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

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Abstract
The antibody titre against Pasteurella multocida antigen using indirect haemagglutination test from colostrum and serum samples was determined. Twenty four pregnant buffaloes were divided into three equal groups: unvaccinated, vaccinated controls and levamisole hydrochloride treated vaccinated. The colostral and serum antibody titres were significantly higher (0.05) in levamisole treated vaccinated group of buffaloes. The calves fed on colostrum from these buffaloes also had higher serum antibody titres. It was concluded that levamisole hydrochloride can be used as an immunomodulator along with antigen in pregnant animals to elevate colostral antibody titre in dams and lactogenic immunity in their neonates.

Introduction
Successful calf raising is the fundamental requirement for profitable livestock industry. Unfortunately, a large number of calves die during the first year of age causing heavy drain on the economics of livestock production. In Pakistan neonatal calf mortality varies from 7.1 to 39.8 percent (Afzal et al., 1983; Khan and Khan, 1991). Jainudeen (1988) indicated the calf mortality as the principal constraint in the development of dairy industry and commended that this mortality rate should be reduced to below 0.5 per cent. Morbidity and mortality rates due to hemorrhagic septicemia are higher in calves than adult (Chaudhry et al., 1993). Vaccination of neonatal calves against infectious diseases, less effective due to compromised immune system (Osborn et al., 1974). Specific resistance of neonatal calves can be enhanced by vaccination of the dam before parturition to stimulate the production of specific antibodies which are then transferred to the newborn via colostrum, to reduce the lactogenic immunity (Spire, 1982). Improvement in the immune response to bacterial and viral antigens has been reported by using immunopotentiators (Rogier et al., 1991), like levamisole hydrochloride (Seplanter, 1982). A number of studies have been carried out on transferring of maternal immunity to the newborn in cattle (Butler, 1974) and buffaloes (Ali et al., 1993). Successful attempts to reduce neonatal mortalities in sows (DeVoy et al., 1985) and cows (Deshpande et al., 1991) have been reported by treating the dams in late gestation with synthetic immunopotentiators. But the similar information in the buffaloes is lacking. The objective of the present study was to determine the effect of vaccination in presence of immunomodulator during late gestation on colostral specific antibody titres and its transfer to buffalo neonates.

Materials and Methods
Experimental 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 septicaemia 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 along with 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 serum 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
biological tests (Wilson and Miles, 1990). A loopful of culture was inoculated into tubes containing 5ml of tryptose broth (TYE) broth (Dilco Lab. Detroit, Michigan). 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. Titerx 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 geometric mean titre (GMT ± SE) were calculated by the procedure described by Thrushfield (1986). The statistical difference between the groups as a 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 unvaccinated control (Fig.1). 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 the 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 up to culmination of experiment (day 84) the serum antibody titre was significantly (P<0.05) higher in levamisole treated group of calves compared with rest of two groups.

Discussion
In the present study, the immunopotentiation with levamisole hydrochloride resulted in significantly high antibody response to HS vaccinum. In bovine experiment involving levamisole hydrochloride as immunomodulator with infectious bovine rhinotracheitis, herpes virus, foot and mouth disease virus and brucellosis, favourable results have been reported (Prior and Porter, 1980; Babluk & Misra, 1982; Kaneene et al., 1981; Schwab & Rosebusch, 1973). Brunner and Muscoplat (1980) reported a significant increased in the level of trypsin inhibiting activity and immunoglobulins in the colostrum of pregnant cows at one week post treatment with levamisole hydrochloride. Flesh et al. (1977) reported most consistent results in the prevention of morbidity and mortality in newborn calves after treatment of pregnant cows with levamisole hydrochloride. The largest experience with levamisole hydrochloride during the last stage of pregnancy resulted in the prevention or reduction in the neonatal disease conditions (Espinasse, 1980).

In the present study the vaccinated control group buffaloes also had higher antibody titre as compared to unvaccinated control group. Valente et al. (1987), Bagley and Call(1975) and Wood et al. (1975) vaccinated pregnant cows against E.Coli and showed the provision of passive protection to their calves via colostrum. Similarly, Ajmal et al. (1987) vaccinated pregnant buffaloes with HS vaccine and reported a significant increase in colostrum antibody titres. Tsunemitsu et al. (1989), Gresham et al. (1984), Myrte and Wood (1982) vaccinated pregnant cows against inactivated bovine Rota virus, Pasteurella multocida and tetanus toxoid vaccine, respectively to achieve higher titres of specific antibodies in colostrum of dams and in sera of their calves after the ingestion of specific colostrum. Presence of specific antibodies in the colostrum of in group buffaloes may have been due to the previous experience and exposure to HS antigen, as has been reported by Oyeniyi and Hunter (1978). It may also be attributed to the local production of antibodies by plasma cells in the sub epithelial connective tissue of the mammary glands (Butler, 1974).

A very low level of GMT in precolostral sera of calves to buffaloes of all groups may be due to the presence of immunoglobulins across the damaged placenta (Menski, 1978), as normally there is no transplacental transfer of immunoglobulins in bovines (Butler, 1974). Ajmal et al. (1990) explained that the precolostral calf sera was showing low levels of antibody titres against the vaccinum.
administered, may actually be the postcolostral samples attributed to the negligence of the attendant. In the present study the first possibility is more likely reason since no attendant was involved in this study.

The higher sera antibody titre throughout the study period as recorded in calves born to dams treated with levamisole hydrochloride. Immunomodulatory effect of levamisole hydrochloride in mycobacterium paratuberculosis infected rabbit was demonstrated through leukocyte migration (Mondal et al., 1993). Giambone and Klesius 1995 reported the effect of levamisole hydrochloride on with coccidiosis vaccination on antibody response in commercial broilers. The group which received no levamisole had 96 per cent mortality with severe clinical symptoms, whereas levamisole treated chicks had only 4 per cent mortality with reduced incidence and severity of coccidiosis. The higher antibody response with brucella strain 19 (Confer et al., 1985) and hemorrhagic septicemia Shulma et al., 1988) in the presence of levamisole hydrochloride resulted in higher sera antibody titre and long lasting immunity. A secondary rise in GMT was recorded in calves born to buffaloes of all experimental groups. It may be due to active antibody response to disease exposure shortly after birth (Gresham et al., 1984). In the present study, there was also an outbreak of HS at the farm, which may have caused the secondary rise in GMT. But interestingly there was a relative increase in GMT instead of similar increase in calves born to all experimental buffalo groups, wherein calves born to levamisole treated group showed greater response as compared to calves of other two groups. Although no plausible explanation can be given but it might be due to the transfer of some unknown information from dam to their calves, that may have modulated 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 used along with 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.

References


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