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Isolation and Identification of *Riemerella anatipestifer* from Ducks in Malaysia

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Abstract

Riemerella anatipestifer is the primary etiological agent of contagious septicemic diseases among ducks. The study is the first attempt to isolate and identify *R. anatipestifer* from ducks in Malaysia. In this study, ten diseased Khaki-Campbell ducks and forty healthy Khaki-Campbell ducks were selected. A pharyngeal swab was collected from each selected ducks. One strain of *R. anatipestifer* was successfully isolated out of the ten diseased ducks and identified using conventional biochemical tests. *R. anatipestifer*was isolated from the healthy ducks. The *R. anatipestifer* isolate was then subjected to antibiotic sensitivity testing using Kirby-Bauer method. The sensitivity of *R. anatipestifer* to penicillin G, enrofloxacin, oxytetracycline, gentamicin, neomycin, and ceftiofur was determined. *R. anatipestifer* was found to be highly sensitive to enrofloxacin, oxytetracycline and neomycin, intermediately sensitive to gentamicin and resistant to penicillin G and ceftiofur.

Keywords: *Riemerella anatipestifer*, Khaki-Campbell ducks, conventional biochemical tests, Kirby Bauer method

Introduction

Economic losses due to *R. anatipestifer* infections in ducks are of significant concern, since the infection can result in significant weight loss, mortality rate of up to 75%, carcass condemnations, and the destruction of colonies as a containment strategy (Zhong *et al*., 2009; Leavitt *et al*., 1997). Mortality can be as high as 95% and is influenced by predisposing viral and bacterial infections (Leavitt *et al*., 1997). Co-infection and adverse environmental conditions can predispose ducklings to disease outbreak (Zhong *et al*., 2009). The disease has a worldwide distribution, and endemic infections are restricted to commercial duck flocks (Singh *et al*., 1983). The infection can be per acute, acute or chronic. The exact route of transmission and the challenge dosage of *R. anatipestifer* are still debatable (Sarver *et al*., 2004).

R. anatipestifer is a gram-negative, nonmotile, nonspore-forming, rod shape bacterium that occurs singly, in pairs and occasionally in pairs. *R. anatipestifer* grows well on blood agar and chocolate agar but is usually non-haemolytic. It shows no growth on MacConkey agar. Its growth is enhanced when incubated at 37°C in a candle jar that provides increased carbon dioxide and moisture. The colonies of *R. anatipestifer* on blood agar when incubated at 37°C for 24 to 48 h are 1 to 2 mm in diameter, convex, transparent, and glistening. *R. anatipestifer* is catalase- and oxidase-positive. It is usually positive for gelatinase test, thus is capable of liquifying gelatin. *R. anatipestifer* is negative for nitrate reduction and has no action on glucose. To date, 21 serovars have been detected using the agglutination test method (Pathanasophon *et al*., 1995).

Ocular and nasal discharges, diarrhea, mild coughing and sneezing, tremors of the head and neck and incoordination are common clinical signs of *R. anatipestifer* infection in ducks. Upon postmortem, the most obvious gross lesion in ducks is fibrinous exudates in the pericardial cavity, air sacs and over the liver surface. Mucopurulent exudate is often detected in nasal sinuses. Pneumonia may be seen. Spleen and liver may be enlarged and mottled. Infection of the CNS can result in fibrinous meningitis. A definitive diagnosis can only be established by isolation and identification of *R. anatipestifer*. It is not yet confirmed whether *R. anatipestifer* may localize and persist in the upper respiratory tract of birds without causing any signs and lesions. Isolation and identification of *R. anatipestifer* in ducks in Malaysia has not been established so far.

Antibiotics can be used to treat *R. anatipestifer* infection. *R. anatipestifer* is reported to be sensitive to enrofloxacin, chloramphenicol, lincomycin, streptomycin and neomycin but is resistant to penicillin G, ampicillin, tetracycline, trimethoprim-sulfamethoxazole, kanamycin and gentamycin (Zhong *et al*., 2009). Identification of the suitable antibiotic for treatment of *R. anatipestifer* infection is significant to treat and control the infection, reduce or prevent mortality of the infected ducks and ensure maximum cost effectiveness by diminishing the unnecessary use of antibiotics to which *R. anatipestifer* is resistant.

Materials and Methods

Sampling

Fifty Khaki Chambell ducks aged one-year were selected from a population of 1000 ducks. Forty healthy ducks and 10 ducks which showed clinical signs suggestive of being diseased such as unsatisfactory or stunted growth, poor feather condition and inactive were sampled. A pharyngeal swab was obtained by encircling the sterile swab around the pharyngeal region for 2 to 3 times. The swab was stored in Aimes® transport medium and transported to Bacteriology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia.

Isolation and identification of Riemerella anatipestifer from ducks

The pharyngeal samples collected were cultured onto blood agar on the day of collection and incubated at 37°C for 24 h. The resultant plates were read after 24 h. Colonies that are highly suggestive of being *R. anatipestifer* was selected and subcultured onto blood agar to obtain a pure culture. At the same time Gram-staining was performed on the suspected colonies. Identification of *R. anatipestifer* via conventional biochemical tests was performed only if a particular secondary culture was confirmed to be Gram-negative rods. Biochemical tests that were performed included catalase, oxidase, nitrate reduction, oxidative fermentative and gelatin liquefaction tests.

Antibiotic sensitivity testing of Riemerella anatipestifer isolated from ducks

The isolated *R. anatipestifer* was tested against penicillin G, enrofloxacin, oxytetracycline, neomycin, ceftiofur and gentamycin to determine its sensitivity against each of the mentioned antibiotic. Twenty microliters (20 μL) of bacterial isolate suspension in 0.5 McFarland concentration was inoculated onto the Muller-Hinton agar surface in at least three directions to obtain uniform growth. A final sweep was made around the rim of the agar. The plates were allowed to dry for 5 min. Commercial antibiotic discs of penicilin, ampicillin, enrofloxacin, tetracycline, kanamycin, and gentamicin were placed onto the bacteria field on the agar plate using sterile forceps. The plates were incubated at 37°C for 24 h. The diameter of the zone of growth inhibition each disk was measured using calipers.

Results

R. anatipestifer was isolated from one out of 10 pharyngeal swabs of diseased ducks and identified via gram-staining and biochemical tests that included catalase, oxidase, nitrate reduction, gelatin liquefaction and oxidative fermentative tests. *R. anatipestifer* was not isolated from the 40 pharyngeal swabs of healthy ducks. *R. anatipestifer* is highly sensitive to enrofloxacin, oxytetracycline and neomycin, intermediately sensitive to gentamicin and resistant to penicillin G and ceftiofur.

Discussion

One *R. anatipestifer* strain was isolated from out of 10 diseased ducks but none from the 40 healthy ducks. Although only one isolate was detected, this finding is significant as the duck carrying that particular isolate may serve as a source of infection and transmission of *R. anatipestifer* to other ducks within the same farm. Under unfavorable stressful circumstances such heat stress, nutrient deficiency, concurrent bacterial or viral infection and other underlying diseases, *R. anatipestifer* can infect and multiply rapidly, resulting in an outbreak of *R. anatipestifer* with a

mortality rate of up to 95%. The farm owner may suffer great economic loss due to weight loss death, and carcass condemnation of the infected ducks.

Based on the findings in this present study, *R. anatipestifer* does not localize in the upper respiratory tract of healthy ducks. Nevertheless, Sarver *et al*. (2001) successfully isolated 44 *R. anatipestifer* strains from 49 clinically healthy ducks. It is vital to take into account that the sample size in this present study is small and sampling was only done in one farm only, hence it is not representative of the duck population in Malaysia.

Knowing the susceptibility of *R. anatipestifer* to various antibiotics is important as it provides valuable information in deciding the choice of effective treatment, control and prevention of *R. anatipestifer* infection. In this present study, when *R. anatipestifer* that was successfully isolated was subjected to antibiotic susceptibility testing using Kirby-Bauer method, it was found to be highly sensitive to enrofloxacin, oxytetracycline and neomycin, intermediately sensitive to gentamicin and resistant to penicillin G as well as ceftiofur.

Upon comparing the findings of present study with that of previous works conducted on antibiotic susceptibility of *R. anatipestifer*, the antibiotic susceptibility of *R. anatipestifer* is found to change with time, thus it is best to perform antibiotic susceptibility test before prescribing and administering the best choice of antibiotic for *R. anatipestifer* treatment, control and prevention.

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