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MYCOPLASMA ISOLATES FROM FERAL BARN SWALLOW; VIRULENCE AND CLINIC-PATHOLOGICAL FEATURES IN CHICKENS.

Ohore Godspower Obarkpor[1], Theophilus Agogo Jarikre[1], Derrick Adu Asare[2], Benjamin Obukowho Emikpe[2]

AFFILIATIONS

1. Department of Veterinary Pathology, University of Ibadan, Ibadan, Nigeria
2. School of Veterinary Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Correspondence: Benjamin Obukowho Emikpe, E-mail: banabis2001@gmail.com, Phone: +233549410841

ABSTRACT

The transmission of a pathogen to a new host is a common cause of a newly emerging infectious illness. Studies of disease ecology and avian pathology must, therefore, pay close attention to the identification of host transfer opportunities and barriers. Using the mycoplasma isolate cultured from the feral barn swallow to infect 3-day-old broiler chickens to assess the pathogenicity and pathology of the isolates alone and concurrently with E. coli. They were grouped as A (inoculated with Mycoplasma only), B (inoculated with Mycoplasma & E. coli), C (E. coli only) and N (control). Parameters assessed included clinical signs, weekly weight gains, full blood count, and gross and histopathology of tissues from infected and control birds. There was moderate pyrexia in chicks infected. No clinical signs were observed in control chicks. There was slight anaemia in the Group B birds (inoculated with Mycoplasma and E. coli) when compared to the other groups. There was moderate leucopaenia in group A and B birds, while severe in group C. There was dullness, closing of the eyes and yellowish-brown mucoid droppings in groups A, B and C chicks from 4dpi. There were congestive and inflammatory lesions in the lungs, trachea, joints and liver. It could therefore be concluded that the mycoplasma isolate from the feral bird was pathogenic in chicken and may contribute to the epidemiology of the disease in poultry.

Keywords: Clinicopathology, Chickens, Mycoplasma Infection, Wild birds.

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INTRODUCTION

The transmission of a pathogen to a new host is a common cause of a newly emerging infectious illness. Research into disease ecology and avian pathology relies heavily on determining the likelihood of host transfer and the factors that might prevent it. Various pathogens can be spread across vast distances by feral birds. (Van et al., 2014). They store and spread a wide variety of pathogens in mechanical and natural ways. The barn swallow (Hirundo rustica) has the widest natural range of any of the passerine birds, making it the most common species of swallow and feral bird worldwide (Turner, 2009). According to Birdlife (2019), barn swallows are a kind of open-country bird that typically nest in man-made structures. There is a high risk of disease transmission due to its intimate connection with people and domestic animals. There is mounting evidence that the host range of Mycoplasma species in the wild is remarkably broad (Sullivan et al 2014, Ley et al 2016).

Both commercial exotic varieties and indigenous breeds of chicken in Nigeria are susceptible to mycoplasmosis, making it one of the major disease issues in poultry production (Mera et al., 2020). The Mycoplasmataceae family is responsible for avian mycoplasmosis (Kleven, 2008), and 20 different mycoplasma serotypes have been isolated from birds, with Mycoplasma gallisepticum (MG) and Mycoplasma synoviae (MS) being the most dangerous. Chronic respiratory illnesses in hens and viral sinusitis in turkeys may both be traced back to MG. Pheasant, parrot, quail, goose, and duck were also shown to harbour this mycoplasma serotype (Lupiani and Reddy, 2009). Upper respiratory tract infections are a common subclinical symptom of MS. When combined with infectious bronchitis and Newcastle disease, however, the condition can have a more serious course, affecting air sacs and the synovial membranes of tendons and joints and resulting in infected synovitis (Yehia et al., 2023). Commercial poultry, as well as game birds including pheasants, chukar partridges, bobwhite quail, Japanese quail, and peafowl, are all susceptible to avian mycoplasma infections. In addition to ducks and geese, yellow-napped Amazon parrots, pigeons, and larger flamingos have all been cultured as hosts for the organism. Wild peregrine falcons have been discovered to have it. Staley et al. 2018, Vinkler et al. 2018, Bale et al. 2020) suggest that M. gallisepticum outbreaks in house finches (Carpodacus mexicanus) have been occurring since 1994. Captive blue jays (Cyanocitta cristata), pine grosbeaks (Pinicola enucleator), evening grosbeaks (Coccothraustes vespertinus), and American goldfinches (Carduelis tristis) have all had this organism confirmed in culture or polymerase chain reaction (PCR) (Grodio, 2013). Additionally, it has been reported that PCR-positive mourning doves (order Columbiformes) were seronegative and culture negative, suggesting that they were infected with a different Mycoplasma species (Grodio, 2013). Serological tests have shown that other species of songbirds are also positive. Some strains have been artificially introduced into captive populations of house sparrows (Passer domesticus) and budgerigars (Melopsittacus undualtus). The mycoplasma species Mycoplasma columborale, Mycoplasma columbum, and Mycoplasma columbinasale have all been isolated from British pigeons (Brown et. al., 2010). However, there are no reports on the pathogenicity of mycoplasma

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isolate from feral birds in poultry in our environment.

Due to the high prevalence of mycoplasmosis in Nigerian poultry industry, and the continuous increasing commercial rearing of chicken in Nigeria, this study was designed with the following objectives; to investigate the pathogenicity of feral avian mycoplasma isolate experimentally in chickens.

MATERIALS AND METHODS

Mycoplasma Isolate

The mycoplasma isolate used was cultured from the droppings of Barn swallow roosting sites within the premises of the University of Ibadan.

Experimental chickens

55-day-old chicks were obtained from reputable hatchery and brooded. The broiler chicks were screened for mycoplasma antibodies using SPA test method. Their body weights were taken for sorting chicks into groups with mean weight of 60.5g at 3-days old, the chicks were divided into 4 groups as follows:

Group 1(A): 15 birds inoculated with mycoplasma isolate oculo-nasally (0.04ml = 40µl as 10µl in each nostril and eye respectively)

Group 2 (B): 15 birds inoculated with mycoplasma isolate oculo-nasally + E. coli infection intratracheally (i.t);

Group 3 (C): 15 birds inoculated with E. coli alone i.t and

Group 4 (N): 15 birds inoculated intranasally with sterile broth.

All groups were housed in separate cages and fed chick mash and clean water ad libitum.

Clinical evaluation and sample collection:

Bi-daily parameters were taken for rectal temperature at 10-11 am every 2 days, other clinical signs of coughing, sneezing, rales, oculo-nasal discharges, diarrhoea, weakness and weekly weight gains were observed daily.

Blood samples were collected via jugular venipuncture weekly for full blood count.

Pathological and microbiological evaluation:

Two (2) chicks were euthanised and sacrificed weekly from the clinically ill birds for monitoring lesions. Birds that died on any day post-infection were also recorded and necropsied Post-mortem examination of exsanguinated birds for lesions with special attention to respiratory, intestinal and articular (joints) tissues. Sterile tissues and swabs were also collected for bacterial re-isolation and identification.

Tissue sample was collected at postmortem from each group at regular weekly intervals post–infection. Samples collected include the proventriculus, lungs, oesophagus, kidney, liver, intestine, tracheal, heart, and joint. These samples were collected into appropriately labelled universal bottles containing 10% neutral buffered formalin of adequate volume for fixing and routine histological processing.

Data Analysis

The clinical data and lesion indices were descriptively presented as Mean ± Standard deviation, and compared with one-way analysis of variance, Bonferroni test at α=0.05 using

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Statistical Package for Social Sciences version 25.

RESULTS

Clinical signs

Rectal temperatures of all chicks' groups are presented in Figure 1. No clinical signs were observed as all chicks were active and alert at 2 dpi and 3 dpi. There was dullness in group C chicks, closing of the eyes and yellowish-brown mucoid droppings in group B chicks 4 dpi. Occasional sneezing was observed in a few chicks from groups A and B 5 dpi. Two chicks were weak and sitting on their hock from group A at 6 dpi. There was mucoid nasal discharge in chicks from group A, B and C and serous nasal discharge in chicks from group N at 7 dpi. There was mucoid nasal discharge in chicks from groups A and C at 14 dpi. About 50% of the chicks showed signs of dullness and occasional sneezing in group B within 28 to 30 dpi. All the birds in groups A, B and C were dull, weak and dehydrated when compared to D at 32 dpi. There was dark greenish discolouration of the ventral abdomen and weight loss in chicks from group A.

There was moderate emaciation in group B chicks at 35 dpi. In addition, there was mucoid nasal exudate, matting of the perineum and blood-tinged droppings at 39 dpi to 42 dpi. There was severe emaciation, and mucoid nasal discharges in chicks from group B at 49dpi.

Haematology

There was slight anaemia in the Group B birds (inoculated with Mycoplasma & E. coli) when compared to the other groups. There was moderate leucopaenia in group A (inoculated with Mycoplasma only) and B (inoculated with Mycoplasma & E. coli) birds, while severe in group C (E. coli) only). There was severe thrombocytopaenia, lymphopaenia and moderate heteropaenia in the inoculated groups (A, B & C), with a remarkable increased Heterophil-Lymphocyte ratio in in the group C birds (Table 1). Weekly, the leucopaenia and thrombocytopaenia were very critical in the Group C birds and also remarkable in the other inoculated birds A & B ($p<0.05$) at 7 dpi. There was also eosinopaenia and persistence of heterophilia in the inoculated groups 7 dpi to 14 dpi (figure 2).

Pathology

Gross lesions

A few chicks showed acute sinusitis (hyperaemic sinuses), yellow cream coloured nodule on abdominal air sac (air sacculitis) and multiple pale foci on the liver (hepatic necrosis) in Group A at 3dpi. The air sacs were cloudy and thickened, mucoid exudate in the trachea in chicks from groups A and B, while there was acute mucoid enteritis in chicks from groups A, B and C at 7dpi. The pleura was thickened and there was mucoid enteritis in chicks from group C at 14dpi. There was acute sinusitis, unilateral pulmonary congestion, air sacculitis, and peri-hepatitis in chicks from group B at 21dpi.

The chicks in group N were well fleshed and bigger than those from A, B and C which also had dark red keel muscles. There was slight to severe acute sinusitis in group A, B and C chicks, cloudy and thickened air sacs (air sacculitis) in A, moderate congestive splenomegaly with a few pale foci in B, acute haemorrhagic enteritis in C and mild caecal tonsillar petechial haemorrhages in A and C at 28dpi.

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There were moderate bilateral hyperaemic sinuses, cloudy and thickened air sacs especially left thoracic and abdominal air sacs, thickened and opaque pericardium, exudative peritonitis, fibrinous perihepatitis with widespread fibrin strands, pneumonia, splenomegaly while the kidneys were soft and friable and there was cheesy exudate of irregular shape attached to thoraco-abdominal wall and over intestine in a chick from group A at 32dpi. There were moderate cloudy and thickened air sacs, moderate congestion, consolidation of the lungs, cheesy plug, moderate hepatic congestion and peri-hepatitis in chicks from group A, B and C, more so fibrinous pericarditis, caseous abdominal exudate and acute enteritis in B at 35dpi.

There was pulmonary congestion, pneumonia, pericarditis, abdominal caseous exudate, haemorrhagic to mucoid enteritis and peri-hepatitis with multiple pale foci in B and C chicks at 42dpi.There was cloudy air sacculitis, pulmonary congestion, pneumonia, pericarditis, haemorrhagic enteritis, caecal tonsillitis at 49dpi.

Microscopic lesions

Group A: There was congestion and inflammatory cell infiltrate consisting mainly of heterophils observed in the trachea, air sacs, lungs, liver and joints. Degeneration and necrosis were observed in the kidney, joint capsule, liver and trachea. Tubular atrophy was observed in the kidney, liver showed atrophy of the hepatic plate. The intestines showed villous atrophy and enteritis. Marked keratinization, hyperkeratosis and hydropic degeneration were observed in the oesophagus of the chickens. Proventricular glandular necrosis and inflammation (Figure 4).

Group B: There was mucous hyperplasia of the nasal turbinates, denudation of the tracheal epithelium, congestion of air capillaries and a few heterophilic infiltrates in the air spaces of the lungs at 7dpi. The liver also showed multiple foci of coagulation necrosis with Kupffer cell hyperplasia. The intestinal villi were atrophic and denuded with heterophilic and mononuclear cellular infiltrate in the mucosa (Figure 4).

Group C: There was vascular congestion, moderate necrosis of mesobronchial epithelium with a few cellular infiltrates and hepatic plate atrophy at 9dpi. There was necrosis of turbinate mucosal epithelium, congestion of submucosal capillaries and heterophillic inflammation. The lung showed capillary congestion, haemorrhage and oedema fluid in air spaces and coagulation necrosis of parabronchial epithelial cells. There was moderate diffuse atrophy of hepatic plates and accentuation of sinusoids at 14 dpi (Figure 4).

Group N: There were no observable lesions in the tissues (Figure 4).

The findings of this study showed the pathogenicity of the mycoplasma isolate experimentally in chicken. Mycoplasmas are most commonly found in the respiratory mucosa, urogenital tract, eyes, mammary glands, and joints of mammals and birds. Mycoplasmas are often thought of as surface parasites that very rarely invade tissues; yet, dissemination to other organs is highly indicative of a transient systemic infection. Successful colonization and subsequent pathogenesis are dependent on mycoplasmas' ability to adhere to host cells (Dawood et al., 2022). Some mycoplasma species may be able to invade cells and live inside of them,

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according to newer data mostly on mycoplasmas that infect people (Benedetti et al., 2020). Mycoplasma species, and perhaps even individual strains, show organ and tissue proclivity. However, this does not mean that the bacterium is absolutely precluded from infecting any other organs. One of the mycoplasma species, Mycoplasma gallisepticum, is a major pathogen that can cause both acute and chronic illnesses at various locations.

Chickens seldom develop severe symptoms of MG infection. Foamy eyes, nasal discharge, and coughing and sneezing are among the common symptoms of MG. Growth retardation and decreased fertility can potentially be effects of MG. When infected hens also have other diseases such as infectious bronchitis, Newcastle disease, and E. coli, as well as when dust and ammonia levels are high in the poultry house, MG can become much more severe. Adult bird mortality from MG is typically minimal, but in hens infected with other respiratory viruses or E. coli, it can reach 30%. Recovered birds may still be contagious (carriers) of MG and may not exhibit symptoms until they are stressed.

Clinical indications in infected chickens were shown to be the result of a wide range of pathologic alterations in different chicken organs, as shown by the results of this investigation. Histological lesions were more common in the lungs, trachea, and liver, and showed an acute pattern at the beginning of MG infection but a chronic one towards the end. Necrosis, haemorrhages, rupture of the lining epithelium, and significant leukocyte infiltration in the sub-mucosal layer with thickening of the mucous glands were seen in tracheal sections. These findings corroborated those of Gowthaman et al. (2020). Conspicuous congestion, haemorrhages, localized necrosis, leukocytic infiltrations mostly with polymorphs nucleated cells, and exudates in alveoli were seen in the lungs (Watkins et al., 2017). It has been shown that some Mycoplasma species may replicate within cells (Gaurivaud et al., 2022). It is hypothesized that avian mycoplasmas' intracellular persistence contributes to their chronicity. Gaining access to resources and maybe avoiding the host's immune system are two obvious benefits of invading eukaryotic cells. There is evidence that mycoplasmas can infiltrate chicken erythrocytes (Vogl et al., 2008), although this has not been tested here. This allows Mycoplasma to avoid being destroyed by the immune system. Infecting erythrocytes is advantageous because it allows the pathogen to spread to other locations. After first colonizing the respiratory system, Mycoplasma spreads systemically via the bloodstream to colonise other organs and develop lesions in other organs and tissues such the liver, kidney, proventriculus, gut, and joint.

Although some mild lesions were observed in the lungs and trachea of the control group, these may be due to other conditions e.g. cold/environmental conditions. The study's clinical, gross, and microscopic findings were consistent with those described for MG and MS infections in birds, either naturally occurring or experimentally induced (Schivaprased et al., 2014). This is because the symptoms of Ornithobacterium rhinotracheale infection, Fowl cholera, Escherichia coli infection, and the Newcastle disease observed in the respiratory system can all be caused by the same pathogen (Ley 2008; Yehia 2023). Culture is the gold standard for diagnosing mycoplasma

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infections, as it is for many other infectious disorders. However, due to the drawbacks of the culture approach, such as the need for antibiotics and the length of time it takes, molecular methods like PCR are now favoured. Researchers have shown that PCR is more sensitive than culture-based methods.

CONCLUSIONS

In conclusion, the findings in chickens infected with mycoplasma isolates in this study correspond to the gross and histological lesions of Mycoplasma gallisepticum in avian species. More studies are underway to further characterize the isolate. Feral birds like the barn swallow serve as reservoirs and /or mechanical vectors for numerous infectious agents. The mycoplasma isolate from the feral bird was pathogenic in chicken and may contribute to the epidemiology of mycoplasmosis in poultry.

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