j.Egypt.vet.med.Assoc 77, no 2. 241 – 246 (2017)

MANNHEIMIA (*PASTEURELLA*) *HAEMOLYTICA* INFECTION IN COMMERCIAL LAYERS; A CASE REPORT

By

Setta, A. [1]*, Refaei, E.[2] and Heba M. Salem[1]

[1]Department of poultry diseases, Faculty of veterinary medicine, Cairo University, Giza, Egypt.

[2]Ceva Santé Animale, Egypt

*Corresponding author: Heba M. Salem, PhD, Lecturer of poultry diseases, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

ABSTRACT

Infection with *Mannheimia haemolytica* (*M. haemolytica*) causes severe economic losses among different animal species as sheep, goat, swine and cattle, while in poultry it is usually seen as secondary bacterial infection to other avian pathogens. The present study highlights the impact of *M. haemolytica* on the health and egg production in White Shaver and Hi-Sex brown layer flocks in Egypt. The clinical signs were recorded as respiratory manifestations, edematous swelling in the comb and fluctuating decrease in egg production. The necropsy findings revealed air saculitis, pericarditis, perihepatitis, congested and flaccid ova with egg peritonitis. A representative samples of spleen, liver lung , trachea and heart were collected to detect the presence of any avian respiratory viruses by using conventional and RT-PCR as well as, testing for bacterial presence by both phenotypic and genotypic methods. The results revealed detection of *M. haemolytica,* in the examined samples. The antibiotic sensitivity test was performed on isolated *M. haemolytica* and the results showed that *M. haemolytica* was resistance to all tested antibiotics.

Key words:

Mannheimia haemolytica, layer, respiratory, PCR, antibiotic resistance.

INTRODUCTION

In birds many bacteria are involved in causing respiratory disease problems including *Pasteurella multocida, Mannheimia haemolytica, Pasteurella gallinarum,* and *Riemerlla anatipestifer* **(Hafez, 2002)**. *M. haemolytica* is a gram negative, coco-bacillus, usually found as commensal in the upper respiratory tract of different animals and can act as an opportunistic pathogen, causing mild to severe respiratory infections under stress conditions **(DeRosa *et al.*, 2000)**. *M. haemolytica* is also a usual flora of the respiratory tract of chicken

and animals and plays a drastic role of opportunist under stress factors by causing the respiratory disease **(Taylor *et al.,* 2010)**. *M. haemolytica* has also been associated with severe pleuropneumonia in sheep and goats **(Zecchinon *et al.,* 2005)**.

M. haemolytica is identified as secondary or co-pathogen in chicken infected with respiratory viral pathogens like Infectious Bronchitis virus (IBV) and Infectious Laryngiotrachitis virus (ILTV) and as a primary respiratory pathogen reported in poultry recently after its isolation from clinically ill and dead chicken in Nigeria **(Antiabong *et al.,* 2006)**.

MATERIAL AND METHODS

Flock problem:

A flock of total population 28614 layer chickens (16581 White Shaver and 12033 Hi-Sex brown chickens) aged 195 days with a history of 6% mortality and 40% reduction in feed intake with severe decline in egg production Fig. (1).

Sample collection:

A total of twenty freshly dead chickens were necropsied (8 White Shaver and 12 Hi-Sex brown) for collection of tissue samples (trachea, lung, liver, spleen and heart) for further laboratory examination. All tissue samples were processed for detection of major respiratory pathogens like Newcastle Disease virus (NDV), Infectious Bronchitis Virus (IBV), Avian Influenza Virus (AIV) (H5) & (H9) as well as *Mycoplasma gallisepticum* (MG), through (RT-PCR).

Bacteriological examination:

The bacteriological examination was also done under complete aseptic conditions by propagation of different samples into Nutrient broth, Brain-Heart Infusion broth and Tetrathionate broth incubated for 14 - 16 hours only. Similarly, same samples were also cultured directly onto 5 % sheep blood agar, Brain-Heart Infusion agar, MacConkey agar and brilliant green agar. Both propagated and cultivated samples were incubated at 37℃ for 24 hrs. Bacteriological positive samples were determined on the basis of morphology using Gram's staining technique **(Brooks *et al.,* 2002; Naowarat, 2007)** as well as, biochemical characters using analytical profile index 20E strips (API-20E, Biomerieux Inc, USA).

PCR:

DNA was purified from overnight cultures grown in Brain Heart Infusion media (BHI) using DNeasy Blood and Tissue Kit (Qiagen). PCR was carried out in 25 µl reaction mixture, using PCR Master (Roche Diagnostics), the *M. haemolytica* glucose-6-phosphate dehydrogenase

gene (*zwf*) primer sequence FWD 5′-TGATGAAGTCGCAAAAGTGC-3′, REV5′-ACGGTTTTCGCCATACTTTG - 3′ with Amplicon size of 671 bp. **(Heaton *et al.*, 2015)** Cycling condition was 5 min denaturation at 96 °C, followed by 30 cycles at 96 °C for 30 s, 55 °C for 30s and 72 °C for 1 min. The run was ended with a final extension step at 72 °C for 10 min.

Antibiotic sensitivity assay:

The antibiotic susceptibility test was performed on pure colonies by disc diffusion method using Mueller Hinton medium. The antibiotic discs of Norfloxacin (10µg), Amoxicillin (10µg), Ciprofloxacin (5µg), Enrofloxacin (5µg) Lincospectin (30µg), Colistin (10µg), Oxytetracycline (30µg), Erythromycin (15µg) and Sulphamethoxazole (25µg) were used. The interpretation was made using standard methods.

RESULTS

Clinico-pathological picture:

Our case report recorded different clinical signs including respiratory manifestations, edematous swelling in the comb with necrotic tips Fig. (2) and fluctuating decrease in egg production. The necropsy finding revealed air saculitis, pericarditis, perihepatitis Fig. (3), congested lung, congested intestine, petechial hemorrhages on coronary fat, hepatomegaly and splenomegaly, congested flaccid ova with egg peritonitis Fig. (4).

Bacteriological Examination:

The bacteriological evaluation showed circular colonies with narrow zone of β-hemolysis on 5 % sheep blood agar and visible growth as lactose fermenting colonies on MacConkey. The isolated bacteria were non-motile and gram negative coccobacilli, similar to *Pasteurella multocida* in morphology. The biochemical test showed reaction of oxidase positive, catalase positive, mannitol positive, Voges - Proskauer (VP) negative, urease negative, and non-acid production from D-mannose which to some extent prove the presence of *Pasteurella* species specially *M. haemolytica*.

Virus detection by RT-PCR in the examined samples:

Negative RT-PCR results were detected to different avian pathogens; AIV. IBV, NDV, MG. Furthermore, conventional PCR was conducted which revealed positive amplification of *zwf* gene indicative for detection of *M. haemolytica* in the suspected samples. On the other hand, *Pasteurella multocida* gave negative result in PCR.

Antibiogram:

The isolates showed resistance against all tested antibiotics.

DISCUSSION

Bacteria of *M. haemolytica* are commensals of the upper respiratory tract of poultry. However, these bacteria play a major role in causing pneumonic pasteurellosis and shipping fever in various animals **(Bavananthasivam *et al.,* 2012)**. Its role in causing disease in poultry is considered as secondary or complicating pathogen especially as co-infecting agent during viral infections **(Hafez, 2011)**. The current study identifies *M. haemolytica* as a primary source of disease in poultry which has been reported to cause a severe respiratory manifestation in layers as well as resulting in significant mortality and decrease in egg production. Other factors, including managemental and environmental stresses have a significant role in enhancing the pathogenesis of these bacteria **(Theurer *et al.,* 2013)**. The antibiotic sensitivity pattern of *M. haemolytica* indicates that, the bacteria are highly resistant to different antibiotics used. So, further studies should have focused on studying the genes which are responsible about this resistance pattern. The improper usage of antibiotics could be the main cause of *M. haemolytica* antibiotic resistance. Data from the present research highlight the importance of prohibition of un-necessary usage of antibiotics in poultry to avoid the risk of antibiotic residues in human food chain. PCR as compared to the conventional methods of bacterial culture and isolation of *M. haemolytica* is an accurate, rapid diagnostic tool for different respiratory pathogens.

CONCLUSION

M. haemolytica may acts as primary respiratory tract infection in layers under stress factors. Further studies should be focused on the antibiotic resistance pattern of *M. haemolytica* as well as vaccine should be developed to avoid such problem.

REFERENCES

Antiabong J, Haruna E, Owolodun J, Yakubu B, Odugbo M, Suleiman I, Ekundayo S and Dalyop, P. (2006): Isolation of *Mannheimia* (*Pasteurella*) *haemolytica* serotypes a2 and a12 from clinically ill and dead chickens: A case report. Tropical Vet, 23: 61 - 64.

Bavananthasivam J, Dassanayake RP, Kugadas A, Shanthalingam S, Call DR, Knowles DP and Srikumaran S. (2012): Proximity-dependent inhibition of growth of *Mannheimia haemolytica* by *Pasteurella multocida*. Appl Environ Microbiol, 78: 6683 - 6688.

Brooks GF, Butel JS, Morse SA (2002): Jawetz, Melnick and Adelberg's Medical Microbiology. 22nd ed. MacGraw Hill, New Delhi, India pp. 197-202.

DeRosa DC, Mechor GD, Staats JJ, Chengappa MM and Shryock TR (2000): Comparison of *Pasteurella* spp. simultaneously isolated from nasal and transtracheal swabs from cattle with clinical signs of bovine respiratory disease. J Clin Microbiol, 38: 327-332.

Hafez MH. (2002): Diagnosis of *Ornithobacterium rhinotracheale*. Int Poult Sci J, 1: 114 -118.

Hafez MH. (2011): Avian adenoviruses infections with special attention to inclusion body hepatitis/hydropericardium syndrome and egg drop syndrome. Pak Vet J, 31: 85-92.

Heaton M P., Gregory P. Harhay, Timothy P. L. Smith, James L. Bono, Carol G. Chitko-McKown (2015): Complete Closed Genome Sequences of a *Mannheimia haemolytica* Serotype A1 Leukotoxin Deletion Mutant and Its Wild-Type Parent Strain. Genome: 3 (3).

Naowarat C. (2007): Eosin-Methylene Blue. Vis. Atl. American Society for Microbiology. http://www.microbelibrary.org/ASMomly/details.asp?id.

Taylor JD, Fulton RW, Lehenbauer TW, Step DL and Confer AW. (2010): The epidemiology of bovine respiratory disease: What is the evidence for predisposing factors Can Vet J, 51: 1095-1102.

Theurer ME, Anderson DE, White BJ, Miesner MD, Mosier DA, Coetzee JF, Lakritz J and Amrine DE. (2013): Effect of *Mannheimia haemolytica* pneumonia on behavior and physiologic responses of calves during high ambient environmental temperatures. J Anim Sci; 91: 3917-3929.

Zecchinon L, Fett T and Desmecht D. (2005): How *Mannheimia haemolytica* defeats host defense through a kiss of death mechanism. Vet Res; 36: 133 - 156.

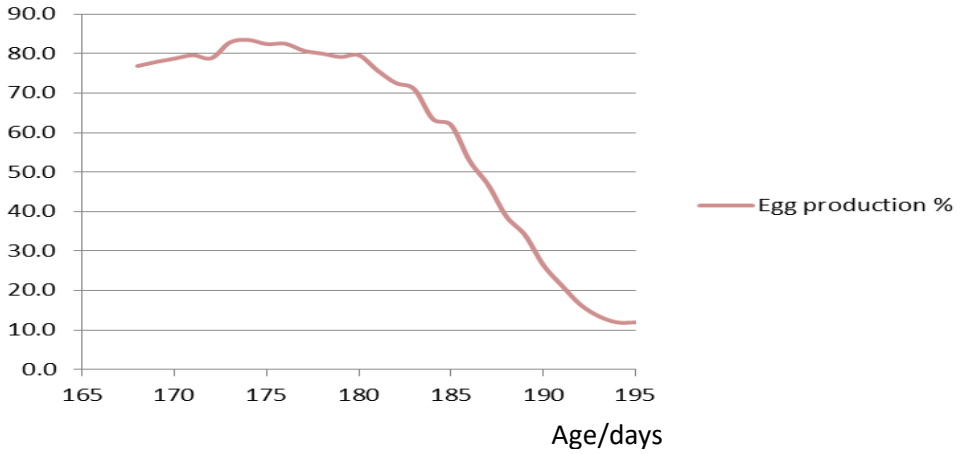

Fig. (1): Egg production curve.

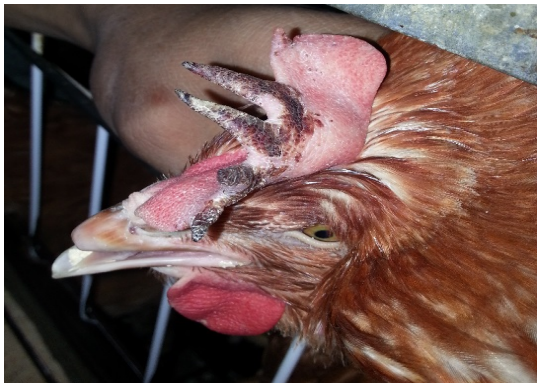

Fig. (2): swelling of comb and wattle.

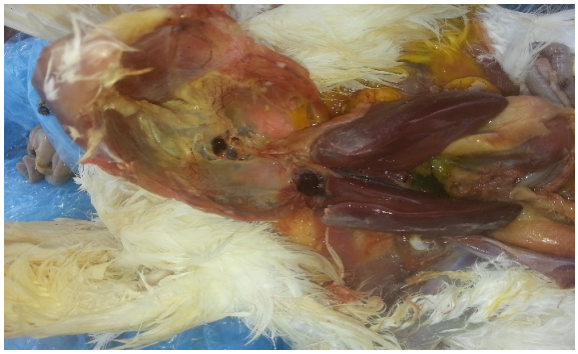

Fig. (3):air saculitis, pericarditis and perihepatitis.

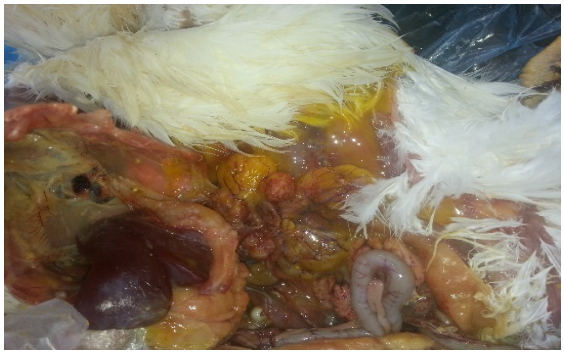

Fig.(4):congested flaccid ovaand egg peritonitis.