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Detection of duck viral enteritis (DVE) by enzyme-linked immunosorbent assay in Jagannathpur region of Jharkhand

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Abstract
Duck farming, like chicken farming, has become a profitable practice worldwide. Ducks are reared for their meat and egg like the chicken industry, but ducks are easier to handle than chickens. Duck plague is another term used commonly in place of Duck viral enteritis (DVE). DVE is one of the few diseases that impose severe economic effects on the duck industry due to high mortality and morbidity. Total 100 random samples were procured from unvaccinated duck of Jagannathpur region of Jharkhand. All samples collected from Jagannathpur region of Jharkhand were detected negative by Enzyme-Linked Immunosorbent Assay (ELISA).

Keywords: Duck viral enteritis, duck plague, enzyme-linked immunosorbent assay

Introduction
Duck farming is an important part of poultry farming in India. Ducks are primarily reared for their meat and eggs. Duck farming in India has gained popularity due to several factors, including the relatively low cost of production, adaptability to various climatic conditions, and the demand for duck products. They require less care, and simple housing, and grow very fast. Being low maintenance, it is quite popular in many Asian countries including India (Rajput *et al*., 2014) [23]. Duck Viral Enteritis is caused by Anatid herpesvirus 1 or Duck Enteritis Virus (DEV). Duck plague is another term used commonly in place of DVE. The term was coined by Bos in 1942 and Jansen and Kunst in 1949 first used it officially. It is a lethal, acute, or chronic, contagious disease of geese, swans, and ducks (Davison *et al*., 1993; Dhama *et al*., 2017) [7, 8] and is not found to harm members of other mammalian and avian species. In several countries, it is called other synonyms like Entenpest in Germany, peste du canard in France, eendenpest in Denmark, and duck plague in the USA (Davison *et al*.; 1993) [7]. The disease is reported globally in domestic and wild waterfowl. The first outbreak of DEV was reported by Baudet in 1923 in Netherland and confirmed by DeZeeuw in 1930 (Friend and Pearson, 1973; Dhama *et al*., 2017) [9, 8]. In 1967, another outbreak was reported in Long Island, New York in the duck industry which led to the loss of $1 million (Plummer *et al*.,1998) [21]. DVE infection occurs by encountering infected and vulnerable ducks by direct or indirect interaction with an infected environment (Sandhu and Shawky, 2003) [23]. Virus transmission occurs by natural water-borne channels. Outbreaks are common in those duck flocks which have easy access to infected water bodies or live with free wild birds. Infected tissues can be administered via parenteral, intranasal, or oral routes to create an experimental infection. In wild birds, a carrier condition is suspected. Recovered birds become latently infected carriers, allowing the virus to be transmitted intermittently. Migratory birds play an integral role in disease transmission. The DVE virus, like other herpesviruses, may fall into latency, and the trigeminal ganglion appears to be a latent location for the virus. Survivors from the natural outbreak exhibited viral shedding up to 4 years of infection. This may lead to latent infection which can often reactivate and serve as a persistent carrier of the disease, maintaining the virus and the disease in the ecosystem. Many birds which are resistant to disease also serve as a carrier of the virus (Plummer *et al*., 1998) [21].
Presently there is no therapy available for this disease. Vaccinations are the only available remedy for this disease. Direct or indirect contact with diseased birds or water sources, as well as wild, free-flying ducks, should be avoided as a preventive measure.

Depopulation, evacuation of diseased birds from the affected area, cleanliness, and disinfection are all used to control the disease. Prevention relies on keeping vulnerable birds in a clean disease-free habitat. Domestic ducklings older than 2 weeks are given the live attenuated vaccine. Annual revaccination of breeding flocks is recommended. As it induces quick protection following immunization, the vaccine can also be utilized in an outbreak. In the case of duck plague, an attenuated vaccine is more useful compared to an inactivated vaccine, which is ineffective to produce immunity against the virus (Shawky and Sandhu, 1997) [25].

Currently, clinical signs and lesions are used as a presumptive diagnostic means while several laboratory methods are available to detect DEV and give confirmatory and differential diagnoses. Various laboratory techniques available are virus isolation, virus neutralization, molecular methods including PCR, in-situ hybridization using a specific oligonucleotide probe (Cheng *et al*., 2008) [6], and LAMP-based nucleic acid amplification (Ji *et al*., 2009; Jiang *et al*., 2012) [13, 14]. Serological techniques also enhance the detection and screening of many birds. neutralizing antibodies against DEV is suitable to use as a tool in the diagnostic assay (Aravind *et al*., 2012; Dhama *et al*., 2017) [1, 8]. The immunological tests commonly used for duck plague include FAT, virus neutralisation assay, and reverse passive hemagglutination test (RPHA) (Sandhu and Shawky, 2003) [23]. Out of these, FAT is considered the second most sensitive, next to virus isolation. Enzyme-Linked Immunosorbent Assay is the most common diagnostic assay used in the laboratory for disease diagnosis from the serum sample. Indirect ELISA for DEV is preferred to detect the antibodies against the duck plague virus. It can be standardized and optimized to make it suitable for screening the prevalence of disease in the area and monitoring the outbreak (Neher *et al*., 2019) [20].

Materials and Methods

Samples

The present study was conducted in CADRAD, ICAR-IVRI, Izatnagar, total 100 random samples are procured from unvaccinated ducks of Jagannathpur region of Jharkhand.

Standardization of ELISA

96 well ELISA plate was coated with 1:500 dilution of purified concentrated gI protein of DEV in Carbonate-Bicarbonate buffer (coating buffer) and incubated at 4 ℃ overnight. Ten-fold serial dilution (1:10 to 1:80) of known infected, negative, and challenged duck sera were prepared in the blocking buffer (3% LAH in 0.05% PBST) to finalize the serum dilutions. ELISA plate is washed 3 times with wash buffer (0.05% Tween-20 in PBS) without any hold time. Each well of the ELISA plate was added with 50 µl of diluted sera sample in duplicates. It was then incubated at 37 ℃ for 1 hour. The plate was then washed using PBST wash buffer 3 times followed by discard. 3 washes were done with a hold time of 3 minutes in each wash. The polyclonal anti-duck HRP-conjugate antibodies were diluted to 1:3000 in the blocking buffer and 50 µl was added to each well. The plate was then incubated at 37 ℃ for 1 hour. Plates were washed using wash buffer 3 times having 3 minutes of hold time at each wash. Substrate solution was added 50 µl in each well. The plate is incubated at 37 ℃ for 15 minutes. The reaction was stopped using 50 µl of stop solution in each well. Reading was taken at 492 nm in an ELISA reader. To validate the developed ELISA assay, diagnostic sensitivity and diagnostic specificity were analysed. The known status of birds before sampling was used for the categorization of the samples. Further, serum samples collected at random (n=100) from Jagannathpur, Jharkhand were tested with the optimized ELISA protocol.

Validation of ELISA

To validate the developed ELISA assay, diagnostic sensitivity and diagnostic specificity were analysed. The known status of birds before sampling was used for the categorization of the samples. Further, serum samples collected at random (n=100) from Jagannathpur, Jharkhand were tested with the optimized ELISA protocol.

Results and Discussion

Based on the findings of the checkerboard titration, the final concentration of the antigens and serum was set at 20 ng/well and 1:20 dilution for I-ELISAs based on recombinant gI. 100% positive samples reacted with the protein whereas 100% negative samples did not produce any OD. No reactivity could be observed with unvaccinated and un-infected samples. The random samples procured from Jharkhand were all negative detected by ELISA.

Conclusion

DEV is a lethal, acute, or chronic, contagious disease of geese, swans, and ducks. The absence of reported cases of DEV in Jagannathpur, Jharkhand is a positive development for the local livestock industry and the overall agricultural landscape. Efforts in disease surveillance, vaccination programs, and biosecurity measures play a crucial role in preventing and controlling DEV. Maintaining DEV-free status is essential for the agricultural economy in Jharkhand, as the disease can lead to significant economic losses through reduced egg and meat production, trade restrictions, and animal suffering. It is important to continue rigorous monitoring and vaccination campaigns to prevent the introduction and spread of DVE in the region.

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