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Effect of Maternal Immunomodulation Alongwith Vaccination on the Production of Colostral Specific Antibodies and Their Transfer to Buffalo Neonates

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bstract

he antibody titre against *Pasturella multocida* antigen using indirect haemagglutination test from colostrum and serum mples was determined. Twenty four pregnant buffaloes were divided into three equal groups: unvaccinated, vaccinated ntrols and levamisole hydorchloride treated vaccinated. The colostral and seral antibody titres were significantly higher <0.05) in levamisole treated vaccinated group of buffaloes. The calves fed on colostrum from these buffaloes also had gher serum antibody titres. It was concluded that levamisole hydrochloride can be use as an immunomodulator alongwith ntigen in pregnant animals to elevate colostral antibody titre in dams and lactogenic immunity in their neonates.

ntroduction

uccessful calf raising is the fundamental requirement for profitable livestock industry. Unfortunately, a large umber of calves die during the first year of age causing eavy drain on the economics of livestock production. In akistan neonatal calf mortality varies from 7.1 to 39.8 per ent (Afzal *et al.*, 1983; Khan and Khan, 1991). Jainudeen 988) indicated the calf mortality as the principal onstraint in the development of dairy industry and commended that this mortality rate should be reduced to elow 0.5 per cent . Morbidity and mortality rates due to aemorrhagic septicaemia are higher in calves than adult Chaudhry *et al.*, 1993).

accination of neonatal calves against infectious diseases less effective due to compromised immune system Osburn *et al.*, 1974). Specific resistance of neonatal calves an be enhanced by vaccination of the dam before arturition to stimulate the production of specific antibodies hich are then transferred to the newborn via colostrum, to roduce the lactogenic immunity (Spire, 1982). nprovement in the immune response to bacterial and viral ntigens has been reported by using immunopotentiators Roger *et al.*, 1991), like levamisole hydrochloride Desplenter, 1982). A number of studies have been carried ut on transferring of maternal immunity to the newborn in attle (Butler, 1974) and buffaloes (Ali *et al.*, 1993). uccessful attempts to reduce neonatal mortalities in sows Debwoy *et al.*, 1985) and cows (Deshpande *et al.*, 1991) ave been reported by treating the dams in late gestation ith synthetic immunopotentiators. But the similar formation in the buffaloes is lacking. The objective of the esent study was to determine the effect of vaccination in e presence of immunomodulator during late gestation on olostral specific antibody titres and its transfer to buffalo eonates.

laterials and Methods

perimental animals: A total of 24 apparently healthy buffaloes 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 selected animals were randomly divided (lottery method) into three equal groups. Animals from group I were neither vaccinated nor treated with immunomodulator and served as unvaccinated control. Animals in group II were vaccinated (Sensitizing dose) with 5ml of commercially available Hemorrhagic septicemia bacterin procured from the Veterinary Research Institute, Lahore, Pakistan. A booster dose was administered 14 d after the sensitizing dose and this group served as vaccinated control. Animals in group III were vaccinated using the same procedure as described for group II. In addition, levamisole hydrochloride (Shahani Labs, Pakistan) was given orally at the dose rate of 0.5mg/kg body weight 7 d before and again alongwith first dose of vaccine. This group served as immunomodulated treatment group.

Sampling protocol: For determination of colostral antibody titre, immediately after calving about 50ml of colostrum was collected at 12 hours interval upto 36 hours. Thus four colostrum samples from each experimental buffalo were collected and kept at -20° C till used for analysis. For seral antibody titres, first blood sample from all the calves born to buffaloes of different experimental groups was collected prior to colostrum feeding (0 hr) and then at an interval of 6 hrs upto 36 hrs. Later the calf blood samples were collected fortnightly until day 84 of age. The serum was separated and kept at -20° C until assayed.

Measurement of antibody profile: Antibody titre against HS antigen in serum and colostrum were measured by indirect haemagglutination (IHA) test as described by Akhtar *et al.* (1991). For preparation of antigen, *P. multocida* (Robert Type-1) was procured from Veterinary Research Institute, Lahore, Pakistan and reconfirmed by different biological and

biochemical tests (Wilson and Miles, 1990). A loopful of culture was inoculated into tubes containing 5ml of tryptose yeast extract (TYE) broth (Dilco Lab. Datroit, Michigon). After incubation at 37°C for 18 hrs, one ml of inoculum was seeded over Roux flasks containing TYE. After incubation at 37°C for 24 hrs, the growth was harvested with 0.89 per cent sodium chloride (Amies, 1951). The growth suspension was subjected to ultrasonic waves (Rapidis 600 No, ultrasonics Ltd, France) for 5x6 minutes. The sonicated material was centrifuged at 2000 rpm for 30 minutes and the supernatant was used as antigen. Titertex 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 rows of wells for positive and negative controls, respectively. All samples were serially diluted as 1:2 through 1:2084. The plates were incubated at 37°C for 40 minutes. The negative samples exhibiting no haemagglutination were manifested by peculiar central settling of erythrocytes. The IHA titre of each serum and colostrum sample was defined as the reciprocal of its end point dilution.

Statistical analysis: The geomean titre (GMT ± SE) were calculated by the procedure described by Thrushfield (1986). The statistical difference between the groups as weekly basis for serum samples were estimated using the analysis of variance procedure for Completely Randomized Designs (Steel and Torrie, 1980).

Results

At 0 hr, the significantly ($P<0.05$) higher GMT was recorded in buffaloes of vaccinated control and vaccinated + levamisole hydrochloride treated group as compared to un vaccinated control (Fig.I). At 12 and 24 hrs, the GMT was significantly higher ($P<0.05$) in levamisole hydrochloride treated group of buffaloes as compared to unvaccinated and vaccinated controls. However, no statistical difference was recorded among all experimental groups at 36 hrs. When the antibody titre of all 12 hourly colostrum samples was taken into account jointly, the levamisole treated group of buffaloes had significantly ($P<0.05$) higher antibody titre compared with those of other two groups.

Serum antibody titres (GMT ± SE) against HS antigen in the dams just after parturition, from which colostrum were fed, were 4.6 ± 1.10, 10.0 ± 1.11, 15.0 ± 1.12 for unvaccinated, vaccinated and vaccinated + levamisole treated buffaloes, respectively. At time 0, all calves had almost same level of seral antibodies against *P. multocida* (Fig.2).

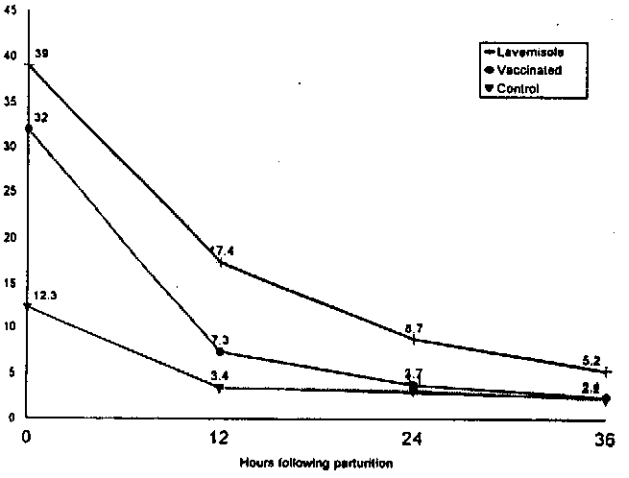
At time 6, 12, 18, 24 and 36 hrs after birth, a significantly higher ($P<0.05$) GMT was recorded in levamisole treated group of calves. Although during this period, the GMT was numerically higher in vaccinated control as compared to calves of unvaccinated control group but statistically th difference was non significant ($P<0.05$). At day 14, secondary rise in antibody titre was recorded in calves of groups with peak values at day 56. From day 14 upto th culmination of experiment (day 84) the serum antibody tit was significantly ($P<0.05$) higher in levamisole treat group of calves compared with rest of two groups.

Discussion

In the present study, the immunopotentiation wi levamisole hydrochloride resulted in significantly high antibody response to HS vaccaine. In bovine experimen involving levamisole hydrochloride as immunomodulat with infectious bovine rhinotreacheitis, herpes virus, fo and mouth disease virus and brucellosis, favourable resu have been reported (Prior and Porter, 1980; Babiuk a Misra, 1982; Kaneene *et al.*, 1981; Schmied a Rosebusch, 1973). Brunner and Muscoplat (1980) report a significant increased in the level of trypsin inhibi activity and immunoglobulins in the colostrum of pregn cows at one week post treatment with levamis hydrochloride. Flesh *et al.* (1977) reported most consist results in the prevention of morbidity and mortality in n born calves after treatment of pregnant cows w levamisole hydrochloride. The largest experience w levamisole hydrochloride during the last stage of pregna resulted in the prevention or reduction in the neon disease conditions (Espinasse, 1980).

In the present study the vaccinated control group buffal also had higher antibody titre as compared to unvaccin control group. Valente *et al.* (1987), Bagley and Call (19 and Wood *et al.* (1975) vaccinated pregnant cows aga E.Coli and showed the provision of passive protectio their calves via colostrum. Similarly, Ajmal *et al.* (19 vaccinated pregnant buffaloes with HS vaccine reported a significant increase in colostrol antibody t Tsunemitus *et al.* (1989), Gresham *et al.* (1984), Myers Snodgrass (1982) vaccinated pregnant cows inactivated bovine Rota virus, Pasteurella multocida tetanus toxoid vaccine, respectively to achieve higher l of specific antibodies in colostrum of dams and in se their calves after the ingestion of specific colostrum. presence of specific antibodies in the colostrum of co group buffaloes may have been due to the pre experience and exposure to HS antigen, as has reported by Oyeniyi and Hunter (1978). It may al attributed to the local production of antibodies by pl cells in the sub epithelial connective tissue of the mam glands (Butler, 1974).

A very low level of GMT in precolostral sera of calve to buffaloes of all groups may be due to the passa immunoglubins across the damaged placenta (Mensik 1978), as normally there is no transplacental trans immunoglobulins in bovines (Butler, 1974). Ajmal (1990) explained that the precolostral calf sera s showing low levels of antibody titres against the va



g. 1: Geomean titre against *Pasteurella multocida* in the colostrum of all experimental buffaloes upto 36 hours postpartum.

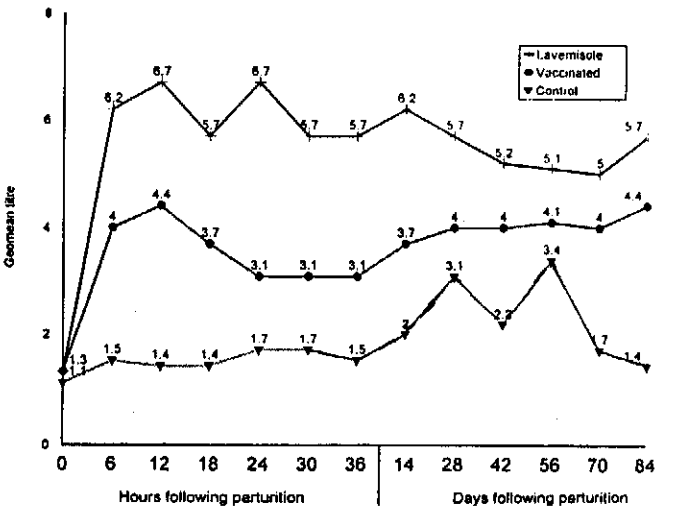


Fig. 2: Geomean titre against *Pasteurella multocida* in calves born to all experimental buffaloes after colostrum feeding

ministered, may actually be the postcolostral samples tributed to the negligence of the attendant. In the present udy the first possibility is more likely reason since no tendent was involved in this study.

ie higher seral antibody titre throughout the study period as recorded in calves born to dams treated with vamisole hydrochloride. Immunomodulatory effect of vamisole hydrochloride in mycobacterium paratuberculosis fected rabbit was demonstrated through leukocyte igration (Mondal *et al.*, 1993). Giambrone and Klesius 985) reported the effect of levamisole hydrochloride ongwith coccidiosis vaccination on antibody response in ommercial broilers. The group which received no vamisole had 96 per cent mortality with severe clinical mtoms, whereas levamisole treated chicks had only 4 per ent mortality with reduced incidence and severity of occidiosis. The higher antibody response with brucella rain 19 (Confer *et al.*, 1985) and hemorrhagic septicemia Sherma *et al.*, 1988) in the presence of levamisole ydrochloride resulted in higher sera antibody titre and long sting immunity. A secondary rise in GMT was recorded in alves born to buffaloes of all experimental groups. It may e due to active antibody response to disease exposure hortly after birth (Gresham *et al.*, 1984). In the present tudy, there was also an outbreak of HS at the farm, which ay have caused the secondary rise in GMT. But nterestingly there was a relative increase in GMT instead of similar increase in calves born to all experimental buffalo roups, wherein calves born to levamisole treated group howed greater response as compared to calves of other wo groups. Although no plausible explanation can be given ut it might be due to the transfer of some unknown nformation from dam to their calves, that may have nodulated the immune response of the calves. Since it has been previously reported that lymphocytes present in the colostrum play a role in the passive transfer of immunity (Tizard, 1977), it may be possible that unknown immunomodulatory factors were also transferred to calf through lymphocytes present in the colostrum.

It was inferred from the present study that levamisole hydrochloride can be use alongwith vaccination to improve the immune response against HS vaccine in buffaloes and their neonates. But whether this procedure can be practiced under field conditions and does it provide a protective level awaits further study.

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