (Table S1, Supplementary Materials). Evaluation of production parameters was done at the end of the grow-out cycle (38 days of age). Chickens were housed in a conventional farm with natural ventilation featuring an age-appropriate environment and kept under ambient conditions using the equipment recommended by the Ross broiler management

handbook [19]. Evaluated parameters included the age of the birds at processing, average daily gain (ADG), feed conversion rate (FCR), livability (LI), and production efficiency factor (PEF).

2.1.2. Source of Animals

Female broiler chickens were hatched at the commercial hatcheries of a Mexican poultry company in Aguascalientes, Mexico. The ROSS® 308 chickens (Aviagen®, Huntsville, AL, USA) came from imported embryos (Keith Smith Farms®, Hot Spring, AR, USA). The reovirus vaccination program for broiler breeders with an active virus was performed, as described, according to age: on day 0 with the 2177 strain (2177®, Merck Sharp and Dohme Corp, Kenilworth, NJ, USA); at 2, 4, and 6 weeks with the inactivated virus strain S1133 (Enterovax®, Merck Sharp and Dohme Corp, Kenilworth, NJ, USA). Vaccination with the inactivated virus was performed at 12 weeks using the inactive S1133, 2408, and SS412 strains (Maximune® 8, Ceva, Libourne, France), as well as the autogenous strain (custom KV: 9802. Elanco, Greenfield, IN, USA), and at 18 weeks with the S1133 (AviPro® 106 REO or KV: 7805, Elanco, Greenfield, IN, USA), and 1733 strains (AviPro® 106 REO or KV: 7805, Elanco, Greenfield, IN, USA), as well as the autogenous strain (custom KV: 9802, Elanco, Greenfield, IN, USA).

2.1.3. Avian Reovirus S1133 Vaccine

The avian reovirus strain S1133 included in the live modified virus vaccine cloned in tissue culture (Enterovax®, Merck Sharp and Dohme Corp, Kenilworth, NJ, USA) was used as the challenge virus with a titer of $10^{6.5}$ median tissue culture infectious dose ($TCID_{50}$)/mL according to the manufacturer's recommendation. Chickens in the vaccinated group received a full dosage of avian reovirus S1133 strain [20] at 1-day-old using a spray cabinet (Spra-Vac II®, Boehringer Ingelheim Vetmedica®, Guadalajara, Mexico).

2.2. Performance Variables

The performance variables were calculated as described in this section. The gain weight of the flock (GWF) was measured in kg at slaughterhouse reception divided by the initial number of chicks (INC), excluding mortalities at chick reception. The gain weight of the chickens (GWC) was calculated from the GWF divided by the INC. The ADG ($g \cdot day^{-1}$) was calculated from the GWC divided by the INC. Mortality was calculated based on chickens received at slaughterhouse reception from the IAC. Livability (LI) was calculated by subtracting the mortality from 100. The feed intake of the flock (FIF) was determined as the difference between the total amount of feed offered and the number of refusals. The feed conversion ratio (FCR) was calculated by dividing FIF with GWF. The production efficiency factor (PEF) was calculated using the following equation:

$$\text{PEF} = \frac{\text{LI (\%)} \times \text{GWC (kg)}}{\text{Age (days)} \times \text{FCR}} \times 100.$$

Cost–Benefit Calculation of Vaccination against Avian Reovirus

A cost matrix was built with variable feed intake·$chick^{-1}$ by average feed cost (462.38 USD), according to the feed program and constant other costs (0.82 USD·$chick^{-1}$), estimated using the chicken production cost of the Mexican Poultry Federation (UNA) [21] (Table S2, Supplementary Materials). The avian reovirus group included an additional cost of 0.0102 USD·$chick^{-1}$. Chick income was obtained in terms of live body weight (kg) by actual price per kg (1.60 USD), as a function of livability. Profiles were calculated as the difference between chicken income and cost [22].

2.3. Sample Collection and Processing

Figure 1 shows the methodology flow chart. At 0, 7, 14, 21, 28, and 35 days of age, 10 chickens from each house were blended (n = 120) from each group. The blood serum

was placed in refrigeration (2 °C). At 14 days of age, one chicken from each chicken house was randomly selected (n = 12) from each group, euthanized by cervical dislocation, and necropsied. Samples of each chicken were taken from the middle parts of the proventriculus (PV), pancreas (PA), proximal duodenal branch (PD), and distal duodenal branch (DD), as well as 3 cm caudally to Meckel's diverticulum in the distal jejunum (DJ) (Figure 2a). The samples were fixed immediately by immersion in 10% neutral buffered formalin. Tissues were then processed and embedded in paraffin using routine histological techniques.

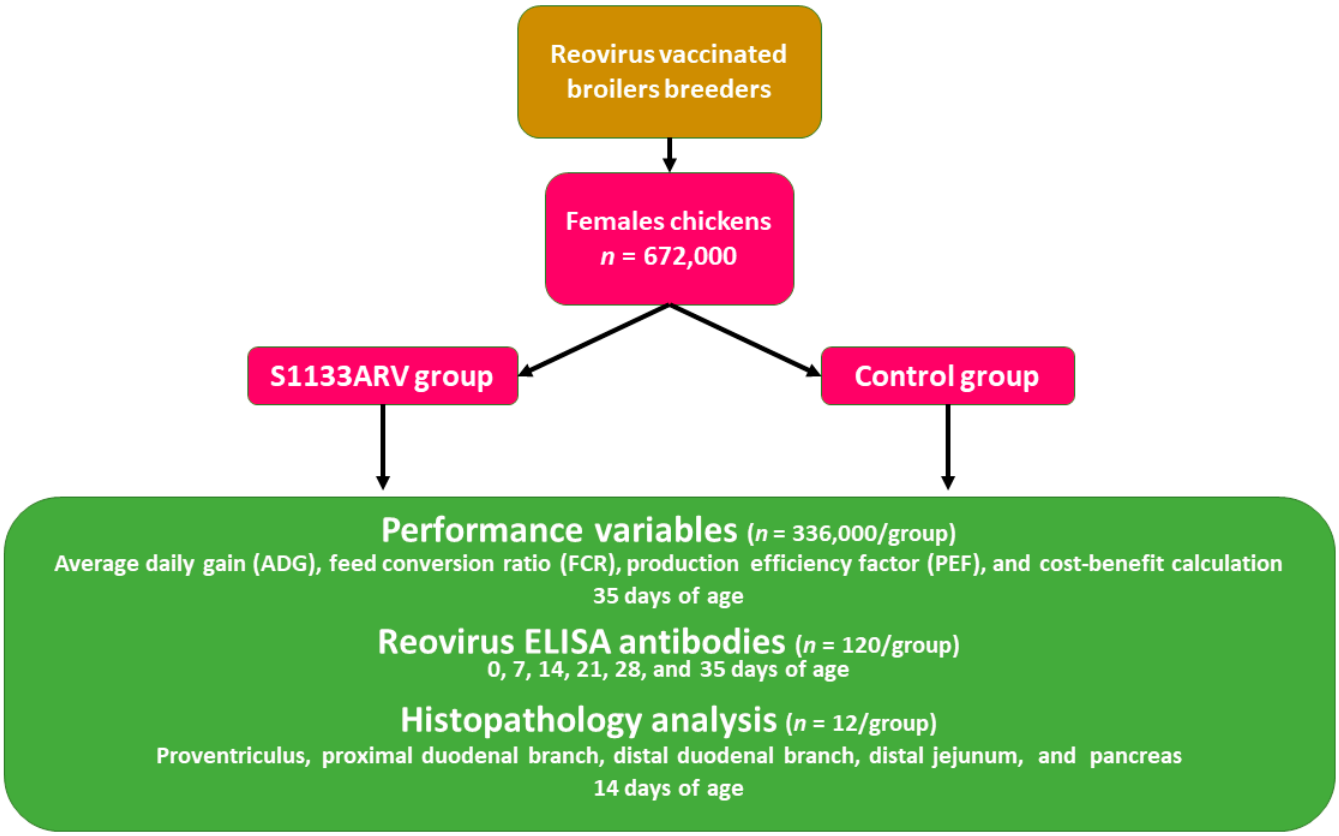

Figure 1. Twenty-four chicken houses with a capacity of 28,000 female broiler chickens were selected from a commercial company (n = 672,000 total chickens). Twelve houses were randomly selected, and chickens were vaccinated at 1-day-old using a spray cabinet with the avian reovirus S1133 strain, whereas the other 12 houses served as the nonvaccinated control group. The variables evaluated were productive performance, reovirus ELISA antibodies, and histopathology analysis.

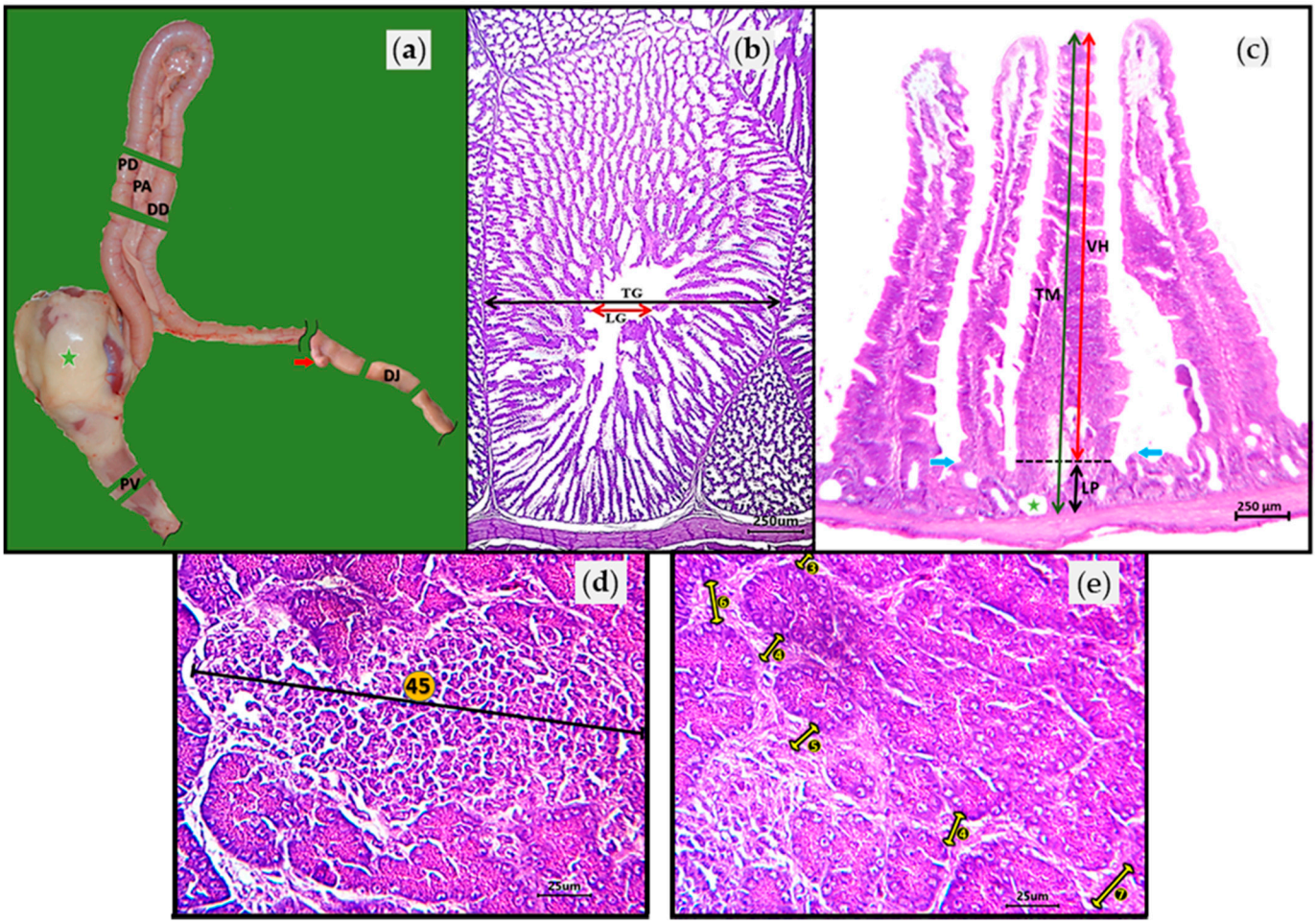

Figure 2. (**a**) Sites where histopathological samples were taken from each chicken from the middle parts of the proventriculus (PV), pancreas (PA), proximal duodenal branch (PD), and distal duodenal branch (DD), as well as 3 cm caudally to Meckel's diverticulum (red arrow) in the distal jejunum (DJ). Green star = ventricle (gizzard); (**b**) PV consisted of measuring the transverse diameter of the total (TG and black arrow) and luminal (LG and red arrow) diameter of the proventricular gland; (**c**) Intestinal evaluation involved measuring the total thickness (TM, green arrow) and lamina propria (LP, black arrow) of the mucosa, as well as the villous height (VH, red arrow). The segmented line indicates the boundary between LP and VH. The blue arrows indicate the upper pole of the enteric glands that comprise the lintel of the LP. The green star indicates an enteric gland cyst; (**d**) Pancreas histopathologic analysis showing the lymphocyte cluster. The largest cross-diameter (black line) of the lymphocyte clusters was used to quantify the number of lymphocyte cell layers (number in orange circle); (**e**) Pancreas parenchyma. The acinar fibrosis/atrophy score was based on the number of fibroblast layers (number in black circle) embedded in bands separating the pancreas acini.

2.4. ELISA for Assessment of Reovirus Antibodies

The obtained serum samples were analyzed for antibodies against reovirus using commercially available enzyme-linked immunosorbent assay tests (Reo ELISA CK100®, BioCheck® UK LTD, Ascot, UK). The ELISA commercial test is widely used for assessing reovirus antibody levels on a flock basis. The test is efficient for the detection of antibodies to avian reovirus in *Gallus gallus*. The ELISA method was developed using whole virus antigen, as well as recombinant σC and σB; thus, the test disallows the differentiation of infected from vaccinated chicks [23].

2.5. Histopathology

Paraffin-embedded tissues were sectioned, mounted, and stained using hematoxylin and eosin (H&E) and examined for lesions; tissues were evaluated by photon microscopy

using the AmScope® 3.7 (Irvine, CA, USA) image analysis program. Each tissue was also assigned a lesion severity score. The proventriculus evaluation consisted of measuring the transverse diameter of the total diameter (TG) and the luminal diameter (LG) of the proventricular gland (Figure 2b). Lymphoid nodules contained in the glandular zone of the proventricular mucosa were also counted. The intestinal evaluation considered the PD, DD, and DJ. From the three intestinal zones, the total thickness (TM) and the lamina propria (LP) of the mucosa were measured, in addition to the villous height (VH) (Figure 2c). Cysts and lymphoid clusters contained in the glandular zone of the intestinal mucosa were also counted. Pancreas analysis consisted of obtaining the percentage tissue degeneration and counting the necrotic foci and lymphoid clusters; the fibrosis/acinar atrophy score was also calculated. The lymphoid infiltrate was evaluated using a digital microscope camera with a field of view (FOV) of 3.4 mm^2 using a 5× objective lens. The largest cross-diameter of the lymphocyte clusters was used to quantify the number of lymphocyte cell layers (Figure 2d). The acinar fibrosis/atrophy score was based on the number of fibroblast layers embedded in bands separating the PA acini (Table 1). The acinar fibrosis/atrophy score was evaluated with a FOV of 0.87 mm^2 and a 10× objective lens (Figure 2e). The acinar fibrosis/atrophy was obtained from the total of 60 scores calculated (five FOVs for 12 tissue cuts). The number of layers was multiplied by the number of clusters, and the average was obtained from the total of 60 scores calculated (five FOVs for 12 tissue cuts).

Table 1. Pancreas acinar fibrosis/atrophy score of the broilers vaccinated with the avian reovirus S1133 strain.

	Percentage (%) of Fibrous Tissue Bands Separating the Pancreatic Acini According to the Number of Fibroblast Layers *		
Score	**1 to 2 Layers**	**3 to 5 Layers**	**More than 5 Layers**
0	0		
0.5	1–5		
1	6–15		
1.5	16–20	1–5	
2	21–35	6–15	
3	36–50	16–20	1–5
4	51–70	21–35	6–15
5	71–85	36–50	16–20
6	86–100	51–70	21–35
7		71–85	36–50
8		86–100	51–70
9			71–85
10			86–100

* The largest number of fibroblast layers observed in the digital microscope camera's field of view in a single column.

2.6. Data and Statistical Analysis

The fibrosis/atrophy PA score was analyzed using the Mann–Whitney U test. The remaining data confirmed normal distribution (Shapiro–Wilk test) and homoscedasticity (Levene test). Consequently, the data were subjected to a parametric test (one-tailed Student's *t*-test). Prior to statistical analysis, the percentage mortality, L/TG, LP/V, and degeneration PA were subjected to an arcsine square root transformation. The statistical significance was set at $p < 0.05$.

3. Results

3.1. Performance Variables

The results of the performance variables of the female broiler chickens vaccinated with the avian reovirus S1133 strain are summarized in Table 2. Significant reductions ($p < 0.05$) in average daily gain and production efficiency factor, as well as an increase in FCR, were observed in chickens that received the avian reovirus vaccine when compared with nonvaccinated control chickens (Table 2).

Table 2. Performance variables of female broiler chickens vaccinated with the avian reovirus S1133 strain.

Broiler Groups	ADG (g·day^{-1})	FCR	LI (%)	PEF
S1133ARV group	43.46 ± 0.53 ^{b}	1.641 ± 0.009 ^{a}	95.16 ± 0.35	253.64 ± 3.17 ^{b}
Control group	44.82 ± 0.46 ^{a}	1.592 ± 0.015 ^{b}	94.60 ± 0.54	266.74 ± 4.68 ^{a}
p-Value	$p = 0.029$	$p = 0.018$	$p = 0.209$	$p = 0.010$

ADG = average daily gain, FCR = feed conversion ratio, LI = livability, PEF = production efficiency factor. Data are expressed as the mean ± standard error. ^{a,b} Different superscript letters within columns indicate a significant difference at $p < 0.05$.

Cost–Benefit Calculation

The two groups presented the same cost of production ($p = 0.4789$). However, the S1133ARV group presented lower income and profits ($p = 0.0229$ and $p = 0.0335$) than the control group (Table 3).

Table 3. Cost–benefit analysis of using the S1133ARV vaccine strain in female broilers.

Broiler Groups	Income (1.60 USD·kg^{-1})	Cost	Profit
S1133ARV group	2.516 ± 0.108 ^{b}	2.076 ± 0.051	0.440 ± 0.116 ^{b}
Control group	2.581 ± 0.073 ^{a}	2.075 ± 0.045	0.506 ± 0.062 ^{a}
p-Value	$p = 0.0229$	$p = 0.4789$	$p = 0.0335$

The amounts are indicated in USD. Data are expressed as the mean ± standard deviation. ^{a,b} Different superscript letters within columns indicate a significant difference at $p < 0.05$.

3.2. Histopathology

Table 4 shows histological measurements of the proventricular gland from broilers vaccinated with the S1133 reovirus strain. Both groups had the same TG of the PV ($p = 0.724$), whereas the LG of the proventricular glands of the S1133ARV-vaccinated broilers was higher than that of the control broilers ($p = 0.017$).

Table 4. Histological measurements of the proventricular gland from broiler chickens vaccinated with the S1133 reovirus strain.

Broiler Groups	TG (mm)	LG (mm)
S1133ARV group	1.442 ± 0.285	0.424 ± 0.193 ^{a}
Control group	1.144 ± 0.254	0.240 ± 0.114 ^{b}
p-Value	$p = 0.724$	$p = 0.017$

TG = transverse diameter of the gland, LG = transverse diameter of the lumen of the gland. Data are expressed as the mean ± standard deviation. ^{a,b} Different superscript letters within columns indicate a significant difference at $p < 0.05$.

The histological measurements of the enteric mucosa from female broilers vaccinated with the reovirus strain S1133 are summarized in Table 5. The VH values from the PD of the control broilers were higher than those of the S1133ARV-vaccinated broilers ($p = 0.00005$). However, both groups had the same VH and TM of the DD and DJ ($p = 0.075$ and $p = 0.066$). The LP of the PD and the DD of the S1133ARV-vaccinated broilers were higher ($p = 0.00005$ and $p = 0.015$) than those of the control broilers. However, both groups had the same LP of the DJ ($p = 0.365$). Both groups had the same TM of the PD ($p = 0.242$), the DD ($p = 0.189$), and the DJ ($p = 0.123$).

Table 5. Histological measurements of the enteric mucosa from broiler chickens vaccinated with the avian reovirus S1133 strain.

Duodenal Areas	VH (mm)	LP (mm)	TM (mm)
Proximal duodenum			
S1133ARV group	1.076 ± 0.257 [b]	0.3235 ± 0.138 [a]	1.400 ± 0.274
Control group	1.269 ± 0.256 [a]	0.1845 ± 0.057 [b]	1.453 ± 0.250
p-Value	$p = 0.005$	$p = 0.00005$	$p = 0.242$
Distal duodenum			
S1133ARV group	0.930 ± 0.223	0.2845 ± 0.072 [a]	1.214 ± 0.247
Control group	1.072 ± 0.357	0.2304 ± 0.086 [b]	1.302 ± 0.354
p-Value	$p = 0.075$	$p = 0.015$	$p = 0.189$
Distal jejunum			
S1133ARV group	0.421 ± 0.203	0.1388 ± 0.075	0.5600 ± 0.247
Control group	0.310 ± 0.147	0.1448 ± 0.043	0.4928 ± 0.144
p-Value	$p = 0.066$	$p = 0.365$	$p = 0.123$

VH = villous height; LP = lamina propria thickness, TM = total thickness of the mucosa. Data are expressed as the mean ± standard deviation. [a,b] Different superscript letters within columns indicate a significant difference at $p < 0.05$.

Table 6 shows the results of the pancreatic histological evaluation of broiler chickens vaccinated with the avian reovirus S1133 strain. No significant changes were observed in terms of degeneration, necrosis clusters, and lymphoid clusters between both groups ($p = 0.171$, $p = 0.612$, and $p = 0.060$). However, the fibrosis scores of the S1133ARV-vaccinated broilers were higher ($p = 0.022$) than those of the control broilers.

Table 6. Pancreatic histological evaluation of broiler chickens vaccinated with the avian reovirus S1133 strain.

Broiler Groups	Degeneration (%)	Necrosis Clusters	Lymphoid Clusters	Fibrosis Score
S1133ARV group	8.18 ± 7.32	0.60 ± 0.89	4.65 ± 5.92	7.82 ± 6.56 [a]
Control group	9.30 ± 3.20	0.40 ± 0.54	5.95 ± 3.78	2.50 ± 1.98 [b]
p-Value	$p = 0.171$	$p = 0.612$	$p = 0.060$	$p = 0.022$

Data are expressed as the mean ± standard deviation. [a,b] Different superscript letters within columns indicate a significant difference at $p < 0.05$.

3.3. Antibody Titers

The results of the antibody titers from broiler chickens vaccinated with the avian reovirus S1133 strain are shown in Figure 3. Chickens in both groups revealed a high maternal antibody titer against the avian reovirus S1133 strain, which is consistent with the vigorous vaccination program of broiler breeders against reoviruses. In both groups, maternal antibody titers showed a progressive reduction on days 7, 14, and 21 of evaluation. Interestingly, on days 28 and 35, the antibody titers in both groups were increased. However, no differences ($p > 0.05$) in antibody titers were found between the two groups across all weeks of evaluation (Figure 3).

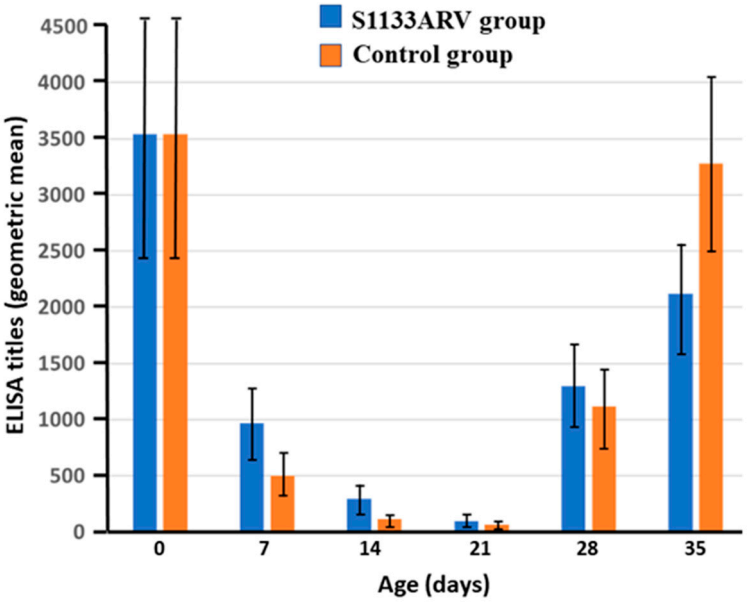

Figure 3. Antibody titers from broilers vaccinated with the avian reovirus S1133 strain ($p > 0.05$).

4. Discussion

The results of the present study indicate that vaccination of neonatal broiler chickens with the avian reovirus S1133 strain has a negative economic and productive impact since replication of the virus induces pathological alterations in the gastrointestinal tract. The use of the vaccine was not justified in the cost–benefit analysis.

The reduction in performance was associated with histopathologic and morphometric changes in the proventricular gland, duodenum, and pancreas of female broiler chickens that were commercially processed at 38 days of life. These findings agree with previous researchers who reported that avian reovirus isolated from intestinal contents of broiler chickens with malabsorption syndrome produced a transient but significant depression in body weight gain when inoculated orally into 1-day-old chicks [24]. In addition, Jones and Georgiou found that resistance to reovirus is age-related because, although reoviruses can infect older birds, the resulting disease is generally less severe, and the incubation period is longer [6].

The decline in the performance of vaccinated broilers can be explained by histological findings. The luminal diameter of the proventricular gland was significantly larger in the vaccinated group. This increase in lumen was due to a reduction in glandular tissue, which caused the ratio of the lumen over the total glandular diameter radius to increase. As is known, glandular tissues produce hydrochloric acid and pepsinogen, which are essential for the digestion of proteins [25].

In the duodenal villi of the vaccinated group, there was a decrease in villus height and an increase in lamina propria such that the ratio of the lamina propria thickness over the total thickness of the mucosa radius increased. Apoptosis is the process via which reoviruses cause epithelial atrophy of the proventricular glands and the epithelium at the tip of the villi [26,27]. This phenomenon may explain the absence of an evident inflammatory process in the tissues observed in this work [28]. These duodenal changes induce a decrease in nutrients, especially in proteins. The highest absorption of proteins in the duodenum occurs in its proximal part, which is the most strongly affected by the vaccine. While the effect of the vaccine virus on enteric villi was lost in the distal jejunum, the absorption of nutrients was lower [25,29].

The pancreas of vaccinated broilers exhibited moderate fibrosis, but this fibrosis was higher in vaccinated chickens compared with nonvaccinated control chickens. An increase in the amount and density of the interstitial connective tissues with compression atrophy of

the acini characterizes the chronic phases of inflammation in the pancreas [30–32]. Typically, bands of mature fibrous tissue separate small lobules of acinar tissues [33]. Pancreatic fibrosis is attributed to selenium deficiency in stunted broilers. One of the limitations of the present study is that the concentration of selenium was not measured. However, a reduction in nutrient absorption is associated with selenium deficiency [34]. It is suggested that the normally low activities of selenium-dependent glutathione peroxidase (SeGSHpx) in the pancreas may predispose that organ to atrophy due to oxidative stress under conditions of nutritional selenium deficiency, resulting in further depletion of SeGSHpx [25]. Pancreatic atrophy seems to be dependent on selenium concentration. In the present study, the pancreatic atrophy was moderate, whereas, in the works of Whitacre et al. [35] and Xu et al. [31], the deficiency and atrophy were severe.

The results of the ELISA revealed a high maternal antibody titer, which is consistent with the vigorous vaccination program of broiler breeders against reoviruses. However, the maternal antibodies did not prevent infection of the live modified avian reovirus S1133 strain in the gastroenteric epithelium. Hence, the replication of the S1133 strain caused damage to the proventricular glands and enterocytes. In the present study, maternal antibodies decreased at 3 weeks of age in both groups. Interestingly, an increase in antibody titers was observed in both groups, presumably, due to a wild reovirus challenge that was not controlled by vaccination, as previously reported by Zhong et al. [13]. Reoviruses can be isolated from healthy birds, and serum antibodies are often found in both affected and healthy birds [3]. Another limitation of the present field trial study is the lack of isolation and characterization of the wild strain(s) responsible for inducing an immune response in nonvaccinated control chickens. However, in the present study, performance parameters of nonvaccinated control chickens were not affected, even though they showed antibody titers against avian reovirus. The ELISA commercial kit used to evaluate antibody titers (Reo ELISA CK100®, BioCheck®) allows for the detection of serum antibodies against all serotypes of avian reovirus in both vaccinated and nonvaccinated flocks; hence, it can be used for screening for field infections, as well as for monitoring vaccination success in poultry. In summary, while the large sample size and per-house randomization schema provide conclusive data regarding the effect of the reovirus S1133 challenge in the present study, limitations to the generalizability of these results compared with other commercial facilities receive short shrift. Further studies to evaluate the use of the reovirus S1133 strain in neonate commercial chickens under different breeder vaccination stratagems that may affect maternal antibody levels or under different background prevalence of ARV infection should be investigated.

5. Conclusions

Strong broiler breeder vaccination programs with the avian reovirus S1133 strain are designed to prevent viral arthritis in breeders and the progeny through passive immunity. Since the virus replicates in the gastrointestinal tissue, regardless of the maternal antibodies, the results of the present study suggest that neonatal vaccination in broiler chickens with the live avian reovirus S1133 strain should be avoided, as it leads to a disruption of gastrointestinal integrity and a decrease in performance. Hence, the cost–benefit analysis demonstrated that the use of this vaccine has a negative impact on company profits.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/vaccines9080817/s1: Table S1. Ingredient composition (kg) and nutrient content of the phases of feed supplied to the experimental female broiler chickens. Table S2. Feeding phase costs and cost breakdown of experimental female broilers.

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Institutional Review Board Statement: This study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Internal Committee for Care and Use of Experimental Animals (CICUAE) of the National Autonomous University of Mexico (UNAM); ethical approval code: CICUAE-FESC C20_06.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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