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Detection of humoral and cell mediated immune responses among breeder ducks vaccinated against riemerellosis#

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Abstract

Riemerellosis is an infectious disease that primarily affects young ducklings, although it can infrequently be manifested in a chronic localised form in older ducks. Though an inactivated vaccine has been found to be most effective in preventing the disease in ducklings, its potency in adult breeder ducks has not been assessed yet. To investigate the same, 200 Kuttanad breeder ducks maintained in the University Poultry and Duck Farm (UPDF), Mannuthy were grouped into two, of 100 birds each, T_1 being the control group and T_2, the treatment (vaccinated) group. The oil-adjuvanted inactivated vaccine, prepared as per the previously standardised protocol, was administered to the T_2 group in two doses, one week apart. Blood samples in serum vials, 20 each from T_1 and T_2, were collected on days 0, 14, 28, 56 and 90 post-immunisation (PI) whereas blood samples in heparin vials, eight each from both the groups, were collected on days 0, 14, and 28 PI. Humoral immunity (HI) and cell mediated immunity (CMI) in the adult ducks were assessed by enzyme-linked immunosorbent assay (ELISA) and lymphocyte proliferation assay (LPA), respectively. The two assays revealed that the inactivated vaccine elicited a good immune response in the adult ducks, with HI noticed until 90th day PI and an increasing CMI that peaked on 28th day PI.

Keywords: *Riemerellosis, duck, inactivated vaccine, humoral immunity*

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Duck rearing holds a pivotal position in the poultry sector, one of the thriving industries in India that contributes significantly to the economic security of the country. Kerala is one among the several states that offer an ideal environment for profitable duck rearing (DAHD, 2019). However, various factors have proved detrimental to the sector, preventing it from achieving the projected growth rates over time, the major one being infectious diseases (George *et al*., 2017). Riemerellosis, caused by *Riemerella anatipestifer*, is one such disease whose occurrence in the state has constantly been on the rise, ever since its first confirmed report in an organised duck farm (Priya *et al*., 2008).

Though riemerellosis primarily occurs as a septicaemic infection in young ducklings, it can also be manifested in a chronic form in adult ducks (Bisgaard, 1995; Sandhu, 2003, Sabnam *et al*., 2017), causing significant economic loss to the farmers in terms of treatment cost, reduced egg production, mortality and carcass condemnation. Over the years, various vaccines have been developed, their efficacy in ducklings studied and the superiority of an oil-adjuvanted inactivated vaccine has been proved (Balan, 2019). But its potency has not been assessed in breeder ducks and hence the same has been attempted in this study.

Materials and methods

Birds used

A total of 200 breeder stock of Kuttanad ducks maintained in the University Poultry and Duck Farm (UPDF), Mannuthy, Thrissur were allocated into two groups of 100 birds each, T_1 being the control group and T_2, the (treatment) vaccinated group.

Immunisation and sample collection

The oil-adjuvanted inactivated vaccine prepared as per the previously standardised and patented protocol, was administered subcutaneously to T_2 group in two doses of 0.5 mL each, one week apart. Blood samples in serum vials, 20 each from T_1 and T_2, were collected on days 0, 14, 28, 56 and 90 post-immunisation (PI) to separate serum, whereas blood samples in heparin vials, eight each from both the groups, were collected on days 0, 14, and 28 PI to separate peripheral blood mononuclear cells (PBMC). The sera and PBMC were made use of to evaluate humoral immunity (HI) and cell-mediated immunity (CMI), respectively.

Assessment of humoral immunity

Indirect ELISA as per the method described by Balan (2019) was carried out in 96-well flat-bottom plates to assess the HI in the ducks. The optimum concentration of the whole cell antigen, primary antibody (serum) and secondary antibody used in the assay were calculated by checkerboard titration. Whole cell antigen, extracted as per the method of Hatfield *et al*. (1987) with modifications in the centrifugation (4000 x g for 20 min.), was diluted to make two-fold dilutions of 1: 50, 1:100 and 1:200. Hyperimmune serum and negative serum maintained in the Department of Veterinary Microbiology, CVAS, Mannuthy, were used as positive and negative controls, respectively. They were diluted in ratios ranging from 1:50 to 1:400. This was followed by the dilutions of secondary antibody (anti-chicken IgY-HRP conjugate) ranging from 1:1000 to 1:8000.

Cut-off value was calculated from an ELISA using negative samples in duplicates. The mean absorbance and standard deviation above the mean were calculated from the replicates. The resultant mean absorbance plus three standard deviation unit was taken as the cut-off value. The samples were screened in serial two-fold dilutions, starting from 1:25 to 1: 51200 and the highest dilution of the test serum showing absorbance more than the cut-off value was taken as the titre (Prakash *et al*., 2005).

Assessment of cell mediated immunity

Cell mediated immune response of the ducks to the vaccine was assessed using lymphocyte proliferation assay (LPA) for which the PBMC were separated from the blood using HiSepTM LSM (Lymphocyte separation medium) and re-suspended in Roswell Park Memorial Institute 1640 (RPMI 1640) medium.

These were then stimulated using pure culture of *R. anatipestifer* (0.5 µg /well) in 96-well flat-bottom plates at 37 °C in five per cent CO_2 for 72 h. Unstimulated PBMC and those added with concanavalin-A, were taken as the negative and positive controls, respectively. After 72 h., MTS tetrazolium (5mg/mL in PBS) was added to each well and the plates were further incubated for three hours under the same conditions. Finally, dimethyl sulfoxide (DMSO) was added after discarding the culture supernatant from each well and the absorbance was measured at 492 nm. The stimulation index (SI), was then calculated using the following formula

Stimulation index (SI) = Mean OD of stimulated culture

Mean OD of unstimulated culture

Statistical analysis

The data were analysed employing independent sample *t*-test using SPSS version 24.0.

Results and discussion

Humoral immunity in the adult ducks

In the checker board titration, the optimum concentration of both the antigen and the primary antibody (sera) was found to be at 1:50 dilution, while that of the conjugate was observed to be at 1:4000. The mean OD of the negative sera was 0.983 with a standard deviation of 0.1104. The cut-off value was calculated to be 1.313. The samples were serially diluted from 1:25 to 1: 512000 and the highest dilution of the test serum with absorbance more than this value was taken as the titre. The data were tabulated and statistically analysed using independent sample *t*-test as shown below.

Statistical analysis of the ELISA titre of adult duck sera revealed a significant difference between the titre values of groups T_1 and T_2 on all the days assessed, except the 0^{th}. This indicates the potency of the vaccine to elicit sufficient HI in the ducks. In a similar study, antibody titre was recorded in White Pekin ducks, in response to an inactivated vaccine, which was found to be maximum on day 48 PI (Lobbedey and Schlatterer, 2003).

Cell mediated immunity in the adult ducks

The CMI was assessed using the LPA. The extent of lymphocyte proliferation was estimated from the SI calculated on days 0, 14 and 28 (Table 2).

Conclusion

Exhaustive investigation of all aspects and parameters of a vaccine is pertinent to exploit its full potential effectively in the target species. Assessment of HI by ELISA and that of

Table 1. Statistical analysis of ELISA titre in control and vaccinated birds

Days	T_1 (Control) group Mean ± SD	T_2 (Vaccinated) group Mean ± SD
0	$0.5123^a \pm 0.64935$	$0.52^a \pm 0.21564$
14	$0.8180^a \pm 0.60047$	$1.6113^b \pm 0.18653$
28	$0.5651^a \pm 0.58085$	$1.5027^b \pm 0.10166$
56	$1.1285^a \pm 0.84144$	$1.4803^b \pm 0.12251$
90	$1.0486^a \pm 0.53144$	$1.5817^b \pm 0.22812$

Mean values bearing different superscripts within the row differ significantly ($p< 0.001$)

Table 2. Stimulation Index

Days	T_1 (Control) group	T_2 (Vaccinated) group
0	0.56	0.54
14	0.61	0.94
28	0.64	1.26

Proliferation of the lymphocytes, in response to the vaccine, was found to increase from day 0 till 28, being the highest on day 28 PI. A similar pattern was observed by Balan (2019) in ducklings.

CMI by LPA in the present study revealed that the oil-adjuvanted inactivated vaccine elicited good immune response in terms of HI and CMI, respectively in the immunised adult ducks when compared to the control birds. This data might pave way for future research on establishment of a vaccination regimen against riemerellosis in ducks and assessment of maternal transfer of antibody from the vaccinees to their offspring via egg yolk. These would aid in effectively curbing the disease at critical checkpoints, thereby saving duck farmers from the huge economic loss inflicted on them by the disease.

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Conflict of interest

The authors declare that they have no conflict of interest.

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