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Short Communication

MOLECULAR DETECTION OF NEW DUCK DISEASE IN DOMESTICATED DUCKS REARED IN A BACKYARD POND

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SUMMARY

Disease onset was reported from a small-scale duck farm on a backyard pond in Nirjuli, Arunachal Pradesh. Considering the characteristic clinical signs and post-mortem findings suggestive of new duck disease, 66 samples comprising of ocular swabs (21), pharyngeal swabs (21), brain (4), lungs (4), liver (4), heart (4), spleen (4) and kidneys (4) were collected from healthy ducks (n=10), ducks with clinical symptoms (n=7) and dead ducks (n=4). Out of these 66 samples, 23 samples revealed isolation of *Riemerella anatipestifer*. Molecular confirmation of the isolates, using *R. anatipestifer* specific Polymerase Chain Reaction (PCR) assay (564 bp) exhibited the target ribonuclease Z gene to be a suitable molecular marker for identification of the isolates as *R. anatipestifer* and suggests that this PCR assay can facilitate fast and accurate identification of new duck disease in ducks during disease outbreaks. Kirby-Bauer disc diffusion test was used to analyze the antibiotic sensitivity of *R. anatipestifer* isolates (n=23) and based on this antibiogram, treatment was provided to the affected ducks.

Keywords: Antibiogram, new duck disease, polymerase chain reaction (PCR), *Riemerella anatipestifer*, treatment

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Duck rearing is an age-old tradition in the North-Eastern Region (NER) of India and efforts are initiated to encourage duckery as an important component in integrated agro ecosystem farming in rural areas. Many duck diseases, other than duck cholera, duck plague and duck hepatitis, are not adequately reported (Shome *et al.*, 2004). New duck disease or duck septicaemia is caused by *Riemerella anatipestifer* (*R. anatipestifer*) a Gram-negative, microaerophilic, non-motile, bipolar bacteria under the family Flavobacteriaceae of the phylum Bacteroidetes (Gong *et al.*, 2020). It is considered to be an economically important disease throughout the world, resulting in high morbidity and mortality rates in ducks. In recent times, frequent outbreaks of the disease in domesticated ducks have been recorded from Kerala and Assam (Priya *et al.*, 2008; Hazarika *et al.*, 2020; Doley *et al.*, 2021). Diagnosis of the *R. anatipestifer* infection based on clinical signs and symptoms and phenotypic characteristics are often difficult due to the resemblance of the organism with many other bacterial species such as *Pasteurella multocida*, *Salmonella* and *E. coli* (Sandhu, 2003; Sandhu, 2008). Kardos *et al.*, (2007) developed a novel PCR assay that proved to be specific for *R. anatipestifer* and capable of correctly identifying it from pure cultures as well as clinical samples from birds.

In the present study, four domesticated non-descript ducks (3-4 weeks of age) from a small scale duck farm (80 ducks) maintained in a bamboo hut on a backyard pond in Nirjuli, Arunachal Pradesh were found dead. As per the details provided by the owners the ducks showed notable signs of respiratory distress and neurological signs, such as trembling of head and neck, paddling of legs and ataxia before death. On further inspection of the flock, 7 ducks were found showing similar clinical signs and symptoms comprising greenish-white diarrhoea, ocular and nasal discharges, offfed, paddling movement, torticollis and incoordination of movement, often accompanied with the tremor of head and neck. A total of 34 samples comprising pharyngeal (17) and ocular swabs (17) from apparently healthy (10), as well as ailing ducks (7) showing typical clinical signs and symptoms, were collected in Hiculture sterile swab (HiMedia) with 0.85 per cent saline. Whereas, the dead ducks (4) collected from the backyard farm outset were subjected to post mortem (PM) examination and the gross lesions were recorded. Tissue samples comprising spleen, liver, kidney, lung, heart and brain were collected aseptically in sterile vials without adding any preservative and transported to the laboratory on ice packs. None of the samples from the healthy, ailing and dead ducks could reveal duck plague infection, confirmed by PCR based detection of duck plague specific DNA directed DNA polymerase (UL-30) gene. The gross lesions observed during the PM of the dead ducks (4) mostly consist of fibrinous perihepatitis, pericarditis, airsacculitis, congestion of lung, enlarged kidney, necrotic foci on liver and enlargement and congestion of the spleen. Similar PM lesions were also reported by Deif *et al.* (2015) during their

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study on duck septicemia. Considering these characteristic clinical signs and PM findings, the samples were subjected to bacteriological examination targeting the presence of *R. anatipestifer* organisms. Collected samples were processed for isolation of *R. anatipestifer* and the preliminary identification of the bacteria was done based on phenotypic attributes considering colony morphology and staining reaction as described by Hazarika *et al.* (2020). Pure colonies with characteristic Gram-negative bipolar short rods were tentatively identified as *R. anatipestifer* and were subjected to molecular confirmation. Template DNA was extracted from all the tentatively identified bacterial colonies by hot cold lysis method (Titball *et al.*, 1989) and were subjected to *R. anatipestifer* species-specific PCR assay (546 bp) with the primer sequence (forward: 5-TTACCGACTGATTGCCTT CTA-3 and reverse: 5-AGAGGAAGACCGAGG ACATC-3) as described by Kardos *et al*. (2007). PCR amplification was carried out in a Thermocycler (Techne, USA) with reaction mixture (final vol. of 25.0 µl) and cycling conditions as mentioned by Sarker *et al.* (2017). A non-template control without template DNA was included as a negative control to rule out any contamination during the PCR reaction. The amplified PCR products were electrophoresed in 1.5 % agarose gel stained with ethidium bromide for 45 min. at 80V and were visualized by UV light in the DNR Bioimaging System Mini Lumi, Gel documentation system (Fig. 1). The amplified DNA was sequenced from Eurofins Genomics India Pvt. Ltd. Bengaluru, India and the sequence result was validated by performing a sequence alignment with *R. anati pestifer* specific gene sequences in GenBank, using genetic analysis software. The sequenced product showed 96.74 % max identity with GenBank accession no. JN578235.1 showing *R. anati pestifer* strain D-26220 RNase Z and xanthosine triphosphate pyrophosphatase genes, partial cds. with zero E-value.

In the present investigation, bacteriological examination of 66 clinical samples under a micro-aerophilic environment revealed 30 samples to be phenotypically positive, yielding 23 (35.0 %) no. of *R. anatipestifer* isolates confirmed by PCR (Table 1). The majority of the *R. anatipestifer* isolates were recovered from pharyngeal (9) and ocular swabs (7) of apparently healthy ducks, clinically affected ducks and dead ducks followed by samples comprising brain (4) and lungs (3) from dead ducks; which may be due to the reason that the bacterium is found as a commensal in the upper respiratory tract of healthy ducks (Ryll *et al.*, 2001). In another study, Cha *et al.* (2015) reported isolation of the organism (69.6%) of pharyngeal swab samples. Pathanasophon *et al.* (1994) isolated the bacteria from liver, lung, spleen and brain tissue samples. According to Gooderham (1996), the most suitable tissue sample for isolation is the brain due to its less chance of contamination with other bacterial species. *In vitro* antibiogram was determined by Kirby-Bauer disk diffusion susceptibility test (Bauer *et al.*, 1966) against 9 commonly used antimicrobials (HiMedia Lab,

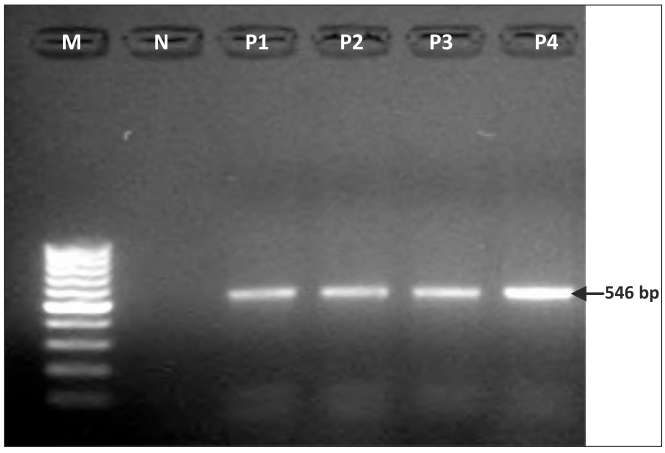

Fig. 1. Detection of *Riemerella anatipestifer* species-specific PCR assay in the field isolates.
M=Marker (100 bp ladder), Lane N= Negative control, Lane P1 to P4=duck isolate positive for *R. anatipestifer* species-specific gene (546 bp).

Table 1

Collection and screening of samples for isolation of Riemerell aanatipestifer isolates

Nature of samples	No. of samples screened for *Riemerella anatipestifer*	No. of isolates phenotypically positive for the *R. anatipestifer*	No. of isolates positive for the *Riemerella anatipestifer* specific gene
1. Healthy Ducks (10)			
Ocular Swabs	10	2	1
Pharyngeal swabs	10	3	2
Sub Total	20	5	3
2. Clinically affected/ailing Ducks (7)			
Ocular Swabs	7	5	3
Pharyngeal swabs	7	6	4
Sub Total	14	11	7
3. Dead Ducks (4)			
Ocular Swabs	4	3	3
Pharyngeal swabs	4	3	3
Brain	4	4	4
Lung	4	4	3
Liver	4	0	0
Heart	4	0	0
Spleen	4	0	0
Kidney	4	0	0
Sub Total	32	14	13
Grand Total	66	30	23(35.0 %)

Figures in parenthesis indicate the percentage rounded off to the nearest value.

Mumbai), *viz.*, Sulfa-trimethoprim (25 mcg/disc), Ciprofloxacin (5 mcg/disc), Enrofloxacin (10 mcg/disc), Ofloxacin (5 mcg/disc), Streptomycin (10 mcg/disc), Neomycin (30 mcg/disc), Lincomycin (15 mcg/disc), Gentamicin (30 mcg/disc) and Cefazolin (30 mcg/disc). Among the 9 different antimicrobial agents used, the isolates were found to be highly sensitive to Sulfa-trimethoprim, Enrofloxacin, Ciprofloxacin, Ofloxacin and Neomycin; intermediately sensitive to Streptomycin and Lincomycin, and resistant to Gentamicin as well as Cefazolin. Based on the antibiogram sensitivity, Biotrim-IM (Sulphadiazine and Trimethoprim injection I.P.) @ 0.2 ml/bird was injected intramuscularly for 5 days in the clinically affected ducks and the rest of the healthy ducks were fed as an oral suspension of Sulcoprim (Sulphamethoxazole and Trimethoprim dispersible powder) for 5 days. The affected ducks responded well to the treatment and other preventive measures like effective cleaning of the bamboo hut, replacement of the litter helped in their faster recovery.

In conclusion, the present investigation is the earliest report of molecularly confirmed isolation of *R. anatipestifer* from ducks of Arunachal Pradesh. The above findings exhibit the presence of *R. anatipestifer* in the domesticated ducks during the disease outset, although it could not establish the pathway of *R. anatipestifer* transmission. Hence, further in-depth studies are necessary to address the knowledge gaps in study of *R. anatipestifer* infection in duck population.

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