RESEARCH

Serological study of agents associated to chronic respiratory syndrome in laying hens

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ABSTRACT

In order to evaluate the serological response to agents associated to chronic respiratory syndrome in poultry, 120 White Leghorn replacement layers, having received all the vaccines of the immunization program currently applied in Cuba, were selected and sampled monthly from the 12th to the 50th week of age. The samples were assayed for antibodies against *Mycoplasma gallisepticum* (by fast serum plate agglutination), *Ornithobacterium rhinotracheale* and Avian Infectious Bronchitis Virus (by ELISA), and Newcastle Disease Virus (by hemagglutination inhibition assay, HIA); comparing the proportion of birds positive to *Mycoplasma gallisepticum*, *Ornithobacterium rhinotracheale*, or with HIA titers higher than 1/8, and performing an analysis of variance for the geometric means of the antibody titers against the Avian Infectious Bronchitis Virus at $p < 0.05$ for statistical significance, as implemented in the Comprop-1 and Statgraphics Plus 5.1 statistical software packages. The results corroborated the presence of *M. gallisepticum* and provided the first evidence of positive reactions to *O. rhinotracheale* in laying hens with chronic respiratory syndrome. The serological kinetics of the bird population vaccinated against avian infectious bronchitis evidenced a second seroconversion event, probably due to the circulation of this infectious agent. No serological responses against Newcastle Disease Virus were detected. Further studies for the isolation and characterization of different *O. rhinotracheale* serovars from laying hens with chronic respiratory syndrome are required.

Keywords: antibody kinetics, *Mycoplasma gallisepticum*, *Ornithobacterium rhinotracheale*, Newcastle disease, avian infectious bronchitis, chronic respiratory syndrome

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RESUMEN

Estudio serológico de agentes asociados con el síndrome respiratorio crónico en gallinas ponedoras. Para evaluar la respuesta serológica de agentes asociados al síndrome respiratorio crónico de las aves, se seleccionaron 120 pollonas de reemplazo de ponedoras, de la raza White Leghorn, que habían recibido todas las vacunas de acuerdo con el programa de inmunización vigente en Cuba. Desde las 12 hasta las 50 semanas de edad, mensualmente se muestrearon estas aves. Las muestras se evaluaron para la detección de anticuerpos contra *Mycoplasma gallisepticum* (mediante seroaglutinación rápida en placa) y *Ornithobacterium rhinotracheale*, contra el virus de la bronquitis infecciosa aviar (mediante ELISA) y el virus de la enfermedad de Newcastle (mediante la inhibición de la hemoaglutinación IHA). Se compararon proporciones de aves positivas a *Mycoplasma gallisepticum*, a *Ornithobacterium rhinotracheale*, y aves reactoras con títulos de la IHA superiores a 1/8, y se hizo un análisis de varianza simple para las medias geométricas de los títulos de anticuerpos contra bronquitis infecciosa aviar. Los niveles de significación en ambos análisis fueron de $p < 0.05$, apoyados en los paquetes estadísticos Comprop-1 y Statgraphics Plus 5.1. Los resultados corroboraron la presencia de *Mycoplasma gallisepticum*, observándose reacciones positivas a *O. rhinotracheale* por primera vez en gallinas ponedoras afectadas por el síndrome respiratorio crónico. La cinética serológica en la población de aves vacunadas contra la bronquitis infecciosa aviar mostró una segunda seroconversión, posiblemente relacionada con la circulación del agente. No se evidenció respuesta serológica a la infección con la enfermedad de Newcastle. Se debe continuar el estudio de aislamiento y caracterización de los diferentes serovares de *O. rhinotracheale* en gallinas ponedoras afectadas por el síndrome respiratorio crónico.

Palabras clave: cinética de anticuerpos, *Mycoplasma gallisepticum*, *Ornithobacterium rhinotracheale*, enfermedad de Newcastle, bronquitis infecciosa aviar, síndrome respiratorio crónico

*I*ntroduction

Chronic respiratory syndrome (CRS) is an infectious disease complex of chicken with a large economic impact on the poultry farming industry worldwide [1, 2]. Recent work has shown that CRS alone is responsible for 20% of the incidence of infectious disease among laying hens in Cuba [3]; additionally, CRS ranks second among the causes of death in this group, according to the epidemiological data provided by the Enterprise Group of the National Poultry Industry Conglomerate (UECAN, from its Spanish acronym) [4]. The symptoms and severity of this affection is influenced by a number of factors, ranging from environmental aspects and farming management practices to the specific nature of the causative bacterial and/or viral agents [5].

Several bacterial species, such as *Avibacterium paragallinarum*, *Pasteurella multocida*, *Mannheimia haemolytica*, *Ornithobacterium rhinotracheale*, *Staphylococcus spp.* and *Escherichia coli* have been involved in the pathogenesis of CRS, although *Myco-*

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1. Rosado I, Ruedas D, Sánchez L, Acosta I, Véliz T, Lobo E, *et al*. Evaluación de la eficacia en condiciones controladas de una bacteria oleosa contra *Mycoplasma gallisepticum*, obtenida en fermentadores de 7L. Rev Cubana Cienc Vet 2003; 28(1):43-5.

plasma gallisepticum has been singled out as its most important etiological agent [6, 7]. However, recent reports have also demonstrated the participation of highly pathogenic strains of *Mycoplasma synoviae* that cause typical CRS lesions [8].

In addition, there are viral entities associated with CRS, including Newcastle Disease Virus (NDV), the viruses for avian influenza, avian Infectious Bronchitis Virus (IBV), avian metapneumovirus (aMPV) and avian Infectious Laryngotracheitis Virus (ILTV) which, when combined with a bacterial agent, result in a more severe clinical condition [9].

The incidence of pneumotropic viruses is often controlled through the implementation of biosafety management practices and the application of live or inactivated vaccines that elicit a specific immune response [10-16]. Cuba has implemented immunization programs against IBV and NDV, administering live or inactivated vaccines to the breeding or laying hen stocks, respectively [17]. Also, there are commercially available inactivated vaccines for the control of pathogenic mycoplasma species that have been tested under natural and experimental conditions [18, 19], and the poultry farming industry has procured vaccine preparations for the control of the main bacterial serovars associated to CRS, such as *P. multocida* [20], *A. paragallinarum* [21] and *O. rhinotracheale* [22]. However, the control of CRS-associated bacterial agents is currently implemented in Cuba through the application of biosafety management practices, which have the added advantage of being applicable not only to endemic, but to exotic infectious diseases as well [23].

A number of diverse methodologies are currently employed for the diagnosis of the main viral and bacterial agents associated to CRS, ranging from the conceptually simple methods such as the isolation of the microorganism itself, to the technically complex methods such as molecular assays. However, serological monitoring is still the method of choice, based on assays such as fast serum plate agglutination (fSPA), the hemagglutination inhibition assay (HIA) and ELISA [24, 25].

Cuba, where the poultry stock undergo extensive immunization following the established mass-scale vaccination programs and where biosafety procedures and practices are enforced, still experiences outbreaks of respiratory disease with high morbidity and low mortality in laying hens. These outbreaks not only affect their genetic, productive and breeding potential, but result in significant economic losses due to decreases in egg and meat production and increases in medication expenses. This paper is therefore aimed at the evaluation of serological response to microbiological agents commonly associated with chronic respiratory syndrome in laying hens.

Materials and methods

Specimen selection

A total of 120 White Leghorn replacement layer pullets, of 12 weeks of age, were selected for this study. They were obtained from a Poultry Production Unit of the province of Havana, and identified by wing bands. After reaching 16 weeks, they were transferred to a Commercial Layers Unit, under a productive system of mixed ages, with a history of outbreaks of respiratory processes. They received a well balanced diet, as indicated by trained technicians or following technical regulations for rearing used in Cuba.

Immunization schedule

The birds were vaccinated according to the Cuban immunization program (Technical Instruction, 2007) (Table). In addition, a fourth dose of the NDV vaccine was applied, due to the low proportion of responding individuals (according to the HIA titers) after the third dose.

Sampling

Blood samples were taken monthly from week 12 and up to week 50, by puncturing the marginal vein of the wing. The samples were drawn into sterile tubes without anti-coagulant and remained at room temperature for clotting, after which they were left overnight at 4 °C. The following day, the serum was obtained by centrifugation of the samples at 3000 rpm for 20 min, and split into 2 fractions of 500 µL that were stored at -20 °C until further evaluation, according to the methodology described by the Food and Drug Administration (FDA) of the United States (2004) [26].

Serology

The serological study for the indirect demonstration of the presence of birds reactive to *M. gallisepticum* and *O. rhinotracheale* was extended until week 38. In the case of NDV and aIBV, it was extended instead until week 50.

Fast serum plate agglutination (fSPA)

The technique used the colored *M. gallisepticum* antigen, available from Intervet Laboratories, and followed the instructions of the manufacturer. Two-hundred microliters of the testing serum were mixed with an identical volume of the specific antigen on a glass plate, which was rotated for 2-3 minutes. The reaction was scored as positive if it yielded visible, defined clumps within that time [23].

Hemagglutination Inhibition Assay (HIA)

The measurement of antibodies against NDV was performed on U-bottomed microplates with the beta method, using a volume of 50 µL for each reagent. The sera underwent serial two-fold dilutions in PBS at pH 7.2-7.4, to which 4 hemagglutination units (HAU) were added, followed by incubation at room temperature (RT) for 30 minutes. Afterwards, 1% chicken erythrocytes were added and the plates were further

2. Burch D. Revisión de la actividad del tiamulin contra el Mycoplasma spp. y su uso en la prevención de la transmisión vertical en reproductoras ponedoras. En: Memoria del XXI Congreso Latinoamericano de Avicultura, 6-10 oct.; Ciudad de La Habana, Cuba; 2009, p. 394-8.

3. Colas M, Santana Y, Merino A, Mojena K, Correa A. Frecuencia de presentación de las principales enfermedades en ponedoras White Leghorn. Rev Cubana Cienc Avíc 2006;30(2):103-6.

4. Informe de balance del Instituto de Investigaciones Avícolas; 2007. Comportamiento de las enfermedades infectocontagiosas en el período 2000-2007, p. 2.

5. Guadalupe A. Implementación del diagnostico de Mycoplasma gallisepticum y Mycoplasma synoviae mediante PCR-RFLP en aves de producción. Tesis en opción al grado de Máster en Ciencias Veterinarias con especialidad en Salud Animal. Universidad Autónoma de Nuevo León; 2005.

6. Kleven SH. Micoplasmosis. En: Primer seminario de actualización en patología aviar. Merial-Cerval, Georgia, Estados Unidos; 2000.

7. Ricci, M. Prevención y control de los miscoplasmas aviares. Avicultura Profesional 2007;25(2):16-8.

8. Cerdá R. Interpretación de los resultados del diagnóstico de micoplasmas aviares. En: Memoria del XXI Congreso Latinoamericano de Avicultura; 6-10 oct.; Ciudad de La Habana, Cuba; 2009. p. 353-7.

9. Icochea E. Diagnóstico diferencial de enfermedades respiratorias en aves bajo condiciones de campo y de laboratorio. En: Memoria del XXI Congreso Latinoamericano de Avicultura; 6-10 oct.; Ciudad de La Habana, Cuba; 2009. p. 413-20.

10. Dave C. Severe acute respiratory syndrome vaccine development: experiences of vaccination against avian infectious bronchitis coronavirus. Avian Pathol 2003; 32(6): 567-82.

11. Osman E. Evaluation of three different vaccination regimes against Newcastle disease in central Anatolia Turk. J Vet Anim Sci 2003; 27:1065-9.

12. Hidalgo H. Influenza aviar: experiencia en el control y erradicación. Avicult Profes 2006;24(1):10-2.

13. Lozano B, Castro F, Suárez A, Gual F, Safati D, Soto E. Presente y futuro de las vacunas aviares de nueva tecnología. Avicult Profes 2006;24(3):14-6.

14. Kannan G. Avian Metpneumovirus: Diagnosis and Prevention. World Poult 2007;23(5):35-7.

Table. Schedule for immunization against avian infectious bronchitis and Newcastle disease in layers and their replacements, using live vaccines manufactured by Labiofam

Type of bird	Age at vaccination (days)	
	Avian Infectious Bronchitis (strain H120)	Newcastle disease (strain La Sota)
Starter Layers	1	7
Replacement hens	35 85	49 90
Laying hens	-	228*

*The fourth dose is not part of the normal vaccination program.

incubated at RT for another 30 minutes. All plates contained control wells of 4 HAU and erythrocytes.

ELISA for the detection of antibodies against aIBV and *O. rhinotracheale*

The assays for the detection of antibodies against aIBV and *O. rhinotracheale* employed commercial kits purchased from Flock Chek, handled according to the instruction of the manufacturer. The 96-well plates were coated with the antigen, followed by the addition of the test serum samples diluted 1/500 as well as positive and negative control sera. This step was followed by the inclusion of an anti-chicken IgG peroxidase conjugate, and the entire reaction was developed for 15 minutes at RT with tetramethylbenzidine as the substrate; the results were read after the addition of a stopping solution on a SUMA PR-521 plate reader at 650 nm. The volume employed for all reactants was 100 µL, each step took 30 minutes, and 5 washes with distilled water were performed between each step. The results were interpreted as instructed by the manufacturer, considering a sample as positive for antibodies against aIBV if the serum titer was higher than 396, and positive to *O. rhinotracheale* if the positive sample coefficient (PSC) was equal to or higher than 0.4, as determined from the absorbance values read from the plate and the formula:

$$CMP = \frac{\text{Absorbance of sample X} - \text{Absorbance of negative control X}}{\text{Absorbance of positive control X} - \text{Absorbance of negative control X}}$$

Statistical analysis

The proportion of individuals positive to *M. gallisepticum* and *O. rhinotracheale* were compared, as well as the proportion of reactive birds with HIA titers higher than 1/8 against NDV. An analysis of variance (Anova) was performed for the geometric means of the titer of anti-aIBV antibodies, using the statistical software packages Comprop-1 and Statgraphics Plus 5.1.

Results and discussion

The examination of the incidence of respiratory processes along the productive chain that goes from replacement layer pullets to laying hens in Cuban poultry farms reveals that these processes are more frequent in the latter; that is, after the 16th week of age [27]. Serological studies by different researchers have revealed the presence of *M. gallisepticum*, as well as pneumotropic aIBV and NDV in samples from birds having complicated respiratory illnesses [28-34].

The present study has detected birds reactive to *M. gallisepticum* and *O. rhinotracheale* (Figures 1 and 2), which suggests the presence of a co-infection with both microorganisms due to the absence of vaccination programs specifically addressing these pathogens.

When studying the proportion of birds reactive to *M. gallisepticum* (Figure 1) from the 16th to the 25th week of age, there is a significant increase of this parameter with time. Similar findings were reported by Rosado in 2001 [25] using ELISA, who demonstrated high levels of circulation of *M. gallisepticum* nationwide, in laying hens with CRS, which seems to have been an important factor in the egg production decrease reported for that year. However, for an infection with *M. gallisepticum* to result in the clinical-pathological process of CRS the presence of other factors are required -such as inappropriate or insufficient diet, inadequate transportation of the birds in the multiple lots, and poor management- which decrease the resistance of the flock and affect the birds at the most critical time of sexual maturity, when egg-laying productivity peaks [35-37]. Additionally, Valencia in 2007 [38] highlighted other factors that could have played a role in the increase in the proportion of *M. gallisepticum*-reactive birds, also revealed in this study, such as high concentrations of dust and ammonia in the sheds, which affect the upper respiratory system and cause ciliostasis and an increase in mucus secretion, the end result being that a larger number of bacteria manage to adhere to the respiratory epithelium and, therefore, a bacterial septicemic process (usually involving *E. coli*, the most prevalent species in the sheds) is started.

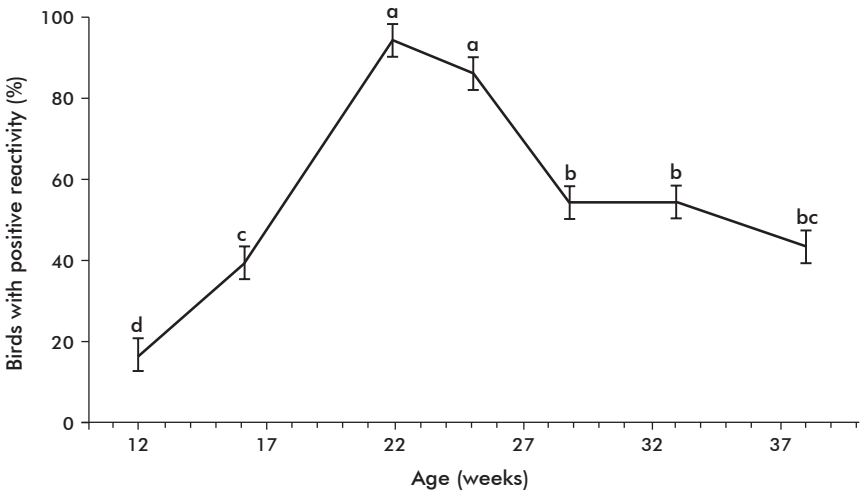

Figure 1. Proportion of reactive birds positive to *Mycoplasma gallisepticum*. A total of 120 samples were analyzed by fast serum plate agglutination, considering as positive the samples with visible clumps. The data are transformed into frequency +/- standard error 0.05. Different letters show statistically significant differences at $p < 0.05$. Proportion Comparison.

15. Alzamora L, Andina I. Prevalencia de la enfermedad de Newcastle en América Latina. Avicult Profes 2008;26(5): 10-3.

16. Aziz T. Laringotraqueitis infecciosa. World Poultry 2009;25(6):28-30.

17. Viamontes O. Impacto económico de una adecuada estrategia de inmunización contra la enfermedad de Newcastle. Memorias del V Congreso de avicultura; 2006. p. 474.

18. Whithear KG. Control de las micoplasmosis aviares mediante vacunación. Rev Sci Tech Off Int. Epiz 2002; 15(4):1527-54. [En línea.] Consultado: 16 de octubre de 2008. Disponible en: http://www.oie.int

19. Malo A. Vacunación efectiva en reproductoras. [En línea.] Consultado: 2 de abril de 2008. Disponible en: http://www.intervet.com.ve/binaries/84_82149.doc

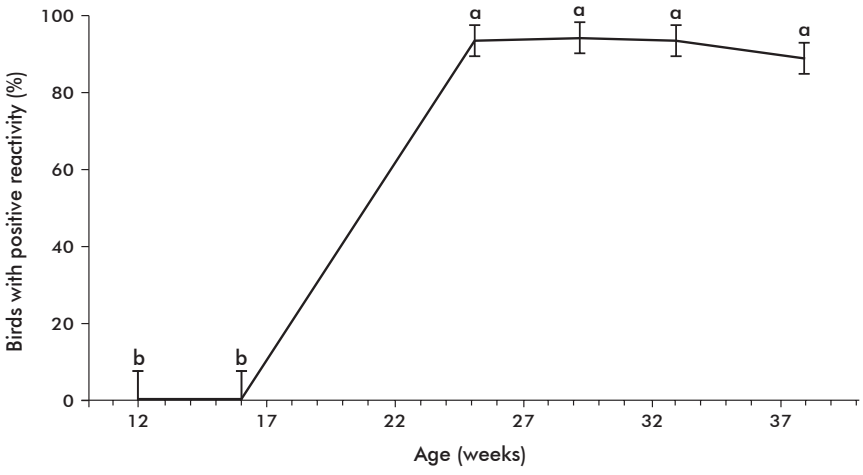

Figure 2. Proportion of reactive birds positive to *Ornithobacterium rhinotracheale*. A total of 120 samples were analyzed with a commercially available ELISA, considering as positive the samples with values larger than 0.4. The data are transformed into frequency +/- standard error 0.05. Different letters show statistically significant differences at $p < 0.05$. Proportion Comparison: 5.

Cerdá *et al.* [39] have pointed out that *O. rhinotracheale* is a Gram-negative pleomorphic bacterium, associated with respiratory diseases in turkey, broilers, breeders and laying hens. Its isolation and identification as a pathogenic agent in commercial poultry farming occurred at the beginning of the nineteen nineties by Vandamme *et al.* [40]. However, no studies have evidenced the presence of this microorganism in Cuban poultry farms.

This study evaluated the positivity for *O. rhinotracheale* among the serum samples using a commercial ELISA test (Figure 2). No positive results for this microorganism were detected during the two first sampling rounds at weeks 12 and 16. However, a significant, persistent increase in positivity for *O. rhinotracheale* was found from week 25 to 38, whichis very similar to that obtained by Cerdá *et al*. [39] during epidemiological studies of *O. rhinotracheale*-positive birds with an ELISA assay in countries with a highly developed poultry industry. Their results revealed that 95% and 96.6% of the laying hens examined from the United States and Germany, respectively, were positive for this pathogen between weeks 14 to 36 of age.

Asymptomatic positive birds kept in a laying hen flock may, however, suffer from a slightly increased mortality rate, a decrease in egg-laying productivity and the deterioration of shell quality [6, 41]. This is also suggested by our data, which revealed a high proportion of asymptomatic laying hens after the 25th week of age.

Several authors have reported that the association of *M. gallisepticum* with *O. rhinotracheale*, when combined with deficient management, can aggravate the presentation of CRS [9, 22, 40]. The large number of individuals reactive to these microorganisms could be related to the eventual appearance, at the 21st week of age, of clinical signs and lesions typical of an acute respiratory process affecting the upper airways, characterized by sero-catarrhal rhinitis, blepharitis, conjunctivitis, facial edema and genital hypoplasia. This process followed a chronic course, affecting the lower airways and resulting in facial tumefaction with a hard consistency leading to loss of vision, muco-fibrinous rhinitis, cachexia, serosal atrophy of subcutaneous fat and the coronary ridge, focal pneumonia of the anteroventral unilateral lobule and diarrhea. These results match those of other authors [42, 43] describing a similar clinical-pathological picture in birds with CRS.

Another important microorganism associated to CRS is the causative coronavirus of infectious bronchitis (IB), which has a special tropism for the respiratory, reproductive and renal tracts. It affects poultry at any age, but its expressions are more severe in young individuals under intensive production systems, causing problems for the adequat application of biosafety management procedures [38]. The control of BI in Cuban flocks in intensive production systems is implemented through the immunization of replacement layer pullets and the evaluation of the serological response of layers, which enables the examination of the level of protection or the detection of viral infections if antibody levels are monitored at different time points after vaccination [33, 34].

The analysis of the geometric means of the titers of antibodies against aIBV (Figure 3) revealed an increase in this parameter starting at week 16 and peaking at week 22. This result coincides with that of Cavanagh in 2003 [44], which suggest that several doses of the aIBV vaccine provide greater protection; and those of Acevedo in 2010 [45], showing that antibody titers can be maintained for a longer period, but start to decline 3 months after vaccination.

However, another significant increase in Ab titers against aIBV is detected at week 33 among otherwise asymptomatic individuals. This may start through an infection with another viral strain rather than from the persistence of the vaccine strain since, coincidentally, a similar complicated respiratory process appeared one week earlier among a different hen batch that had been laying for 9 to 10 months. Using clinical samples from these birds, which had high antibody titers against aIBV, Acevedo *et al*. in 2010 [45] isolated chick embryos and identified through reverse transcription-polymerase chain reaction (RT-PCR) an aIBV strain that may differ from the vaccine strain. Valencia, in 2007 [38], pointed out that different variants of aIBV continue to appear, and many of them are found circulating among otherwise healthy chickens.

Shane [46], on the other hand, suggests viral persistence as one of the factors contributing to the appearance of variant aIBV strains, since some recent studies prove that the dissemination of the virus from the trachea and cloaca continues for up to 70 days post-immunization. In addition, it is possible to isolate viable IB virus from lungs and kidneys of previously vaccinated birds for a period of up to 170 days, regardless of their isolation status.

In our study the proportion of reactive birds with HIA titers for NDV higher than 1/8 evidenced that this reactivity is not caused by an ongoing infection with this virus (Figure 4). The relevance of the post-vaccination response was shown by the fact that 100% of the positive, reactive individuals were protected after the fourth immunization. This confirms the efficacy of the vaccine, as underscored by Viamontes *et al*. in

20. Leotta GA, Vigo GB, Chinen I, Prieto M, Callejo R, Rivas M. Identificación, biotipificación y caracterización de cepas de Pasteurella multocida aisladas en la Argentina. Rev Argent Microbiol 2006; 38(3):125-9.

21. Terzolo HR. Revisión de coriza infecciosa. Propuesta de Investigación 2005. [En línea.] Consultado: 1 marzo 2008 Disponible en: www.produccion-animal.com.ar

22. Chung-Hsi CA, Shin-Ying LA, Chiou-Lin CB, Hsiang-Jung, T. Use of Random Amplified Polymorphic DNA Analysis and Single-Enzyme Amplified Fragment Length Polymorphism in Molecular Typing of Ornithobacterium rhinotracheale Strains. Avian Dis 2009;53:108-14.

23. Merino LA. Conferencias de bioseguridad. Curso de patología y epizootiología aviar. Folletos 2009. p. 1-30.

24. Crespo del Pilar M. El diagnóstico viral por el laboratorio. Rev Colombia Médica 2000;31:135-50.

25. Rosado I. Obtención y evaluación de un candidato vacunal contra la infección por Mycoplasma gallisepticum. Tesis en opción al grado científico de Doctor en Ciencias; Universidad Agraria de La Habana. Centro Nacional de Sanidad Agropecuaria; 2001.

26. FAO. Chapter 3. Vaccination. A Technology Review: Newcastle Disease. 2004. p. 21-7.

27. Boado E, Laurent R, Herrera C, Quintero D, Cánovas A. Prevalencia de las principales enfermedades en las diferentes categorías de aves durante las épocas del año. Rev Cubana Cienc Avíc 1991;18(3): 257-62.

28. Guilarte O, Viamontes O, Cánovas A, Acosta I, González R, Trujillo A, Núñez L. Identificación de los niveles de anticuerpos contra el virus de la bronquitis infecciosa y el virus de Newcastle en aves afectadas con la enfermedad respiratoria crónica. Rev Cubana Cienc Avíc 1985;12(1):15-22.

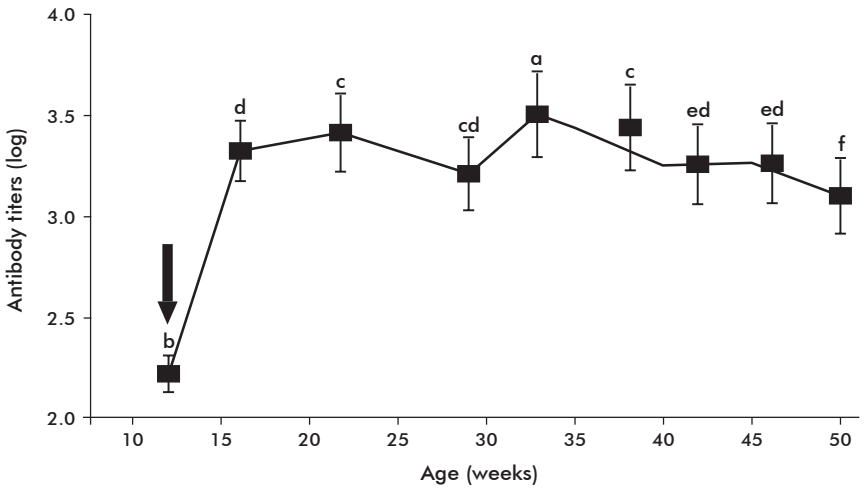

Figure 3. Logarithm of the geometric means of the antibody titers to the avian Infectious Bronchitis Virus. A total of 120 samples were analyzed with a commercially available ELISA. The arrow point at the time of application of the third dose of the vaccine. Different letters show statistically significant differences at $p < 0.05$ Anova. Statgraphic Plus.

1991 [47], when pointing out that the vaccine inhibits or reduces the excretion or multiplication of the virus, thereby decreasing mortality rate and improving productive parameters. In the light of this result, we concluded that no association of NDV with CRS could be detected in the population analyzed.

The present study confirmed the presence of *M. gallisepticum* and demonstrated, for the first time, the presence of reactive birds positive to *O. rhinotracheale* among layers with chronic respiratory syndrome. The serological study performed in birds vaccinated against aIBV revealed a pattern of seroconversion that suggests that this infectious agent is circulating among the sample population, a finding with potentially detrimental implications if not addressed promptly. The HIA titers measured when evaluating the birds for NDV do not suggest an ongoing infection, but rather demonstrate the efficacy of the vaccine. As a whole, these results help to better characterize the Cuban epidemiological situation and can be used to define the sanitary procedures required for the control of these diseases. We consider that further studies on the different serovars of *O. rhinotracheale* in particular, and on chronic respiratory syndrome in birds, in general, are warranted.

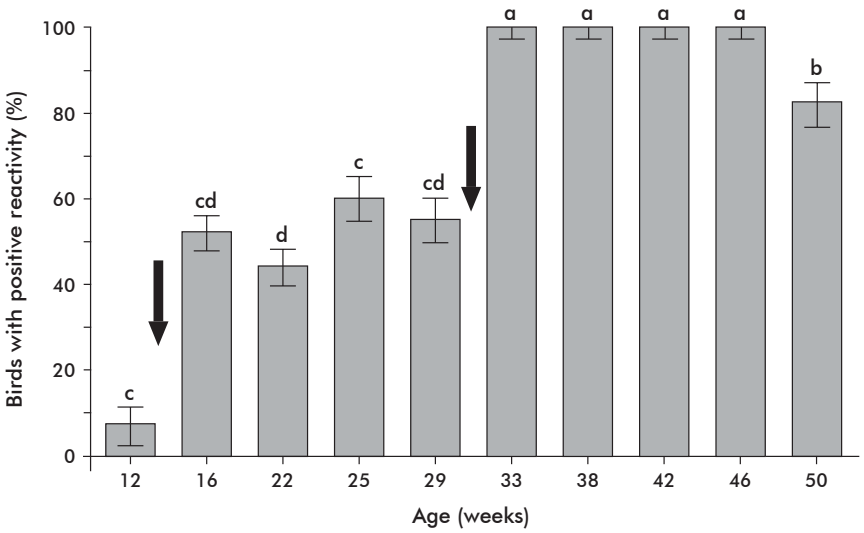

Figure 4. Proportion of reactive birds positive to Newcastle Disease Virus (NDV), i.e. with HIA titers larger than 1/8. A total of 120 samples were analyzed with a hemagglutination inhibition assay (HIA). The arrow point at the times of application of the third and fourth doses of the NDV vaccine. The data are transformed into frequency +/- standard error 0.05. Different letters show statistically significant differences at $p < 0.05$. Proportion Comparison: 5.

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29. González R, Viamontes O, Fonseca C. Análisis de los resultados serológicos de la prueba de HI en ponedoras vacunadas por aspersión con la cepa La Sota del virus de la enfermedad de Newcastle. Rev Cubana Cienc Avíc 1986;13(2):129-36.

30. Quintero D. Las pruebas de la hemoaglutinación e inhibición de la hemoaglutinación en la serología de la enfermedad de Newcastle. I. Rev Cubana Cienc Avíc 1989;16(1):81-9.

31. Masdeu V, Acosta I, Rejo T, Espinosa V, Herrera C, Hernat R, Díaz G. Características de los procesos respiratorios mediante el diagnóstico serológico y el cuadro clínico lesional. Rev Cubana Cienc Avíc 1997;21(1):11-6.

32. Cuello S, Noda J, Perera CL, Alfonso P. Diagnóstico de bronquitis infecciosa aviar por diferentes técnicas serológicas. Revista Cubana Cienc Avíc 2000; 24(2):104-9.

33. Cuello S, Noda J, Alfonso P, Perera CL. bronquitis infecciosa aviar. Cinética de anticuerpos posvacunales en reproductoras y su transferencia a la progenie. Rev Salud Anim 2004;26(1):42-7.

34. Noda J, Cuello S, Alfonso P, Casañas J. Bronquitis infecciosa aviar: obtención de antígeno y detección de anticuerpos por inhibición de la hemoaglutinación. Rev Cubana Cienc Avíc 2002;26(2):89-95.

35. Parra T. La calidad de la materia prima y el alimento terminado. Rev Acont Avíc 1999;7(50):26-8.

36. Idoate A. Micoplasmosis. Enfermedad respiratoria crónica. Julio 2000 [En línea.] Consultado: 5 de abril de 2009. Disponible en: http://www.oie.int/esp/publicat/rt/e_rt15_4.htm

37. Sánchez A, Luzbel Y, Lamazares MC, López W, Soca M, Pérez E. Uso del programa computarizado EPIZOOIND en el análisis de un brote de coriza infecciosa. En: Memorias del Congreso de Veterinaria. Universidad Agraria de La Habana, Facultad de Medicina Veterinaria. 2007. p. 27-32.

38. Valencia B. Bronquitis infecciosa: Soluciones prácticas. En: Memorias del Congreso Nacional de Avicultura 2007. [En línea.] Consultado: 6 de febrero de 2010. Disponible en: http://www.amevea-ecuador.org/memorias.htm

39. Cerdá R, Uriarte J, Origlia J, Petruccelli M. Ornithobacterium rhinotracheale: Su importancia como patógeno respiratorio en pollos y su asociación con Mycoplasma synoviae. [En línea.] Consultado: 16 de febrero de 2010. Disponible en: http://www.vetanco.com.ar/pdf/ornithobacterium_rhinotracheale_capia.pdf

40. Vandamme P, Sergers M, Vancaneyt K, Van Hover R, Mutters J. Description of Ornithobacterium rhinotracheale gen. nov. isolated from the avian respiratory tract. Int J Syst Bacteriol 1994;44:24-37.

41. Van Veen L. Conoce usted el real impacto de la infección por Ornithobacterium rhinotracheale en las aves. Infovet. 2003. Mayo-junio: 1-5.

42. Fernández A. La bioseguridad y su importancia económica en los países de América Latina. Ediciones Midia S.A. de C.V. México. 1997; p. 63-9.

43. Kleven SH. Complejo respiratorio de las aves. Cuarto curso de actualización AVIMEX. Department of avian medicine, Universidad de Georgia, Atenas, Estados Unidos; 1999.

44. Cavanagh D. Survey acute respiratory syndrome vaccine development: Experience of vaccination. Avian Pathol 2003;32(6):567-82.

45. Acevedo AM, Burgher Y, Colas M, Relova D, Correa A, Bacallao E, *et al*. Detección en muestra clínica e identificación de aislados del virus de la bronquitis infecciosa aviar por un ensayo de reverso transcripción acoplado a reacción en cadena de la polimerasa. Rev Salud Anim. [In press, 2010.]

46. Shane M. Las enfermedades respiratorias: retos actuales en el mundo. 2005. [En línea.]. Consultado: 16 de febrero de 2010. Disponible en: http://labs.intelart.com.mx/mj/avimex/cursos/x1-10.doc

47. Viamontes O, Cánovas A, Guilarte O, Acosta I. Evaluación serológica de reemplazos de aves de reproductores que recibieron la vacuna tetravalente oleosa contra las enfermedades Newcastle, bronquitis y Gumboro. Rev Cubana Cienc Avíc 1991;3:228-31.

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