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Characterization of *Mannheimia haemolytica* by culture and biochemical tests of isolates from breeder birds

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Abstract
Pasteurellosis is a contagious disease of domestic and wild birds; the course of the disease occurs in acute, septicemic and chronic form. Mortality usually occurs in deep litter floor housed among commercial layers and breeding flock, Outbreak of Pasteurellosis associated with the environment or managemental stress may result in a drop in egg production among breeder and layer flocks causing an economic significance loss for poultry farmers. This study aimed to investigate and characterize the organism associated with birds' mortality with predominant signs of respiratory distress. The major necropsy findings include air sacculitis, congested lungs, pericarditis, fibrinous perihepatitis, necrotic foci scattered on the surface of liver, flaccid ova and egg yolk peritonitis. Lungs and liver samples were collected and all samples were cultured onto Blood and Mac Conkey agars. Further the pure cultures were subjected for biochemical tests for identification of the organism. The study revealed the significant involvement of *M. haemolytica* in respiratory infection resulting in mortality in breeder birds.

Keywords: Characterization, culture, biochemical, breeder, *Mannheimia haemolytica*

Introduction
Pasteurellosis is distributed across the globe; endemic outbreak of pasteurellosis is mostly encountered in intensive poultry farming system, chronically infected birds are considered to be a major source of infection (Antonio Zanella. 2007) [2]. Intraflock transmission is enhanced by handling birds for vaccination *(*Simon M. Shane.1997) [12]. *Mannheimia haemolytica* a non-motile cocco-bacillus usually resides in upper respiratory tract present as commensal, can act as an opportunistic pathogen causes respiratory tract infection under stress conditions (DeRosa *et al*., 2000) [6]. *M. haemolytica*, a normal inhabitant of the respiratory tract of chicken and under stress factors, causing respiratory distress (Taylor *et al*., 2010) [14]. Co infection with other viral respiratory diseases of poultry like Infectious Laryngotracheitis and Infectious Bronchitis can occur simultaneously (Antiabong *et al.*, 2006) [4]. *M. haemolytica* as a primary source of infection causes severe respiratory distress in poultry birds besides causing mortality and loss of production (Ali *et al.*, 2015) [1]. The incidence of *Mannheimia haemolytica* infection increases with the advancement of age in birds (M Bisgaard, 1977) [5]. Successful Isolation of *M.haemolytica* organisms from sporadic outbreaks with the involvement of respiratory tract, digestive tract and visceral organs is recorded in poultry (Greenham and Hill, 1962) [7]. Setta, A. *et al* 2017 [11] recorded post mortem finding of air sacculitis, pericarditis and perihepatitis, flaccid ova and egg yolk peritonitis in birds affected with *Mannheimia haemolytica* infection.

Materials and Methods
Dead birds around 30 weeks of age from a breeder farm with a history of respiratory distress, decrease in egg production and mortality were received in the laboratory. Based on the lesions in necropsy examination samples were collected in separate plastic bags, labelled and kept cooled in the ice-box.

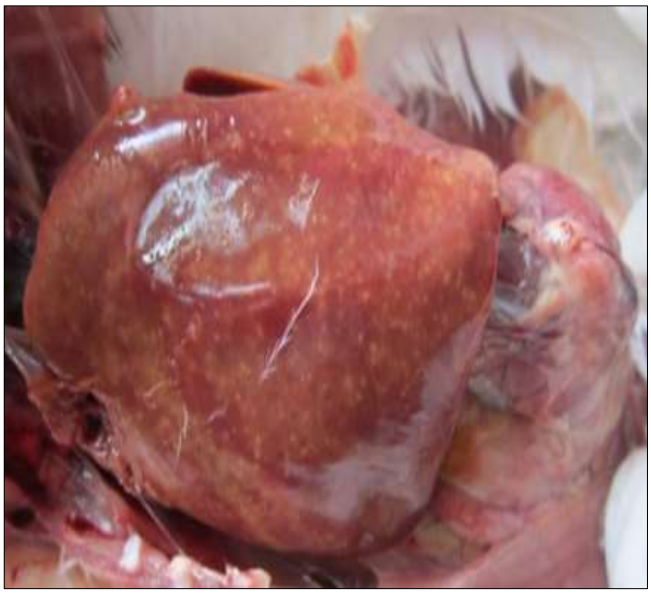

Fig 1: Necrotic white foci on liver

Fig 4: congested lungs

Fig 2: Air Sacullitis, Pericarditis and Hepatitis

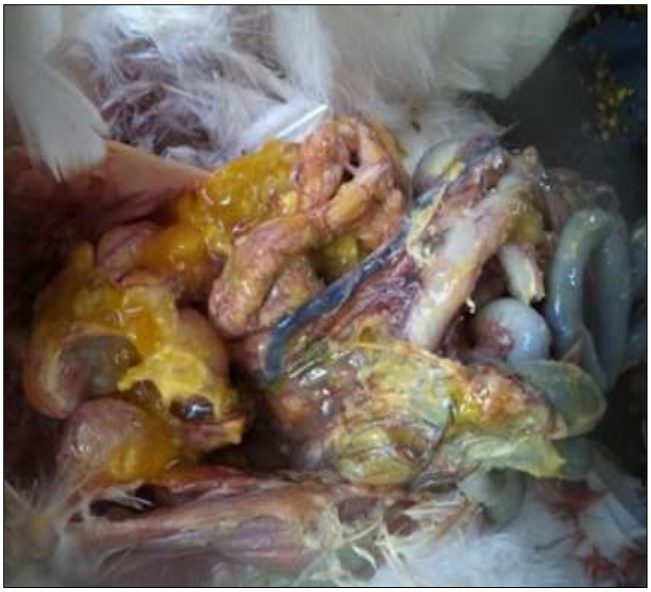

Fig 5: Egg yolk peritonitis

Fig 3: Flaccid ova

Results and Discussions

In necropsy examination, the major lesions observed were necrotic white spot on liver (fig-1), air sacculitis pericarditis and perihepatitis (fig-2), flaccid ova, congested lungs and egg yolk peritonitis as depicted in (fig-3), (fig-4) and (fig-5). Some of these findings are very similar to that of fowl cholera infection.

Colony characteristics on blood agar the colonies showed regular, smooth and greyish colonies of 1-2mm in diameter after 24-48 hrs of incubation (fig-6). Red pin point colonies observed on Mac conkey agar (fig-7) similarly Hawari *et al.*, 2008 observed that *M. Haemolytica* were able to grow on Mac Conkey agar.

Fig 6: Colonies on 5% sheep blood agar

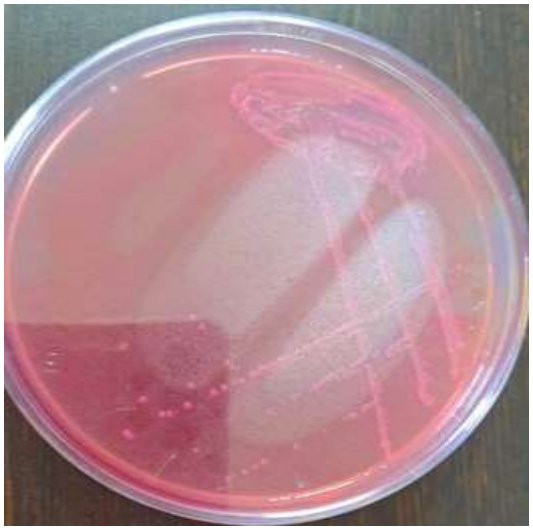

Fig 7: Colonies on Mac conkey agar

Isolation and identification of the organism

The samples were inoculated onto Mac Conkey agar and 5% sheep blood agar. Inoculated plates were incubated at 37 °C for 24 and 48 hours, the well-separated pure colonies were picked up and subjected to standard morphological and biochemical tests

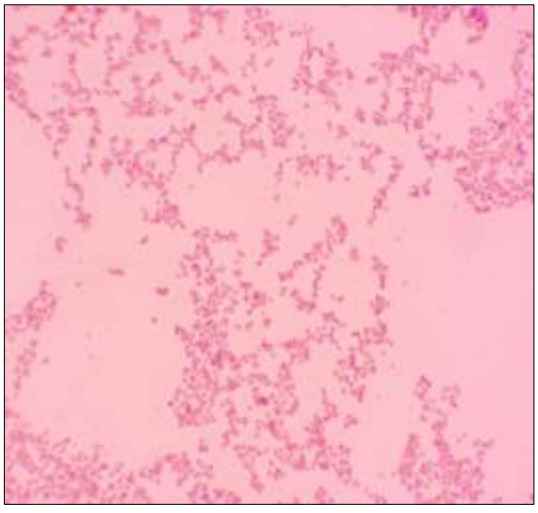

Fig 8: Gram negative cocco-bacilli

Microscopy revealed Gram-negative, non-motile, small rods as depicted in (Fig- 8). Smith and Phillips, 1990 described the organism as Gram-negative, evenly stained short rods. While the biochemical tests (Table-1) reactions showed positive for Sucrose, Fructose, Maltose (fig-9) and negative for Mannose, Dulcitol, Urease and Indole (fig-10), fermented Glucose, lactose and sucrose with no production of H2S (fig-10). Quinn PJ *et al.*, 2001 describes the differentiation between *Pasteurella* and *Mannheimia* organisms through culture test and biochemical tests, Hawari *et al.*, 2008 also recorded that isolates presumed to belong to *M. haemolytica* did not produce Indole.

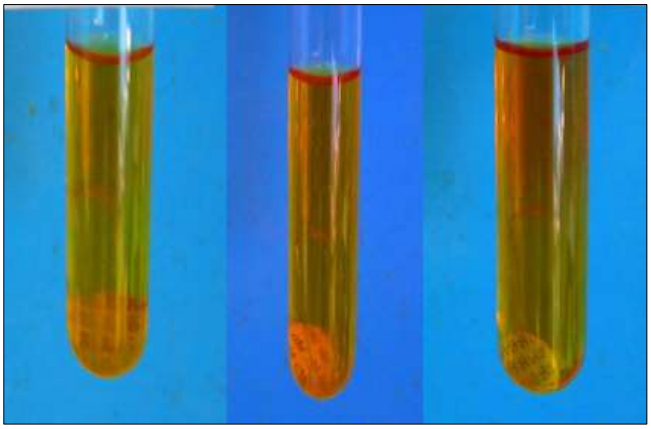

Fig 9: Maltose, fructose and sucrose test

Fig 10: Indole & TSI

Table 1: Biochemical tests result

Name of the Test	Result
Maltose	Positive
Mannose	Negative
Fructose	Positive
Dulcitol	Negative
Sucrose	Positive
Urease	Negative
Indole	Negative
TSI	Yellows slant/butt, no H2S

Mannheimia organisms are able to ferment glucose, lactose and sucrose without gas production but give a negative reaction for Indole and Urease tests. Catalase and oxidase tests are usually positive (Smith and Phillips, 1990) [13]. Ali *et*

al., 2015 [1] isolated *Mannheimia haemolytica* from adult commercial poultry flocks, initially reported with severe respiratory distress, similarly Kuotsu *et al*., 2019 [9] reported *Mannheimia haemolytica* infection among growing flocks from field condition, Based on the morphological and biochemical examination, the isolated organism was confirmed for *Mannheimia haemolytica.*

Conclusions

Serological tests are generally of little diagnostic value in the majority of the diseases caused by Pasteurellae and *Mannheimia* species, as the necropsy findings in *Mannheimia haemolytica* infection in poultry are very similar to those findings in Fowl cholera infection, definitive diagnosis can be difficult unless it is distinguished by cultural and biochemical characteristics, hence culture and biochemical tests can be used as reliable methods which is relatively less expensive as compared to other molecular diagnostic tools, however molecular techniques like Polymerase Chain reaction (PCR) assay can be used as a valuable tool for rapid and specific detection of *Mannheimia haemolytica* infection in a clinical sample.

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