Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; SP-8(2): 133-137

Received: 12-01-2020
Accepted: 14-02-2020

C Majhi
M.V.Sc. scholar, Department of Veterinary Clinical Medicine, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology CVSc. & AH, OUAT, Bhubaneswar, Odisha, India

GR Jena
Assistant professor, Department of Veterinary Clinical Medicine, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology (CVSc&AH, OUAT), Bhubaneswar, Odisha, India.

L Das
Scientist, (Animal Science), Krishi Vigyan Kendra, Keonjhar, Odisha University of Agriculture and Technology (CVSc&AH, OUAT), Bhubaneswar, Odisha, India.

D Kumar
Scientist, Regional centre, ICAR-Central Avian Research Institute, Bareilly, Uttar Pradesh, India

SK Mishra
Scientist, Regional centre, ICAR-Central Avian Research Institute, Bareilly, Uttar Pradesh, India

A Mishra
VAS, Department of F & ARD, Govt. of Odisha, Odisha, India

MA Elmorsy
Ph. D scholar, Department of Veterinary Clinical Medicine, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology CVSc and AH, OUAT, Bhubaneswar, Odisha, India

MR Das
Professor and head of Department of Veterinary Clinical Medicine, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology CVSc and AH, OUAT, Bhubaneswar, Odisha, India

Corresponding Author:
C Majhi
M.V.Sc. scholar, Department of Veterinary Clinical Medicine, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology CVSc. & AH, OUAT, Bhubaneswar, Odisha, India

Isolation and identification of *Riemerella anatipestifer* from Duck in Odisha, and its susceptibility to antibiotics and therapeutic management

C Majhi, GR Jena, L Dash, D Kumar, SK Mishra, A Mishra, MA Elmorsy and MR Das

Abstract
This study was conducted in the Department of Veterinary Clinical Medicine, C.V.Sc & A.H, OUAT, Bhubaneswar, Odisha, India, for isolation and identification of *Riemerella anatipestifer* from duck in Odisha by culturing, staining and biochemical methods. Also, detection of its susceptibility to antibiotics with reference to therapeutic management and preventive vaccination were done. One hundred and twenty-six samples were collected from twenty-two different farms in Odisha. Results showed that eleven out of twenty-two susceptible farms were positive. The in-vitro antibiotic sensitivity of the organism showed high sensitivity to Ciprofloxacin, Norfloxacin, Gentamycin, Chloramphenicol and Polymyxin-B, moderate sensitivity to Doxycycline, and resistance to penicillin-G, Metronidazole, Sulphadiazine, Methicillin, Ampicillin, Cefuroxime and Erythromycin. Chloramphenicol application at therapeutic doses either through feed or water with supportive therapy like immune modulator multivitamin and hepatic stimulants reduced the mortality rate. As a preventive measure, ring vaccination strategy was adopted and gave good results in prevention of further spread of the disease.

Keywords: *Riemerella anatipestifer*, stain, culture, Antibiotic, sensitivity, biochemical, chloramphenicol, hepatoprotective, vaccination

1. Introduction
Duck rising is a lucrative livestock industry in the globe because of its egg, meat and feather. Like chicken, ducks are reared for eggs and meat. There are over 10 million duck population exists in our India and ranks the 2nd in the world after Indonesia. In India, Kerala, West-Bengal, Odisha and Andhra Pradesh are the States where ducks are predominant. The availability of vast river beds and enormous water bodies are contributing large scope in the development of duck industry.

Infectious diseases like Duck plague (Duck Viral Enteritis), Duck Cholera (Pasteurellosis) and Duck Viral Hepatitis are commonly encountered in village flocks. Most of the farmers ranked duck plague or duck viral enteritis as the most important disease, followed by duck cholera, duck septicemia, botulism and duck viral hepatitis [1]. Surveillance and monitoring programmes are the first steps towards providing an appropriate level of understanding of the health status of duck population. Hence, disease surveillance of duck population is urgently needed to detect the presence of infectious and non-infectious causative agents and to swiftly adopt counter measures to improve the productivity of ducks to assess the importance of common duck diseases and the level of control practices.

Pasteurellosis (Duck cholera) caused high mortality in both domestic and wild water fowl in India [2]. Duck cholera occurs as a relatively common disease, recorded in worldwide including India, and outbreaks occur mainly in young and adult birds with 5 to 100% morbidity and 0.5 to 80% mortality rates. Economic loss to the duck industry from this disease is due to mortality, with rates ranging from 5% to 75%, as well as weight loss and condemnations. Typically, ducklings of 1 to 8-weeks old are highly susceptible. Ducklings under 5-weeks old usually die within 1 to 2 days after appearance of clinical signs, but older birds may survive longer. Stress factors such as concomitant disease or adverse environmental conditions predispose to outbreaks of the disease in ducklings. *Riemerella anatipestifer* is important in veterinary medicine, as it is reported worldwide as the cause of epizootic infectious

Polyserositis of domestic ducks, it is also pathogenic for turkeys, and has been isolated from chickens, pheasants, and waterfowl. Synonyms of Riemerellosis disease are new duck disease, duck septicemia, anatipestifer syndrome, anatipestifer septicemia and infectious serositis [3].

Till date, there was no report recorded on *Riemerella anatipestifer* infection in coastal regions of Odisha and hence, the present investigation was carried out to study the recent outbreak in coastal districts of Odisha.

2. Materials and Methods

The present research work was undertaken to observe the behavioural characteristics of field isolates of *Rimerella anatipestifer* recovered from outbreak samples of different costal and non-costal districts of Odisha. The isolates were studied for cultural, morphological, biochemical characteristics followed by drug sensitivity against commonly used antibiotics. The work of the present study was done at Department of Veterinary Clinical Medicine, C.V.Sc & A.H, OUAT and Regional Centre CARI, Bhubaneswar.

2.1. Cultural and Morphological Characteristics

The test isolates were first inoculated in Nutrient broth at 37º C for 36-48 hours. Then the organism was inoculated on Nutrient agar at 37 ºC for 24 hours, and then on BHI agar, EMB agar and blood agar (BA) at 37 ºC for 24 hours. Next day, single non-haemolytic colony from BA was stained by Gram's method, and then transferred to MacConkey agar (MCA) and incubated at 37ºC for 24 hrs.

2.2. Biochemical Characterization

Test isolates of *Riemerella anatipestifer* were identified by biochemical tests *viz*., Oxidase, Catalase, Indole, Methyl Red, Voges-Proskauer and Carbohydrate fermentation (Dextrose, Galactose, Lactose, Fructose, Sucrose, Xylose, Mannose, Maltose, Sorbitol, Dulcitol, Adonitol, Inositol, Salicin, Inulin, Arabinose and Trehalose) with some minor modifications [4].

2.2.1. Oxidase test

Standard oxidase discs were used to perform the test. The loopful culture from single colony was just touched on the disc. Immediate development of blue colour was considered as positive.

2.2.2. Catalase test

This test was performed by taking 2-3 drops of 3% H2O2 on a clean grease free glass slide and a single colony was mixed with the help of wire loop. Immediate formation of gas bubbles was considered as positive test.

2.2.3 Indole test

Few drops of xylene were added on a two days old growth of the isolate in two ml of tryp tone water, and mixed thoroughly to dissolve indole, and then about 0.2 ml of Kovac's reagent was added. Absence of pink ring of xylene was considered as negative reaction.

2.2.4. Methyl Red Test

1-2 drops of methyl red indicator were added on 2 ml of a 24 - 48 hours old broth of the organism under investigation. Acid produced due to fermentation of glucose by bacterial culture gives red colouration to the medium and gives a positive result, while negative reaction is indicated by development of yellow colour.

2.2.5. Voges-Proskauer Test

6 drops of 5% alpha-naphthol and 2 drops of 40% potassium hydroxide were added on 2 ml of a 24 - 48 hours old broth of the organism under investigation. Absence of pink-red colour at the surface within 30 min was considered as negative.

2.2.6. Carbohydrate fermentation tests

Fermentation reactions of eleven sugars *viz*., Dextrose, Galactose, Lactose, Fructose, Sucrose, Xylose, Mannose, Maltose, Sorbitol, Dulcitol, Adonitol, Inositol, Salicin, Inulin, Arabinose and Trehalose were studied. One per cent of each sugar in peptone water base with 1% Andrade's indicator was used. Few drops of one day old growth of the isolate in peptone water were inoculated into each sugar, and a thin layer of sterile liquid paraffine was spread over the medium. The tubes were incubated at 37 °C for seven days, and readings were recorded after every 24 hrs. Production of pink or red colour was considered as positive reaction.

2.3. In-vitro antibiotic drug sensitivity

For deciding the resistance pattern, the test isolates were grown in BHI broth at 37° C for 8 hours. The plates of BA were seeded with about 1 ml of inoculum, and then allowed to dry. The antibiotic discs were placed on the inoculated agar surface at about two cm apart, and then plates were incubated at 37º C overnight. The diameters of the zones of inhibition were measured where the measurements were compared with the zone size interpretative chart furnished by the manufacturer, and the zones were graded as sensitive, intermediate and resistant.

In-vitro antibiotic sensitivity tests were carried out on 13 commonly used antibiotics such as Chloramphenicol (C30), Doxycycline (DO30), Ciprofloxacin (CIP30), Norfloxacin (NX10), Gentamycin(G50), Polymyxin-B (PB300), Methicillin (MET 100), Sulfadiazine (SZ100), Penicillin-G (P10), Metronidazole (MT5), Ampicillin (AMP10), Cefuroxime (CXM30) and Erythromycin (E15).

2.4. Therapeutic study

As a therapeutic measure, Chloramphenicol was given at the dose of 1 gm / 5 litre of drinking water or 50 gm / 100 kg feed by oral routes along with anti-stress and hepatoprotective supportive therapy.

2.5. Ring vaccination

As a preventive measure, the ring vaccination strategy with inactivated (killed) duck cholera vaccine was applied around the area of study.

3. Result and Discussion

Results of bacterial isolation of 126 samples from 22 farms showed that 11 farms were affected with *Riemerella anatipestifer*, and this represented 50% of the examined farms.

Smears from heart blood stained by Leishman's stain revealed the presence of bipolar organisms, and this was in agreement with the findings of Pillai *et al.* [5] and Heba *et al.* [6].

In the present study impression smears from different organ were taken and were stained by Gram's stain which revealed the Gram-Negative character of the organism, and this was consistent with Heba *et al.*, (2015) [6], Mouahid *et al.*, (1992) [7], Leavitt and Ayroud, (1997) [8] and Priya *et al.*, (2008) [9].

The results of samples culturing showed different morphological characteristics of bacteria on different media

after incubation at 37° C for 24hours. The findings in this study revealed the cultural characteristics like smooth, circular dew drops like colonies on Brain Heart Infusion agar medium (Fig. 1), non-haemolytic colonies on Blood agar medium (Fig. 2), Smooth, Grey, glistening and dewdrop like colonies on Nutrient agar medium (Fig. 3), the bacteria grew but did not produce metallic sheen on EMB agar (Fig. 4), diffused turbidity in Nutrient broth (Fig. 5) and no visible growth in McConkey's broth medium (fig. 6). These observations were similar to those described by Leavitt and Ayroud, (1997) [8].

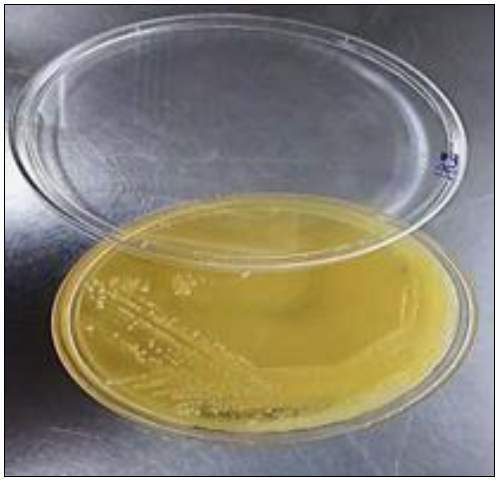

Fig 1: BHI Agar; Smooth circular dew drops like colonies

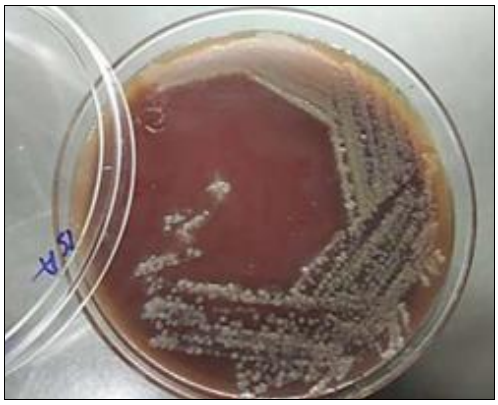

Fig 2: Blood Agar-Smooth, circular, non-haemolytic colony

Fig 3: Nutrient agar –Smooth, Grey, glistening and dewdrop like colonies

Fig 4: EMB agar, The bacteria grew on but did not produce metallic sheen

Fig 5: Nutrient broth- Diffused turbidity.

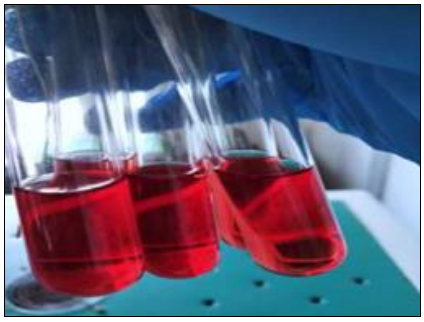

Fig 6: McConkey,s brooth. Didn't grow on McConkey;s broth medium.

The biochemical characters of the test isolates of this study were found negative for Indole (Fig. 9), Voges-Proskauer test (Fig. 11), while positive for Oxidase test (Fig. 7), Catalase test (Fig. 8), Methyl Red test (Fig. 10), and this was in harmony with results of Priya *et al*., (2008) [9] and Heba *et al*., (2003) [10]. Carbohydrate fermentation test revealed that the bacteria fermented mostly lactose, maltose, dextrose and sucrose

Fig 7: Oxidase Test Fig positive

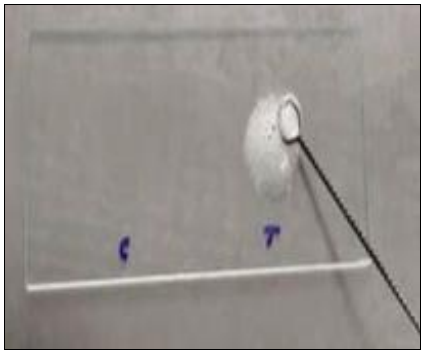

Fig 8: Catalase Test positive

Fig 9: Indole Test Negative

Fig 10: Methyl Red Test positive

Fig 11: VP test negative

In the present study, the test isolates were subjected to commonly used antibiotics for knowing in-vitro sensitivity. The test isolates showed highest sensitivity to Ciprofloxacin followed by Gentamycin, Polymyxin-B and Chloramphenicol, and moderate sensitivity to Doxycycline, but resistant to Methicillin, Sulfadiazine, Penicillin-G, Metronidazole, Erythromycin, Cefuroxime and Ampicillin. This was in agreement with the findings of Heba *et al.,* (2015) [6], Chang *et al.,* (20003) [10] and Zhong *et al.,* (2009) [11]. Similar findings were there, but some contrary results like that Neomycin & Polymyxin B were resistant for this isolate [8]. Maity *et al.,* (2012) [12] Reported similar results as that of the present study, but he found that the organism was resistant to Ciprofloxacin and Lomefloxacin.

As a therapeutic measure, a suitable antibiotic (Chloramphenicol) was given to the ducks. Chloramphenicol was given at dose of 1 gm / 5 lit of water or 50 gm/100 kg feed along with anti-stress and hepatoprotective supportive therapy, and good results were obtained represented in reduction in the mortality rate of the ducks.

As a preventive measure, the ring vaccination strategy with inactivated (killed) duck cholera vaccine was found to be a beneficial preventive measure against this disease.

4. Conclusion

The in-vitro drugs susceptibility of the organism showed high sensitivity to Ciprofloxacin, Norfloxacin, Gentamycin, Chloramphenicol, Polymyxin-B with moderate sensitivity to Doxycycline, and resistance to penicillin G, Metronidazole, Sulfadiazine, Methicillin, Ampicillin, Cefuroxime, Erythromycin. As a therapeutic measure, mortality rate was reduced after application of a susceptible antibiotic (chloramphenicol) with some supportive therapy like immune modulators multivitamin therapy and hepatic stimulants. As a preventive measure, the ring vaccination strategy with inactivated (killed) duck cholera vaccine was found to be a beneficial preventive measure against this disease.

5. References

1. Rahman MA, Ahasan MS. Pasteurellosis in Domestic Ducks: Duck cholera status, Identification, Characterization Bangladesh Paperback, 2010;
2. Kumar AA, Shivachandra SB, Biswas A, Singh VP, Vijendra PS, Srivastava SK. Prevalent serotypes of *Pasteurella multocida* isolated from different animal and avian species in India. Veterinary Research Communications. 2010; 28:657-667.
3. Sandhu TS, Leister ML. Serotypes of *Pasteurella anatipestifer* isolated from poultry in different countries. Avian Pathology. 1991; 20(2):233-9.
4. Cowan ST, Steel KJ. Manual for the Identification of Medical Bacteria. Cambridge University Press, New York, 1965.
5. Pillai RM, James PC, Punnose KT, Sulochana S, Jayaprakasan V, Nair GK. Outbreak of Pasteurellosis among duck population in Kerala. Journal of Veterinary and Animal Sciences. 1993; 24:34-39.
6. Heba N, Samir DA, Mohamed KF, Jakee JE. Identification of Duck Septicemia. Egypt Global Veterinaria. 2015; 15(4):397-400.
7. Mouahid M, Hinz KH, Mutters R, Mannheim W. Characterization of Haemophilus Paragallinarum by Analysis of Whole Cell Carbohydrates, Fatty Acids and Phospholipids. 1992; 21(1):127-36.

8. Leavitt S, Ayroud M. Animal Health Laboratory-Airdrie, Alberta Agriculture, Food and Rural Development. Canine Veterinary Journal, 1997, 38.
9. Priya PM, Pillai DS, Balusamy C, Rameshkumar P, Senthamilselvan P. Studies on outbreak of New Duck disease in Kerala, India. International Journal of Poultry Science. 2008; 7:189-190.
10. Chang CF, Lin WH, Yeh TM, Chiang TS, Chang YF. Antimicrobial susceptibility of *Riemerella anatipestifer* isolated from ducks and the efficacy of ceftiofur from ducks in Singapore: a proposal of new serotypes. Avian Patholog. 2003; 21:453-459.
11. Zhong CY, Cheng AC, Wang MS, Zhu DK, Luo QH, Zhong CD. Antibiotic susceptibility of *Riemerella anatipestifer* field isolates. Avian Diseases. 2009; 53(4):601-607.
12. Maity DK, Chatterjee A, Guha C, Biswas U. Pasteurellosis in duck in west Bengal. Exploratory Animal and Medical Research. 2012; 2:119-123.
13. Eman M EL-Rawy, Afaf A Khader, Manal S Mahmoud, Eman SA Zaki, Selim S Salma, Wafaa S Abd El-Moneim, *et al*. Preparation and evaluation of combined oil adjuvant vaccine against duck pasteurellosis and *Riemerella anatipestifer* infection in ducks. Int J Vet Sci Anim Husbandry. 2020;5(1):18-21.