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Identification of Duck Septicemia in Egypt

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Abstract: Duck septicemia is a constant threat to duck farming caused by *Riemerella anatipestifer* (*R. anatipestifer*) During 2014; Examination of 120 samples collected from ducks and ducklings showing anorexia, pyrexia, ataxia, white watery droppings, nasal discharge from different Egyptian farms in Giza, Qalyubia, Beni Sueif and Elbehera Governorates. The samples were collected from blood as well as liver, heart and spleen. The results revealed a recovery of 20 *R. anatipestifer* isolates with a prevalence rate 16.7%. Out of 69 samples collected from diseased ducks (more than 7 weeks old) revealed isolation of 14 *R. anatipestifer* isolates (11.7%). While out of 51 samples collected from diseased ducklings (1-7 weeks old), revealed isolation of 6 *R. anatipestifer* isolates (5%). Comparing the results obtained from ducks and ducklings showed a higher prevalence rate of ducks (11.7%) than ducklings (5%). *Omp*A gene specific for *R. anatipestifer* was detected among all isolates by Polymerase Chain Reaction (PCR).

Key words: *Riemerella anatipestifer* · Duck Septicemia · PCR · *Omp*A Gene

INTRODUCTION

R. anatipestifer is a Gram negative, rod-shaped, non-motile, non-spore-forming bacterium [1], previously known as *Moraxella anatipestifer* or *Pasteurella anatipestifer, R. anatipestifer* is the causative agent of a wide spread bacterial disease of ducks. Till now, 21 serotypes have been identified [2]. It causes great losses to duck industry and high treatment costs. It is a critical problem in intensive ducks production since 1982 in Asia [3]. *Riemerella anatipestifer* infection was reported to cause disease in pheasants, domestic geese, swans and turkeys [4, 5]. Cha *et al*. [6] detected *R. anatipestifer* in wild birds in South Korea.

The main clinical signs of the infected ducks and turkeys are mainly nasal discharge, swollen sinuses, dyspnea, diarrhea and neurologic disturbances and the most obvious postmortem lesion is fibrinous exudate of serosal surfaces [7]. Outer membrane proteins play a very important role in virulence and induce a strong antibody response [8]. *Omp*A gene of *R. anatipestifer* is an important target to differentiate, between *R. anatipestifer* and other bacterial species [9]. The present study aimed to detect *R. anatipestifer* among ducks and ducklings in different Egyptian farms during 2014 using conventional standards and PCR.

MATERIALS AND METHODS

Clinical and Postmortem Signs: During 2014; a total of 120 ducks and ducklings samples collected from different Egyptian farms in Giza governorate were examined against *R. anatipestifer*. Some birds were apparently normal, others showed variable clinical signs varies from local to systemic infection; anorexia, weakness, restlessness, growth retardation, coughing, nasal discharge. Some birds showed nervous manifestations as pyrexia, ataxia and tremors. Other birds showed joints and limb swelling, white watery droppings. Postmortem examination revealed typical perihepatitis, pericarditis, air sacculitis and septicemia. Liver and spleen samples were collected and analyzed bacteriologically and biochemically.

Bacteriological Examination: [10] Applying of Leishman's stain for blood films prepared from each sample, Cultivation of the liver and/or spleen impression smears on MacConkey agar; blood agar (Difco, USA) containing 7% sheep blood and incubation at 37°C for 18-24hrs in microaerophilic conditions, the suspected colonies were then examined microscopically and morphologically after staining by Gram stain. Pure cultures were then examined biochemically against

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catalase, oxidase, indole production, gelatin liquefaction and ornithine decarboxylase [11], then sub-cultured on brain heart agar (Difco, USA) and preserved in semisolid agar for further identification.

PCR for detection of *R. anatipestifer* isolates: Oligonucleotide primers used for amplification of *R. anatipestifer Omp*A gene using primer pairs (*R. anatipestifer Omp*A F1 (CTGCTCAGACTACTAGCAATC) and *R. anatipestifer Omp*A R1 (GTTCAATGAAGCTGACGCTTG) (This study). Genomic DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturers' instructions from pure cultures suspected colonies. PCR assay was performed using Emerald Amp® GT PCR Master Mix (Takara Bio Inc., Clontech Laboratories, Japan). The reaction was a mixture of Verso one step Master Mix (2X)12.5 µl, 1 µl Enzyme mix (5 units), Forward primer (F)1 µl, Reverse primer (R) 1 µl, Extracted DNA 5 µl, Nuclease free water 4.5 µl to reach a final volume of 25 µl in micro-amplification tubes.

Thermal cycling profile was consisting of; initial denaturation step at 94°C for 5 min. cDNAs were then amplified with 40 cycles of 94°C for 30 seconds, annealing at 50°C for 1 minute and extension at 72°C for 1 minute, followed by a final extension step at 72°C for 10 min. The amplified products (5µl) were loaded onto 1.5% agarose gel containing 0.5 µg/ml ethidium bromide for nucleic acid visualization. Electrophoresis was conducted using 1x TAE buffer and PCR products were visualized under UV trans-illumination in comparison to 100-1000 molecular size marker (Abgene).

Table 1: Isolation of *R. anatipestifer* from ducks and Ducklings

Type of sample	No. of examined samples	Positive samples	Isolation%
Ducks	69	14	11.7%
Ducklings	51	6	5%
Total	120	20	16.7%

RESULTS

Necropsy: The most common growth lesions of the isolates were congestion of internal organs (liver and heart), mild to severe pericarditis and perihepatitis.

Isolation and Identification of *R. anatipestifer* from Ducks and Ducklings: Leishman's stain for blood films prepared from positive sample revealed typical bipolar coccobacilli in between the RBCs. Colonies of *R. anatipestifer* were smooth, non-motile, convex and transparent. The Gram's stain showed Gram-negative, coccobacilli. All the isolates were positive for catalase, oxidase and gelatin liquefaction but negative for indole, ornithine decarboxylase. None of the isolates grew on MacConkey's agar. Bacteriological examination of 69 samples collected from the blood, liver and spleen of diseased ducks (more than 7 weeks old) revealed isolation of 14 *R. anatipestifer* isolates (11.7%). While 6 *R. anatipestifer* isolates out of 51(5%) samples collected from blood as well as liver, heart and spleen of diseased ducklings (1-7 weeks old), were detected (Table 1).

PCR of *Omp A* gene: From each *Riemerella* isolate, a product of 342bp of the *Omp*A gene was amplified (Figure 1).

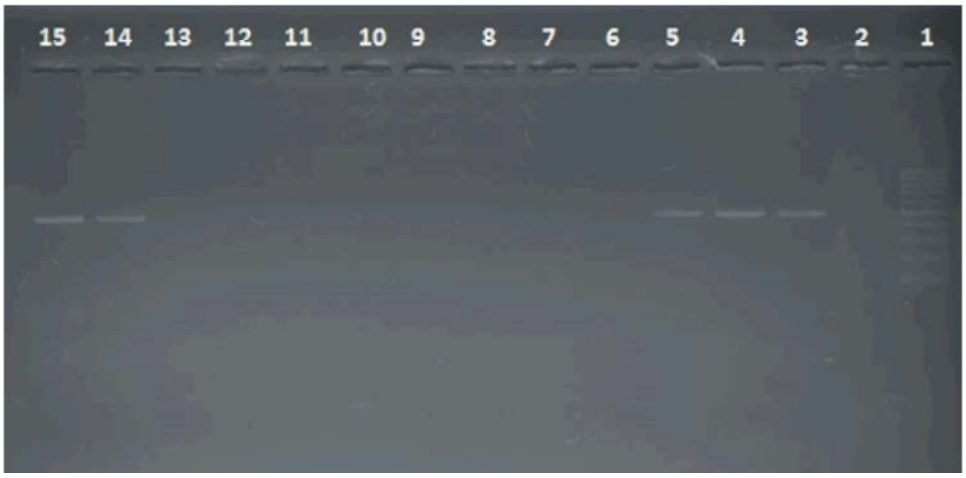

Fig. 1: Results of PCR using specific primers for *R. anatipestifer Omp*A gene. Lane1: 100bp marker (Abgene), Lane 2: Negative control, Lane 3: Positive control (*R. anatipestifer* ATCC# 11845), Lanes 4,5,14 and 15: Positive samples, Lanes 6,7,8,9,10,11,12 and 13: Negative samples.

DISCUSSION

Several trials were done to explore *R. anatipestifer* mechanism of transmission since its first report by Riemer, 1904 [12]. This study reported several outbreaks of infection caused by *R. anatipestifer* in commercial ducks. The clinical signs were mainly characterized by depression, anorexia and severe nervous manifestations [13, 14]. The affected birds showed variable clinical signs and some ducklings were died one or two days after appearance of clinical signs. Little information about *R. anatipestifer* might be due to it's difficult to be isolate from infected birds, especially if they passed the acute stage of the disease [15]. So, it is recommended to cultivate samples from several organs and initial isolation should be done under microaerobic conditions [16]. Diagnosis of *R. anatipestifer* is difficult due to absence of selective and/or indicative media for isolation [17], in addition, its morphological similarity to *Pasteurella* spp. [18]. Pillai *et al.* [19] showed that the bipolar organisms revealed by Leishman's staining of blood smear were larger than *P. multocida*. All blood films prepared from blood smears of the infected samples showed bipolar coccobacilli in-between RBCs when stained by Leishman's stain. The cultural and biochemical characteristics of the isolated organism in this study are suggestive of *Riemerella anatipestifer*.

For ducklings infected by *R. anatipestifer* before 5 weeks old, mortality varies from 5% to 75% and morbidity is usually as high as 100%. Older waterfowls usually suffer from chronic, non-fatal or subclinical disease [7]. It is therefore difficult to exclude *R. anatipestifer* infection from waterfowls in a farm rearing multi-age birds. From the examined ducks (69 samples) and ducklings (51 samples) 14 and 6 *R. anatipestifer* isolates could be isolated with a rate of 11.7% and 5%respectively.

Characterization of *R. anatipestifer* by traditional methods is often not sufficient because of phenotypic diversity. So, other methods should be used for further accurate and rapid diagnosis; such as gene analysis. The PCR amplification of the partial region of the *Omp*A gene done in this study was found to be highly beneficial and was proofed to be a fast way to confirm identification of *R. anatipestifer*. OmpA is known to stimulate a strong antibody response [20]. PCR assay for partial *Omp*A gene was used for confirmation of the isolates and it was amplified from all *R. anatipestifer* isolates.

CONCLUSION

Duck septicemia causes great loss to duck industry. Using genetic based diagnostic tool for diagnosis of *R. anatipestifer* is very important. *Omp*A gene as a target gene for diagnosis of *R. anatipestifer* is a very accurate choice but further genetic studies about *R. anatipestifer* should be done.

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