VETERINARSKI ARHIV 70 (2), 59-66, 2000

Effect of levamisole hydrochloride on serum and colostral antibody titres against foot and mouth disease virus in vaccinated buffaloes (*Bubalus bubalis*)

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QURESHI, Z. I., L. A. LODHI, H. JAMIL, M. NAWAZ: Effect of levamisole hydrochloride on serum and colostral antibody titres against foot and mouth disease virus in vaccinated buffaloes (*Bubalus bubalis*). Vet. arhiv 70, 59-66, 2000.

ABSTRACT

A study was conducted to ascertain whether post-vaccination antibody titres in serum and colostrum against foot and mouth disease virus in pregnant buffaloes (*Bubalus bubalis*) could be enhanced by simultaneous administration of levamisole hydrochloride. Twenty-four pregnant buffaloes were divided into three groups of equal size: unvaccinated, vaccinated control, and levamisole-treated vaccinated. The vaccination was given 10 weeks prior to expected parturition. Weekly serum samples were collected until parturition, while colostrum samples were obtained within 1h after parturition. Passive haemagglutination test was applied to determine antibody titres. Levamisole-treated animals showed a progressive rise in antibody titre until week 6, reaching a peak value of 70.0 ± 4.3 (SD) during that same week. Levamisole hydrochloride significantly ($P<0.05$) increased antibody titres in serum, although results of statistical analysis of colostral antibody titres were not significantly different between experimental groups.

Key words: buffalo, *Bubalus bubalis,* antibody titre, foot and mouth disease virus, immunomodulation, levamisole hydrochloride

Introduction

Foot and mouth disease (FMD) is an important disease of farm animals in Pakistan and causes important economic losses through

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ISSN 0372-5480
Printed in Croatia

reduced milk production and loss of working capacity (KAZIMI and SHAH, 1980; ANONYMOUS, 1992-1993). Severe symptoms of FMD have been recorded in buffalo calves (REDA and HEGAZI, 1985) resulting in significant calf mortality (AHMED et al., 1986). The protection of buffalo calves against infectious diseases depends upon passive immunity obtained through ingestion of colostrum (BRAMBELL, 1970). The failure of transfer of maternal antibodies to the newborn is an important predisposing factor for health problems.

Bivalent FMD virus vaccine (serotypes A and 0) is used for the control of FMD in the country, but this confers a relatively low level of immunity and for a short duration (AKHTAR and HAQ, 1993). Moreover, vaccination of neonatal calves against infectious diseases is less effective (OSBURN et al., 1974). Specific resistance of neonatal calves could be enhanced by vaccination of the dam to stimulate the production of specific antibodies, which are then transferred to the newborn via colostrum, producing "lactogenic" immunity (SPIRE, 1982).

Levamisole hydrochloride (a commonly used anthelmintic) is an immunostimulating agent in animals and man (SYMOENS and ROSENTHAL, 1977; CONFER and ADLIUGER, 1981; WEST, 1982). Its effects on humoral and cell-mediated immune response in several diseases have been examined (LEJAN, 1981; FLESH et al., 1982; CONFER et al., 1985). However, there is little information on this subject in the buffalo, especially under the environmental conditions obtaining in Pakistan. The present paper thus evaluates the combined effect of levamisole hydrochloride and FMD vaccine on serum and colostral antibody titres in buffaloes.

Materials and methods

Experimental animals and treatment schedule

Twenty-four clinically healthy buffaloes (*Bubalus bubalis*) in their last trimester of pregnancy, ranging from 4 to 8 years in age, kept at the Livestock Production Research Institute (LPRI), Okara, Pakistan, were included in this study. All buffaloes were kept under similar feeding, housing and management conditions. The animals at LPRI had been routinely vaccinated against FMD twice a year.

The selected animals were randomly divided into three groups of eight. Animals from group I were neither vaccinated nor treated with any immunomodulator, and served as unvaccinated controls. Animals in group II were vaccinated (sensitising dose) with 5 ml of commercially available

bivalent (types A and 0) FMD vaccine grown on bovine hamster kidney (BHK) cell line. The vaccine was procured from the Veterinary Research Institute, Lahore, Pakistan and administered *i/m* 10 weeks prior to the expected date of parturition. A booster dose was administered 14 days after the sensitising dose and this group served as vaccinated controls. Animals in group III were vaccinated using the same procedure described for group II. In addition, levamisole hydrochloride (Shahani Labs, Pakistan) was given orally at a dose rate of 0.5 mg/kg body weight 7 days before, and again together with the first dose of vaccine. This group served as the immunomodulated treatment group.

Passive haemagglutination

Prior to vaccination and immunomodulation, a blood sample was collected from each of the experimental animals. Post-vaccination and immunomodulation serum samples were collected at weekly intervals until parturition. About 50 ml of colostrum was obtained within 1h of parturition (first milking) from buffaloes in all 3 groups. Serum and colostrum samples were stored at -20 °C until determination of antibody titre.

Antibody titres against FMD types "A" and "0" in serum and colostrum were measured by passive haemagglutination test as described by AFAQ et al. (1992). FMD antigens were purified from the FMD virus type "A" and "0" grown on BHK cell line. After harvest, the suspension was centrifuged at 19,000 g for 15 min to remove cell debris. The supernatant was ultracentrifuged at 48,000 g for 30 min (Preparative ultracentrifuge, L5-50B, Beckman USA) to obtain pelleted virus particles. The pellets, suspended in phosphate buffered saline and sonicated (Lab Sonic, 2000, Shipley, England), served as antigen. For preparation of antigen sensitised erythrocytes, one volume of 1 per cent gluteraldehyde solution prepared in phosphate buffered saline (PBS) was mixed with two volumes of prepared antigen and one volume of 10% sheep erythrocytes. The suspension was incubated at 25 °C for 60 minutes with repeated agitations (every 15 minutes during incubation). The suspension was then centrifuged at 1,500 rpm for 5 minutes, when the supernatant was discarded. The sensitised erythrocytes thus obtained were washed twice in PBS solution at 1,500 rpm for 5 minutes each. The erythrocytes were finally re-suspended in PBS solution to obtain 1.5% suspension of sensitised sheep erythrocytes (TOKUDA and WARRINGTON, 1970). Titertek microtitration plates (Flow lab. U.K) containing 8 rows (A to H) and 12 columns (1 through 12) of U-shaped wells were used to measure the antibody titres. In each microtitration plate, 6 samples were titrated

row-wise at a time, leaving the last 2 rows of wells for positive and negative controls, respectively. All samples were serially diluted as 1:2 through 1:2048. The plates were incubated at 37 °C for 2 h. Negative samples exhibiting no haemagglutination were manifested by the central settling of erythrocytes. The passive haemagglutination antibody titre of each serum and colostrum sample was defined as the reciprocal of its end-point dilution. Thus, the passive haemagglutination antibody titres of all samples were recorded in comparison with positive and negative controls.

Statistical analysis

The geometric mean titres (GMT)±SD were calculated using the procedure described by THRUSFIELD (1986). The statistical differences between the groups on a weekly basis were estimated using the analysis of variance procedure for completely randomised designs (STEEL and TORRIE, 1980). The means were compared using Duncan's multiple range test (DUNCAN, 1955).

Results

The geometric mean passive haemagglutination titres recorded against FMD type "A" of all experimental buffaloes are shown in Fig. 1. Buffaloes in all groups were seropositive at the beginning of the experiment, with similar antibody titres. There was a progressive rise in GMT against FMD type "A" in buffaloes from groups II and III, with peak values of 21.0±4.27 and 70.0±4.32 during weeks 5 and 6, respectively. From weeks 3 through 10 following vaccination, antibody titres were significantly higher in levamisole-treated buffaloes (group III) compared with buffaloes from the other 2 groups. Antibody titres also differed significantly between buffaloes from groups I and II during weeks 3 to 10 in favour of group II.

For FMD type "0" there was a progressive rise in GMT in buffaloes in groups II and III until week 3. However, statistical evaluation on a weekly basis did not reveal any difference between the 3 groups throughout the experimental period. A 2- to 3-fold non-significant ($P<0.05$) increase in colostral antibody GMT was recorded in levamisole-treated buffaloes (Fig. 2.)

Discussion

Augmentation of the host's defence mechanism with a variety of biological immunostimulants and adoption of vaccination of pregnant dam

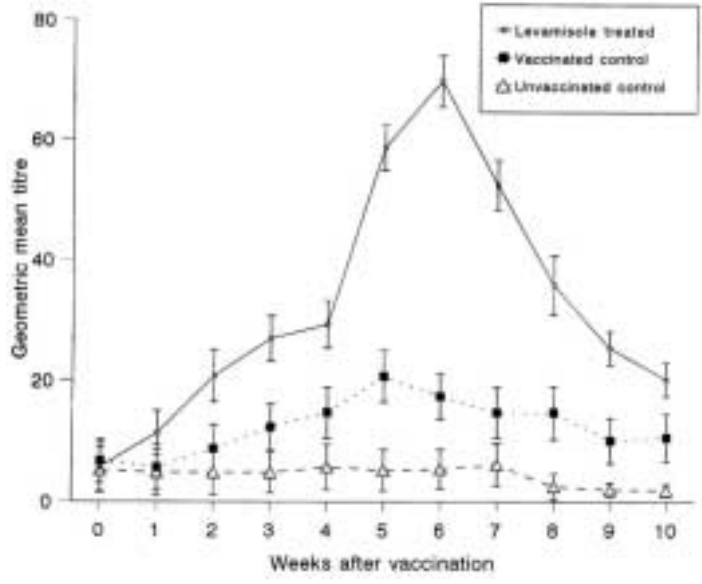

Fig. 1. Serum antibody titres (GMT±SD) against FMD type "A" of all experimental buffaloes measured by passive haemagglutination test

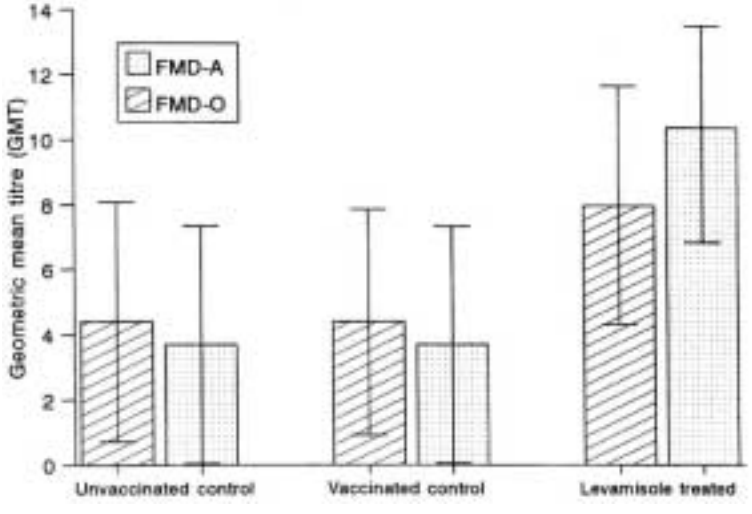

Fig. 2. Colostral (GMT±SD) antibody titre against FMD type "A" and "O" of all the experimental buffaloes measured by passive haemagglutination test

to produce colostral immunity or specific colostrum for protection of neonates in early life, is a good approach in immunobiology (LOWE, 1980; MAGNUSSON and FOSSUM, 1992). The immunostimulating activity of levamisole has been well documented in several experimental and clinical studies (NEVEU, 1970; SYMOENS and ROSENTHAL, 1977; DEBOWY et al., 1985; SHERMA et al., 1988).

In the present study, significantly higher serum antibody titres in the presence of levamisole corroborate the findings of BABIUK et al. (1982), FERNIE et al. (1983), and GIAMBRONE and KLESIUS (1985). Antigenic stimulation with *Brucella abortus* strain 19 (CONFER et al., 1985) and haemorrhagic septicaemia (SHERMA et al., 1988) in the presence of levamisole hydrochloride also resulted in higher serum antibody titres and long-lasting immunity. The gradual decline we saw after the sixth week post-vaccination is probably a reflection (at least in part) of a transfer of these antibodies to colostrum. BUTLER (1974) and TIZARD (1982) have provided convincing evidence that direct transfer of immunoglobulins from serum to colostrum takes place prior to parturition.

The seropositivity and the presence of specific antibodies in the colostrum of the control group of animals in the present study may have been due to previous exposure to FMD virus. The colostral antibody titres in the present study did not differ significantly between the 3 groups. Unfortunately, no information about the protective level of immunoglobulins in the colostrum against FMD vaccine could be traced in the literature. However, it is generally accepted that a higher concentration of colostral immunoglobulins provide more protection against infectious diseases (GAY et al., 1965; RADOSTITS and ACRES, 1980).

The exact mechanism by which levamisole could enhance serum antibody response to infective agents is not known. It has been reported that levamisole enhances macrophage and T-lymphocyte function and reduces suppressor T-cell function (HERSEY and WERKMEISTER, 1981). Because antibody formation to most infectious agents is T-lymphocyte-dependent, the augmentation of the helper functions of these cells could enhance antibody production (BABIUK and MISRA, 1982).

In conclusion, under the conditions of the present study, levamisole given 7 days before, and again together with vaccination, enhanced antibody response to FMD virus in serum. However, such treatment failed to raise colostral titres compared to those of the unvaccinated buffaloes.

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Received: 25 June 1998
Accepted: 18 April 2000

QURESHI, Z. I., L. A. LODHI, H. JAMIL, M. NAWAZ: Učinak levamisol hidroklorida na titrove protutijela za slinavku i šap u krvnom serumu i kolostrumu u cijepljenih bivolica (*Bubalus bubalis*). Vet. arhiv 70, 59-66, 2000.

SAŽETAK

Istraživanje je provedeno da se utvrdi da li se može post-vakcinalni titar protutijela za slinavku i šap u serumu i u kolostrumu gravidnih bivolica povečati istovremenim davanjem levamisol hidroklorida. Dvadeset i četiri gravidne bivolice (*Bubalus bubalis*) su podijeljene u 3 jednake skupine: necijepljene, cijepljene kontrolne i cijepljene uz tretman levamisolom. Cijepivo je davano 10 tjedana prije očekivanog telenja. Tjedni uzorci seruma su uzimani do telenja, a kolostruma 1 sat nakon telenja. Za utvrđivanje titra protutijela rabljen je test pasivne hemaglutinacije. Životinje tretirane levamisolom pokazale su progresivan rast titra protutijela do šestog tjedna kada su dosegle najvišu vrijednost od 70,0±4,3 (SD). Levamisol hidroklorid je značajno ($P<0,05$) povisio titar protutijela u serumu ali se rezultati statističke analize titra kolostralnih protutijela nisu značajno razlikovali među skupinama pokusnih životinja.

Ključne riječi: bivol, *Bubalus bubalis*, titar protutijela, virus slinavke i šapa, imunomodulacija, levamisol hidroklorid