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ORIGINAL ARTICLE

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Effects of oral administration of levamisole on non-specific immunity, serum proteins and health in normal colostrum-fed neonatal dairy calves

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Abstract Soon after levamisole became widely used as a veterinary anthelmintic, it was recognised that concurrent enhancement of immune responsiveness sometimes accompanied anthelmintic treatment, especially in old or chronically ill animals. There are many reports concerning the immunomodulatory effects of levamisole in immunocompromised and vaccinated animals, but information about the effects of levamisole in immunocompetent animals is limited and controversial. Thirty Holstein dairy calves were randomly divided into two groups: test ($n = 15$) and control ($n = 15$). Blood samples were taken from the jugular vein, between 24 h and 48 h after birth, and put into EDTA-containing tubes and plain tubes; levamisole was then administrated orally at a dose of 2 mg/kg body weight three times at 1-day intervals in test groups. Blood sampling from all the calves was continued at days 7, 14, 21 and 28. The weight of calves was measured only at days 1 and 28 (before feeding). The levels of packed cell volume (PCV) (microhaematocrit), total white blood cells (WBCS) and differential leukocyte count (manual standard methods), total serum protein (colorimetry), beta and gamma globulins (electrophoresis), albumin:globulin (A:G) (calculated) and disease occurrence were measured and recorded. Appropriate statistical methods were used for data analysis, and $P \leq 0.05$ was considered as significant. No significant differences were seen for PCV, total serum protein, WBC count and health between groups. In the test group, neutrophil level at day 14 and monocyte level at day 21 were significantly higher than in the control group ($P < 0.05$). Electrophoresis showed no significant differences for beta globulin level and A:G between groups, but gamma globulin level at day 28 was significantly higher in the test group ($P < 0.05$).

Keywords Dairy calves · Health · Leukogram · Levamisole · Serum proteins

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Introduction

Levamisole is a highly acceptable anti-nematodal drug because of its broad range of activity in a large number of hosts (sheep, cattle, horse, pig, dog, chicken). Soon after tetramisole and, subsequently, levamisole became widely used as veterinary anthelmintics, it was recognised that concurrent enhancement of immune responsiveness sometimes accompanied anthelmintic treatment, especially in old or chronically ill animals (Courtney and Roberson 1995).

The high mortality of infant cattle, horse, sheep and pigs has been a serious problem in farm animal breeding for many years. Such losses are mostly caused by infectious diseases, especially in the neonatal and postnatal periods.

Intensive studies have recently been initiated to assess methods of preventing and treating infectious diseases. One of these methods has been non-specific immunoprophylaxis with natural or synthetic immunomodulators. Successful results have already been obtained in the prophylaxis of diseases of young farm animals after non-specific stimulation of the pregnant adults (Deshpande et al. 1991; Kotowski 1991; Krakowski et al. 1999; Wawron and Szczubial 2000).

These immunologically enhancing treatments suggested that the drug might be of value in promoting cell-mediated and humoral immunity in neonatal dairy calves. Most previous reports studied the effects of levamisole administration on immunocompromised or vaccinated animals (Irwin et al. 1976; Babiuk and Misra 1981, 1982; Roth and Kaeberle 1984; Ivanov et al. 1987; Ogunbiyi et al. 1988; Sharma et al. 1990), but there are

limited data concerning the effects of orally administered levamisole in normal immunocompetent neonatal calves. The present study was performed to reveal the effects of levamisole on leukogram, globulin levels and health in normal colostrum-fed neonatal dairy calves.

Materials and methods

The study was conducted in a dairy herd with approximately 400 calves per year at Mashhad suburb (northeast of Iran). This herd consisted of pure-bred animals of the Holstein breed. The herd was totally confined in free-stall housing without access to pasture. Cows were fed with alfalfa hay, concentrate and corn silage.

The cows were dried 2 months before their expected time of parturition and transferred to a separate stall. As the time of parturition approached, they were moved to straw-bedded maternity pens. Prompt assistance was given to cows with dystocia. Following parturition the umbilicus of each calf was treated with pavidone iodine, and the calf was weighed and transferred to an individual pen. Within the first 6 h of life 2.5 kg of the dam's colostrum was bottle-fed to the calf, and colostrum feeding was continued every 12 h for 48 h. After 48 h herd milk was used for feeding twice daily (2 kg every 12 h) until the calf was 10 days old. After this time concentrate, high quality alfalfa and water were allowed ad libitum. The calves were weaned at 45 days of age. The heifer calves were mainly used as herd replacements.

Thirty calves were used in the present study. They were divided into two groups (test $n = 15$; control $n = 15$). Based on the gender and parity of dams, the number of calves in each group was approximately identical. In the test group oral levamisole was administered at a dose of 2 mg/kg body weight three times at 1-day intervals. Samples were taken from the jugular vein between 24 h and 48 h after birth (before levamisole administration in the test group) and on days 7, 14, 21 and 28. The blood was added to EDTA-containing tubes for haematological measurements and plain tubes for serum extraction and measurement of total protein and serum protein electrophoresis. The serum was harvested after centrifugation at 3,000 rpm for 10 min and stored at −20°C until required for analysis.

The haematocrit [packed cell volume (PCV)] levels were obtained by microhaematocrit methods. White blood cell (WBC) measurements were taken manually by standard methods (Dacie and Lewis 1984). Differential leukocyte counts were performed on routinely prepared Giemsa-stained blood films by the cross-sectional technique (Jain 1986). One hundred leukocytes were identified. Serum total protein was determined by Biuret colorimetric method with a spectrophotometer (Jenway 6105, Jenway, Felstead, England). Percentage and absolute levels of beta and gamma globulins were determined by cellulose acetate electrophoresis and densitometry (Helena system). Disease occurrence was also recorded during the experiment.

The SAS program was used for data analysis. After testing normal distributions of the data we used a parametric independent *t*-test to investigate significant difference between groups at various sampling times. The chi-square test was also used for comparison of disease occurrence between groups. $P \leq 0.05$ was considered as significant.

Results

The results are shown in Tables 1 and 2. No significant differences were seen for PCV, total serum protein and WBC count between groups. In the test group, neutrophil level at day 14 and monocyte level at day 21 were significantly higher than the control group ($P < 0.05$). Electrophoresis showed no significant differences for beta globulin level and albumin:globulin (A:G) between groups but gamma globulin level at day 28 was significantly higher in the test group ($P < 0.05$).

During the study, in the test group, six cases of diarrhoea were recorded. In the control group ten cases of diarrhoea occurred. The chi-square test showed no significant difference between groups for disease occurrence.

Discussion

There are reports concerning the effects of levamisole administration on the immune response of various animal species (Hunter et al. 1981; Singh and Dhawedkar 1993; Sopinska and Guz 1993; Wisniewski et al. 1993; Asif et al. 1995; Cabaj et al. 1995; Keskar et al. 1996; Findly and Munday 2000; Qureishi et al. 2000; Zia-ul-Rahman et al. 2003). For calves, most of the papers studied the immunomodulatory effects of simultaneous administration of levamisole and various vaccines. However, the information about the effects of levamisole in normal intact neonatal dairy calves is limited and controversial.

There are few reports concerning the alterations of leukocyte number after levamisole administration. Asif et al. (1995) reported no significant changes in the leukocyte, neutrophil, eosinophil and basophil counts in Sahiwal heifers after oral administration of levamisole. In another study on buffalo heifers, Zia-ul-Rahman et al. (2003) reported an increase of WBCs on day 1 after levamisole administration, which returned to pre-dose levels at 7 and 14 days after dosing. Neutrophil percentage decreased and lymphocyte percentage increased on days 7 and 14 after drug administration, but an increase in monocyte count was observed on days 7 and 14 of the experiment. Our results are consistent with those of Asif et al. (1995) and Zia-ul-Rahman et al. (2003), with regard to WBC count, and Zia-ul-Rahman et al. (2003) for monocyte level. Paulik et al. (1992) showed increased levels of neutrophil percentage in mice after levamisole administration. Nalini-Kumari and

Table 1 Mean ± SE of some haematological parameters and statistical comparisons between trial groups

Timepoint	PCV (%)		WBC(10^9/l)		Neutrophils (10^9/l)		Band(10^9/l)		Lymphocytes (10^9/l)		Monocytes (10^9/l)		Eosinophils (10^9/l)	
	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
24-48 h	35.11 ± 0.02	33.31 ± 0.01	10.21 ± 1.08	9.74 ± 0.86	6.04 ± 0.59	6.64 ± 0.70	0.000	0.004 ± 0.004	4.04 ± 0.65	2.93 ± 0.26	0.080 ± 0.049	0.086 ± 0.043	0.014 ± 0.006	0.053 ± 0.035
P value	0.76		0.87		0.52		0.4		0.31		0.78		0.38	
First week	32.01 ± 0.01	32.11 ± 0.01	8.39 ± 0.74	10.73 ± 1.18	3.44 ± 0.62	5.08 ± 1.02	0.005 ± 0.005	0.006 ± 0.006	4.69 ± 0.32	5.22 ± 0.80	0.081 ± 0.022	0.055 ± 0.025	0.016 ± 0.009	0.011 ± 0.011
P value	0.62		0.28		0.32		0.1		0.74		0.28		0.37	
Second week	30.41 ± 0.01	31.00 ± 0.01	8.66 ± 0.98	8.22 ± 0.68	3.74 ± 0.59	2.36 ± 0.50	0.000	0.006 ± 0.006	4.84 ± 0.48	5.53 ± 0.48	0.12 ± 0.044	0.070 ± 0.029	0.000	0.000
P value	0.78		0.98		0.02		0.36		0.32		0.34		–	
Third week	29.81 ± 0.01	29.31 ± 0.01	9.38 ± 0.78	8.18 ± 0.59	2.60 ± 0.42	2.25 ± 0.34	0.000	0.000	6.48 ± 0.48	5.54 ± 0.41	0.17 ± 0.071	0.049 ± 0.043	0.000	0.015 ± 0.009
P value	0.84		0.23		0.07		–		0.12		0.01		0.09	
Fourth week	29.31 ± 0.01	31.11 ± 0.02	10.09 ± 0.91	9.68 ± 0.74	3.63 ± 0.49	3.05 ± 0.43	0.000	0.000	6.39 ± 0.61	6.50 ± 0.41	0.077 ± 0.035	0.10 ± 0.029	0.000	0.000
P value	0.82		0.82		0.39		–		0.59		0.35		–	

Choudhuri (1986) have studied the levamisole effects, together with rinderpest vaccination, in buffalo calves. They reported a non-significant increase in leukocyte count, with significant increase in lymphocyte percentage that was associated with a decrease in the neutrophil level. These changes occurred 1–3 weeks after levamisole administration. Goranov and Bonovska (1987) reported no changes in the total count of leukocytes in sheep after levamisole injection, although a rise in the phagocytic activity of neutrophils was seen. In the present study there were significant differences between neutrophil and monocyte levels at the second and third weeks of the trial between groups, respectively ($P < 0.05$). These findings are consistent with those of Paulik et al. (1992) but are in contrast with the results of Nalini-Kumari and Choudhuri (1986) and Goranov and Bonovska (1987).

This study revealed significant difference in gamma globulin levels between groups, with higher values in the test group ($P < 0.05$). Since most of the immunoglobulins migrate in the gamma globulin zone, this suggests that levamisole administration induced antibody production in the test group.

Some apparently conflicting evidence also exists with regard to the effect of levamisole on antibody production. The results of the present study are consistent with Bekere's findings (1988), indicating that levamisole given to neonatal cattle increases the value of humoral factors of natural immunity, and levamisole could, therefore, be used for improving the formation of natural immunity and as a preventive against hypoproteinaemia in newborn cattle. Zia-ul-Rahman et al. (2003) reported increased levels of total serum protein on day 7, whereas globulin increased on days 7 and 14 after levamisole administration in buffalo heifers. Our results are consistent with those of Zia-ul-Rahman et al. (2003) for gamma globulin level. The difference between times of increase is probably related to species difference or dose and number of administrations. Singla and Juyal (1992) observed an increase in serum gamma globulin levels in calves infected with *Trypanosoma evansi* and in calves infected with *T. evansi* and simultaneously treated with levamisole, but, compared with untreated uninfected controls, the increase was significant only in the infected/treated group. Furthermore, there are reports concerning augmented humoral immune responses after levamisole administration following vaccination against *Pasteurella multocida* and infectious bovine rhinotracheitis virus in calves and cattle, respectively (Babiuk and Misra 1982; Sharma et al. 1990). Similar results were obtained in suckling rats immunised against sheep red blood cells (Hunter et al. 1981). Sheep given vaccinations against clostridial diseases and levamisole had higher anti-clostridial antibody titres than sheep given only vaccine (Hogarth-Scott et al. 1980). On the other hand, in a further study, blood derivative containing 10% bovine serum proteins (60% immunoglobulins and 40% albumin) and 10 mg levamisole per ml, or levamisole alone, was given i.m. to fifteen 3-week-old calves at a levamisole dose of 10 mg/kg body weight, the

Table 2 Mean ± SE of serum proteins measurements with statistical comparisons between trials groups

Timepoint	Total protein(g/l)		Beta globulin(g/l)		Gamma globulin(g/l)		A:G	
	Test	Control	Test	Control	Test	Control	Test	Control
24–48 h	64.8 ± 2.7	60.10 ± 1.7	9.6 ± 0.8	9.8 ± 1.1	17.1 ± 2.9	13.4 ± 1.6	1.00 ± 0.13	1.03 ± 0.09
P value	0.15		0.74		0.3		0.74	
First week	64.6 ± 1.9	64.9 ± 1.2	14.2 ± 0.6	14.4 ± 0.7	13.7 ± 1.7	13.6 ± 1.4	1.00 ± 0.08	0.92 ± 0.04
P value	0.91		0.79		0.97		0.41	
Second week	63.2 ± 1.4	61.8 ± 1.1	10.8 ± 0.9	10.1 ± 0.6	12.5 ± 1.9	11.9 ± 0.9	0.99 ± 0.08	1.00 ± 0.03
P value	0.46		0.11		0.98		0.88	
Third week	64.2 ± 1.4	62.1 ± 1.3	10.6 ± 0.5	9.2 ± 0.7	12.4 ± 1.5	12.4 ± 1.5	0.98 ± 0.06	1.06 ± 0.07
P value	0.26		0.09		0.87		0.47	
Fourth week	63.6 ± 1.7	61.6 ± 0.8	9.6 ± 0.3	9.8 ± 0.4	16.1 ± 1.6	10.7 ± 0.5	0.85 ± 0.05	0.96 ± 0.06
P value	0.55		0.72		0.02		0.06	

dose being repeated 5 days later. Neither preparation had any effect on humoral immunity (Paulik et al. 1989). Babiuk and Misra (1981) suggested that administration of levamisole and attenuated infectious bovine rhinotracheitis vaccine in vivo did not elevate cellular or humoral responses. Cabaj et al. (1995) reported similar results in lambs injected with human erythrocytes and ovalbumin 1 day after levamisole administration.

Most drugs' actions on the immune system are modulated by the interaction between the T cell recruiting efficacy of the sulphur moiety and the cholinergic effects of the imidazole ring (Renoux 1980). Levamisole appears to alter cyclic nucleotide phosphodiesterases, decreasing cyclic guanosine monophosphate (cGMP) degradation and increasing cyclic adenosine monophosphate degradation (Boothe 2001). Stogause and King (1995) suggested that, in the rat, levamisole administration caused lower levels of corticosterone than in controls and probably this reduction increased immune function. Szeto et al. (2000) reported that levamisole induces interleukin 18, a recently characterised cytokine with potent activity in stimulating interferon gamma and shifting the immune balance toward a type 1 response.

In conclusion our results indicated that, in normal neonatal dairy calves, levamisole can promote non-specific immune responses, although further studies using more precise methodology will be necessary.

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