

Original Research Article

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## Assessment of Cell Mediated Immune Response to *Riemerella anatipestifer* Vaccines

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### ABSTRACT

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New duck disease / riemerellosis, reported in Kerala from 2008 onwards from various parts of the state, causing severe economic loss in the duck industry. A Gram negative bacterium, *Riemerella anatipestifer* was identified as the causative agent. Due to the presence of 21 serotypes of the organism with little/ no cross-protection, development of an effective vaccine is the only solution to curtail the disease. The present study was conducted to assess the cell mediated immune (CMI) response elicited by inactivated and subunit vaccines against riemerellosis, employing lymphocyte proliferation assay (LPA) and leukocyte migration inhibition test (LMIT). The inactivated vaccine evoked significant CMI response in host system and LPA could be identified as an effective tool for assessing it.

### Introduction

Riemerellosis is a bacterial disease among ducks, caused by *Riemerella anatipestifer*, which has been well documented as a cause of considerable economic loss to the duck production in Kerala. At least 21 serotypes of

the organism have been identified globally. Hence, vaccination is the mainstay for the control of the disease. Various workers employed ELISA to screen antibodies against riemerellosis (Huang *et al.*, 2002; Lobbedy and Schlatterer, 2003). Shancy (2015) extracted outer membrane protein (Omp) from

*R. anatipestifer* isolate of Kerala and standardised Omp based ELISA. Ahmad (2017) evaluated Omp and recombinant Omp A (rOmpA) antigens for the serodiagnosis of *R. anatipestifer* infection in ducks and found that rOmpA based ELISA was highly sensitive and better choice than Omp based ELISA. Cellular immunity was also important for the evaluation of immune response in addition to humoral immunity. Information on the effects of currently available *R. anatipestifer* vaccines on CMI is scanty. Hence, the present study was conducted to assess the CMI response elicited by inactivated and subunit vaccines against *R. anatipestifer* infection, employing LPA and LMIT.

## Materials and Methods

### Preparation of bacterial suspensions for vaccine preparation

*Riemerella anatipestifer* isolate (designated as RA1) maintained in the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, as lyophilized form was revived by adding brain heart infusion broth (BHIB) supplemented with bovine serum albumin (BSA) at five per cent concentration. It was streaked onto the blood agar (BA) plates, incubated for 24 to 48 h at 37°C in a candle jar.

### Vaccination programme

A total of 52, day-old ducklings were divided into three treatment groups with ten birds each. They were injected with 0.5 mL of different types of vaccine subcutaneously. Group I (T1) served as control with 22 birds including six birds each for challenge control of inactivated and subunit vaccine. Group II (T2) was injected with an inactivated vaccine ( $7 \times 10^9$  cfu/ mL), which was prepared as per the protocol standardised in the Department of

Veterinary Microbiology and group III (T3) and group IV (T4) were administered with different antigen concentration of subunit vaccine (equal quantity of the rOmpA protein (250 µg and 500 µg) and montanide), respectively. A booster dose was given at third week post-primary vaccination to T2, T3 and T4.

### Assessment of cell mediated immune responses

The CMI response was assessed by separating peripheral blood mononuclear cells (PBMC) from blood collected from control and vaccinated ducks at 0, 14 and 28 days primary immunization (PI) by LPA (Lee *et al.*, 2010) and LMIT as per Bendixen (1977).

### Lymphocyte proliferation assay (LPA)

To determine the extent of antigen-specific lymphocyte proliferation, PBMC ( $10^5$  cells/well) from immunized ducks was incubated with *R. anatipestifer* on 96-well plates for 72 h at 37°C and 5 per cent CO<sub>2</sub> tension. RPMI-1640 (Roswell Park Memorial Institute) medium was used as the negative control and 2 µg/ mL Con A (Sigma, CA, USA) was used as the positive control.

At the end of incubation, 20µL of MTS tetrazolium (Promega, Madison WI, USA), were added to each well and the plates were read at 492 nm and stimulation index (SI) was calculated as

$SI = (\text{Mean OD of stimulated culture}) / (\text{Mean OD of unstimulated culture})$ .

### Leukocyte migration inhibition test (LMIT)

Leukocytes were isolated from the blood using GranuloSep™ GSM 1119 and HiSep™ LSM 1077 (Hi-Media Laboratories, Mumbai). Agarose (two per cent w/v in distilled water)

was boiled and cooled to 45°C and equal quantity of double concentration of Hank's balanced salt solution (HBSS), pH 7.2, containing one per cent lactalbumin hydrolysate, 0.4 per cent yeast extract, 20 per cent foetal calf serum (FCS), streptomycin (200 µg/ml) and penicillin (200 IU per ml) was added.

The agarose medium, kept at 45°C, was poured into Petri dishes to a height of three millimeter thickness and was incubated at 37°C for one hour prior to use.

Leukocyte suspension prepared from the peripheral blood was divided into two equal parts and to one portion 0.1 mL of antigen was added and to the other portion 0.1 mL of sterile normal saline (NS) was added.

Contents of each tube were thoroughly mixed and incubated for one hour at 37°C with occasional shaking to avoid cell clumping. The contents of each tube were filled in six wells of three millimeter diameter, cut eight millimeter apart in the agarose gel. The charged plates were incubated at 37°C in a humid chamber for 20 h.

At the end of incubation period, the cells were fixed to the glass surface by flooding the plates with methanol acetic acid fixative (seven parts methanol + one part acetic acid + two parts distilled water) for 15 min. The agarose gels in the plates were then partially dried to facilitate their peeling off from the plates.

Migration area of leukocytes was measured by taking average diameter of the opaque zone around the wells. The migration index was calculated as the average area of migration of cells treated with antigen divided by the average area of migration of cells treated with NS. A LMIT index less than 0.8 was considered as a positive reaction.

## **Results and Discussion**

### **Preparation of vaccines**

All the prepared vaccines were homogeneous suspensions, which facilitated subcutaneous administration. They were sterile and did not cause any side effects.

### **Measurement of cellular immune responses**

#### **Lymphocyte proliferation assay**

The proliferation of lymphocytes was higher on 28th day of post-vaccination when compared to 0th and 14th days. The SI of the PBMCs isolated from T2 group was maximum on 14th and 28th day followed by treatment groups that received 500 µg rOmpA (T4) and 250 µg rOmpA (T3) (Table 1 and Fig. 1).

#### **Leukocyte migration inhibition test**

No opaque zones were noticed at the end of incubation period both from control and vaccinated birds on day 0, 14 and 28 days PI.

In the present study, CMI response was measured at 0, 14 and 28 days PI by LPA and LMIT. Saravanan *et al.*, (2003) found that *in vitro* MTT assay was found to be a simple and convenient method for assessing the cell activation rate and growth.

Direct migration inhibition assay under agarose was proved to be a convenient, rapid and easily reproducible method for LMIT (Clausen, 1971; Bendixen, 1977; Azadegan *et al.*, 1981). The advantage of this technique is that the plates can be fixed, stained and evaluated at any time and/ or save as a permanent record in addition that the test does not require any expensive equipment.

Lymphocytes were isolated from vaccinated and control groups at various days post-

vaccination and cultured for 72 h. The SI of lymphocytes from vaccinated birds was higher than from non-vaccinated ones. The mean SI values of all the vaccinated groups were higher on day 28 when compared to on day 14. Highest SI was noticed in birds inoculated with inactivated vaccine when compared to subunit vaccine groups. Chu *et al.*, (2015) evaluated the immune response and protective efficacy provided by a subunit vaccine containing rOmpA and plasmid constructs containing CpG ODN. The SI of the PMBC from the rOmpA + CpG ODN vaccine group was significantly higher than that of the rOmpA group by LPA, on both day 14 and 28 after immunisation.

On LMIT, no migration zones were noticed during pre and post-vaccination periods. Surya (2009) carried out LMIT to assess the CMI response of whole cell and membrane protein vaccines against *Mycoplasma gallisepticum* and noted the leukocyte migration index to be above 0.8 from the samples collected at third week post booster vaccination. This is in contrary to the results of the present study wherein, no zone of inhibition could be documented against *R. anatipestifer* vaccine during this time period. The difference in time required to achieve the peak response in both the studies might be attributed to the difference in the bacterial spp. and host system, which would have contributed to different patterns of immune response.

**Table.1** Stimulation index (SI)

| SI                         | 0      | 14     | 28     |
|----------------------------|--------|--------|--------|
| <b>Inactivated (T2)</b>    | 0.1188 | 0.9803 | 1.2314 |
| <b>rOmpA (250 µg) (T3)</b> | 0.1188 | 0.1896 | 0.2419 |
| <b>rOmpA (500 µg) (T4)</b> | 0.1188 | 0.2101 | 0.2784 |

**Fig.1** Lymphocyte proliferation assay



Cell mediated immune response was assessed by separating PBMC from blood collected at 0, 14 and 28 days post vaccination by LPA

and LMIT. On LMIT, no migration zones were noticed at the end of incubation period during pre and post-vaccination periods.

On LPA, maximum proliferation of lymphocytes was achieved on 14th and 28th post-vaccination in T2 group when compared to subunit vaccine groups (T3 and T4). It was concluded that *R. anatipestifer* inactivated vaccine evoked significant CMI response in host system and LPA could be an effective tool for assessing the same.

## References

- Ahmad, S. M. 2017. Development of recombinant outer membrane protein A based ELISA for the diagnosis of *Riemerella anatipestifer* infection in ducks. *M. V. Sc thesis*, Kerala Veterinary and Animal Sciences University, Pookode, 82 p.
- Azadegan, A., Kaneene, J. M. B., Muscoplat, C. C. and Johnson, D. W. 1981. Development of a migration inhibitory factor assay under agarose of bovine mononuclear leukocytes using an antigen of *Brucella abortus*. *Am. J. Vet. Res.* 42: 122.
- Bendixen, P. H. 1977. Application of the direct leucocyte migration agarose test in cattle from a *Mycobacterium paratuberculosis*- infected herd. *Am. J. Vet. Res.* 38: 66-70
- Chu, C., Liu, C., Liou, J., Lee, J. and Cheng, L. 2015. Development of a subunit vaccine containing recombinant *Riemerella anatipestifer* outer membrane protein A and CpG ODN adjuvant. *Vaccine.* 33: 92-99.
- Clausen, J. E. 1971. Tuberculin-induced migration inhibition of human peripheral leukocytes in agarose medium. *Acta Allerg.* 26: 56-63.
- Huang, B., Kwang, B.J., Loh, H., Frey, J., Tan, H.M. and Chua, K. L. 2002. Development of an ELISA using a recombinant 41 kDa partial protein (P45N') for the detection of *Riemerella anatipestifer* infection in ducks. *Vet. Microbiol.* 88: 339-349.
- Lee, J.W., Lin, Y.M., Yen, T.Y., Yang W.J. and Chu, C.Y. 2010. CpG oligodeoxynucleotides containing GACGTT motifs enhance the immune responses elicited by a goose parvovirus vaccine in ducks. *Vaccine.* 28 (50):7956-7962.
- Lobbedey, L. and Schlatterer, B. 2003. Development and application of an ELISA for the detection of antibodies against *Riemerella anatipestifer* antigens in egg yolk of vaccines and in serum of their offspring. *J. Vet. Med.* 50: 81-85.
- Saravanan, B. C., Sreekumar, C., Bansal, G. C., Ray, D., Rao, J. R. and Mishra, A. K. 2003. A rapid MTT colorimetric assay to assess the proliferative index of two Indian strains of *Theileria annulata*. *Vet. Parasitol.* 113: 211-216.
- Shancy, C. 2015. Standardisation of outer membrane protein based ELISA to detect antibodies against new duck disease. *M. Sc thesis*, Kerala Veterinary and Animal Sciences University, Pookode, 79p.
- Surya, S. 2009. Development and evaluation of whole cell and membrane protein vaccines against *Mycoplasma gallisepticum*. *Ph.D. thesis*, Kerala Veterinary and Animal Sciences University, Pookode, 186p.

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