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# ANTIBODY RESPONSE OF RABBITS TO COMBINED HEMORRHAGIC SEPTICEMIA AND FOOT & MOUTH DISEASE VIRUS VACCINE

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## ABSTRACT

Infectious diseases particularly Foot and Mouth Disease (FMD) & Hemorrhagic septicemia (HS) are challenge for livestock and dairy industry all over the world and cause great economic losses. Vaccination programs using monovalent vaccines against the diseases are effective but the repeated vaccination induces stress to the animals and increase cost of vaccination for the farmer. In this study, efficacy of bivalent FMD+HS vaccine was compared to monovalent FMD and HS vaccines in rabbits. Previously isolated and characterized FMD virus "O" serotype and *Pasteurella multocida* were used in the experimental design. Alum precipitated FMD, HS and FMD+HS vaccines were prepared and injected in experimental rabbits (6 months of age) at 0 and 14th day. The serum samples were collected on 0, 14, 28, 35 and 48 days post-priming. Antibody titer was measured by Virus Neutralization (V/N) for FMD "O" virus and Indirect Hemagglutination (IHA) test for lipopolysaccharide (LPS) of *Pasteurella multocida*. The results showed that peak level of Geometric mean antibody titer (50.8 VN units & 40.3 IHA units) was obtained on 35th day in both the monovalent FMD and HS vaccine, respectively whereas in bivalent FMD+HS vaccine peak level of GMT antibody titer (161.3 VN units +50.8 IHA units) was detected on 48th days post-priming. It is concluded that combine bivalent vaccine produced better antibody titer that persisted for longer time.

**Key words:** Food and Mouth Disease, *Pasteurella multocida,* Monovalent, Bivalent.

## INTRODUCTION

Bacterial and viral diseases cause enormous economic loss through morbidity and mortality to livestock which contribute 39% of agriculture value added and about 9.4% of GDP. About 35 million people are deriving 20-25% income from livestock.

Hemorrhagic septicemia (HS) is an important bacterial disease of cattle and buffaloes occurring in many Asian and African countries, resulting in high mortality and morbidity. In many Asian countries like Pakistan, disease outbreaks mostly occur during the change in climatic conditions typical of monsoon. The disease is caused by *Pasteurella multocida*, a gram-negative coco-bacillus and aerobic residing mostly in the upper respiratory tract of animals. Foot and Mouth disease (FMD), a highly contagious viral disease affecting primarily cloven footed animals and is of most concern all over the world (Pereira, 1981). The causative agent (virus) belongs to the genus apthovirus of Picornaviridae (Dietzschold and Ahl, 1970*)*. In Pakistan, the most prevalent serotypes are O (70%), Asia-I (25%) and A (4.67%) causing a loss of Rs. 6.00 billions to farmers annually (Awan *et al.,* 2009; Khawaja *et al.,* 2009*)*.

Vaccination is an important tool to control the infectious diseases and is considered as effective method to control the diseases (Salt *et al.,* 1999; Sutmoler, 2003*)*. When an animal is subjected to vaccination, both humoral and cellular wing become activated and memory against them is induced. Later on when infectious agent enters the body, it effectively eliminates these agents from the vaccinates before the occurrence of the disease *(*Twomey *et al.,* 1995).

Routine vaccination program of dairy animals against infectious diseases in developing countries is important for increasing milk production and reducing economic losses. Combined vaccination helps to reduce the cost of vaccination program per annum. In large animal, different combinations like FMD + HS (Joseph and Hedger, 1984), FMD+ Rinderpest (Hedger *et al.,* 1986), FMD and rabies antigens (Palanisamy *et al.,* 1992) have been tried effectively. In many South and South-East Asian Countries FMD, HS and Black Quarter (BQ) vaccines are commonly used (Srinivasan *et al.* 1997). In combine vaccine trial, the antibody response of vaccinate to either of pathogen is either better or equivalent. The results indicate that the antigens in combined vaccines are compatible to each other.

## MATERIALS AND METHODS

**Preparation of FMD Vaccine:** Cryopreserved BHK-21 cells were obtained from Quality Operations Laboratory (QOL) University of Veterinary and Animal Sciences (UVAS), Lahore and revived for culturing in strict

sterilized condition. The live cell percentage was calculated using hemo-cytometery method as described by Ghori *et al.* (2011). The cell suspension was diluted using growth medium to $2.5x10^5$ cells per ml and 15 ml of the diluted cell suspension was transferred to each of the 25 $cm^2$ roux flask. The flasks were incubated at 37°C for 48 hours. The flasks having complete monolayer were selected for the propagation of previously isolated and characterized FMD "O" type virus (OIE 2006). Biological titer (Tissue culture dose 50-$TCID_{50}$) of "O" serotype of FMD virus was determined as described by Reed and Munch (1938). The FMD virus "O" type suspension was inactivated using binary ethyleneimine (Bahnemann, 1975). The inactivated virus suspension was admixed with Thiomersal sodium at rate of 0.05 percent and then with aluminum hydroxide gel to obtain 2% final concentration in the vaccine (Ilyas *et al.* 2004). Quality control procedures were carried out as described by Muhammad *et al.* (2011). The vaccine thus prepared, was transferred into properly labeled vials and stored at $4^0$ C in the refrigerator till use.

**Preparation of Hemorrhagic Septicemia Vaccine:** The pure, identified and characterized culture of *Pasteurella multocida* was inoculated into the fermenter containing casein sucrose yeast (CSY) broth (OIE 2009; Sarma, 1980). The prepared medium in fermenter vessel was sterilized by autoclaving at 121$^o$C for 15 minutes and vessel was attached with bio-fermenter. The temperature, pH, BOD was adjusted at $37C^0$, 7.4 and 100%, respectively, using control panel of the fermenter. The fermenter was run for 24 hours. Biological titer of the bacteria was determined in terms of dry mass and total viable bacterial count. For inactivation of the bacterial culture formaldehyde (37%) was added in the culture at the rate of 0.5% and the culture flask was incubated at 37 $^0$C for 24 hours. Quality control procedures were carried out as guided by OIE, (2009). Alum based Hemorrhagic Septicemia Vaccine (AHSV) was prepared from the inactivated mass culture by the technique as described by Ali *et al.* (2000).

**Preparation of Multivalent Vaccine:** Inactivated Foot & Mouth Disease Virus suspension and *Pasteurella multocida* culture were mixed together at the rate of $10^6$ units of $TCID_{50}$ and 2.2mg/dose, respectively at $4C^0$ for 10 minutes. Thiomersal sodium was mixed at the rate of 0.05% of the final volume. Alum gel was added as described in Muhammad (2012). Finally, the total volume was adjusted with sterilized phosphate buffer saline at the pH of 7.2-7.4.

**Vaccination Trial:** Twelve rabbits (6 months of age) were divided into four groups. Each group contained three rabbits. Vaccines i.e. alum precipitated *Pasteurella multocida* (AHSV), Foot and Mouth Disease Virus "O" type Vaccine (FMDV), and multivalent HS and FMD vaccine (HS-FMD) was injected in the rabbits of group A, B, C, respectively while the fourth group (D) was control one (without vaccination).

Blood sample from each rabbit was collected on 0, 14, 28, 35 and 48 days post-priming. The serum from each of the blood sample was separated and stored in properly labeled vials at -40 $^0$C for antibody titration through VN and IHA test as described by Lewis *et al,* (1991) and Das *et al.* (1998). The statistical analysis was performed by calculating the Geometric Mean Titer (GMT) of antibodies against each of the vaccine.

## RESULTS AND DISCUSSION

Antibody response of rabbits (group A) to FMD vaccine was determined through V/N test using reference serotype obtained from UVAS, Lahore, Pakistan and results are shown in Table 1. The geometric mean anti-FMDV "O" V/N antibody titer on 0, $14^{th}$, $28^{th}$, $35^{th}$ and $48^{th}$ day post priming were 0, 10.1, 50.8, 20.2 and 12.7 units, respectively. The FMD vaccine when injected in the rabbits induces antigen presenting cells (APC) in the nearest lymph-node. The APC's present the antigen on their surface in association with self immune associated (Ia). The T cells recognize the antigen and transform into lymphoblast and populate in different lymph nodes and other lymphoid organs such as spleen, mucus membrane associated lymphoid tissues such as peyr's patches, etc., (Tizzard, 1998, Vanio *et al.* 1988; Muhammad *et al. 1993*). The lymphocytes secrete cytokines which potentiate cell mediated immunity (Kenneth *et al.,* 2004) as well as humoral immunity.

The serum antibody titer of the rabbits (group B) against LPS of *P. multocida* was determined and results are shown in Table-2. The antibody titer on 0, $14^{th}$, $28^{th}$, $35^{th}$ and $48^{th}$ day post priming was 0, 6.4, 42.3, 21.3 and 10.6 IHA units, respectively. LPS of *P. multocida* is T cell independent antigen. The antigen does not present on surface of APC along with Ia of the animal body and hence the specific B cell alone respond to the LPS and transform into plasma cells. The B cell do not get cooperation of the T-cells, hence response of the plasma cells is primary and poor. The antibody response is of short duration (Abbas *et al.,* 1991).

Antibody titers of rabbits (group C) against each of the immunogen of combined FMD+HS vaccine were measured and shown in Table 3. These were 0, 10.1, 32, 50.8 and 50.8 IHA units against LPS of *P. multocida* and 0, 12.7, 80.6, 101.6 and 161.3 units of VN antibody titer against FMD virus on 0, $14^{th}$. $28^{th}$ $35^{th}$ and $48^{th}$ day post priming. The control group without vaccination showed zero titer against FMD virus and LPS of *P. multocida*. The antibody response of the rabbits against each immunogen of combined FMD+HS vaccine was higher than that of rabbits receiving monovalent vaccines. These finding are in agreement to that of Rajesh (2003), Afzal

and Muneer (1990) who demonstrated that specific B cells respond directly to LPS of *P. multocida* and transform into plasma cells that secret immunoglobulin M (IgM). Protein antigens of FMD virus are processed through APC along with Ia antigen and stimulate the Th cells for production of cytokines whose production is antigen specific but their action is antigen non-specific so cytokines activate the response of LPS stimulated B cells. In this way LPS specific B cells proliferate, differentiate, and transform into plasma cells and memory cells. The plasma cells secret higher level of immunoglobulins and animals may show boosting response to second immunization. The cytokines produced in response to FMD virus proteins augment humoral as well as cellular immunity against FMD and LPS of *P. multocida* (Lu *et el.,* 1991). Combined vaccine containing FMD virus, rabies virus, *P. multocida* and *Cl. Chauvoei* antigens are effective to reduce the frequency and cost of vaccination Srinivasan *et al.,* 2001).

**Table 1: Antibody response of rabbits to FMD "O" virus vaccine**

| S# | Post-Priming Sampling Day | Anti-LPS-IHA antibody titer | Anti-FMD-VN antibody titer |
|---|---|---|---|
| 1 | 0 | 0,0,0 (0) | 0,0,0 (0) |
| 2 | 14 | 0,0,0 (0) | 16,8,8 (10.1) |
| 3 | 28 | 0,0,0 (0) | 64,32,64 (50.8) |
| 4 | 35 | 0,0,0 (0) | 16,32,16 (20.2) |
| 5 | 48 | 0,0,0 (0) | 16,8,16 (12.7) |

**Table 2: Antibody response of rabbits to HS vaccine**

| S# | Post-Priming Sampling Day | Anti-LPS-IHA antibody titer | Anti-FMD-VN antibody titer |
|---|---|---|---|
| 1 | 0 | 0,0,0 (0) | 0,0,0 (0) |
| 2 | 14 | 64, 64, 64 (6.4) | 0,0,0 (0) |
| 3 | 28 | 64, 32, 32 (42.3) | 0,0,0 (0) |
| 4 | 35 | 16,16,32 (21.3) | 0,0,0 (0) |
| 5 | 48 | 8,8,16 (10.6) | 0,0,0 (0) |

**Table 3: Antibody response of rabbits to combined FMD and HS vaccine**

| S# | Post-Priming Sampling Day | Anti-LPS-IHA antibody titer | Anti-FMD-VN antibody titer |
|---|---|---|---|
| 1 | 0 | 0,0,0 (0) | 0,0,0 (0) |
| 2 | 14 | 16,8,8 (10.1) | 16,16,8 (12.7) |
| 3 | 28 | 32,32,32 (32) | 128,64,64 (80.6) |
| 4 | 35 | 64,32,64 (50.8) | 128,64,128 (101.6) |
| 5 | 48 | 64,64,32 (50.8) | 128,256,128 (161.3) |

Note: Figure in parenthesis indicate the geometric mean titer of antibodies

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