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Prevalence of Theileriosis in Sheep in Okara District, Pakistan

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Abstract.- Ovine theileriosis is an important tick-borne disease of sheep in the tropical and subtropical regions of the world, caused by *Theileria hirci* (*T. lestoquardi*). The disease is economically important in small ruminants, particularly in sheep, causing clinical illness and mortalities in Middle East, west Asia, Indian subcontinent, parts of Africa and Mediterranean Basin. The present study was done to determine the infection rate of theileriosis in Lohi sheep and to investigate an economical and effective remedy for this protozoal problem. Another object was to study the effect of this disease on various blood parameters. A total of 400 sheep were examined for the presence of Thieleria in blood smears. Clinical signs were recorded and lymph node biopsy smears were also examined. On the basis of Giemsa stained blood smears, 16.5% sheep were found positive of which 65.1% were clinically symptomatic and showed schizonts of thielerial parasite in their lymphocytes. For treatment buparvaquone was found to better (90% success rate) than oxytetracycline (30% success rate).

Key Words: *Theileria hirci*, ovine oxytetracycline, buparvaquone.

INTRODUCTION

Theileriosis is a tick-borne disease of wild and domestic animals in the tropical and subtropica regions of the world. The etiologic agent of ovine theileriosis is *Theileria hirci* (*T. lestoquardi*), which is transmitted by ticks of the genus *Hyalomma*. The ovine theileriosis is an acute, subacute, or chronic disease characterized by pyrexia, malaise anorexia, lacrimation, digestive disturbances, emaciation, dyspnoea, swelling of the superficial and internal lymph nodes, enlargement of the spleen and liver, lymphoid infiltration of the kidneys, ulceration of the abomasum, icterus and transitory haemoglobinurea. In contrast, *T. ovis*, transmitted by different species of tick including *Rhipicephalus evertsi* and *Ixodes ricinus*, is apathogenic or causes only a mild disease in sheep (Hooshmandrad and Hawa, 1973). Ovine theileriosis is an economically important disease of small ruminants, particularly sheep and causes clinical illness and mortalities. The disease occurs in south-eastern Europe, North Africa, the Near and the Middle East, India and China and considered as an important cause of disease and mortality in the Mediterranean Basin, West Asia, the Indian subcontinent and possibly parts of China (Uilenberg, 1997).

Bovine theileriosis has been extensively studied worldwide but information concerning ovine theileriosis is sporadic. Recently, interest has arisen in sheep-infecting *Theileria* parasites. Of these, *Theileria hirci* is considered to be highly pathogenic (Jianxung and Hong, 1997; Yin *et al.*, 2002). A number of theileriocidals including tetracyclines, buparvaquone and halofuginone lactate have been used for the treatment of this disease (Radostits *et al.*, 2007).

Keeping in view the importance of sheep in Pakistan and adverse effect of theileriosis on it, the present study was designed to determine (i) the infection rate of theileriosis in sheep, (ii) evaluate and compare the efficacy of oxytetracycline and Buparvaquone in sheep and (iii) to study the effect of theileriosis on various blood parameters of infected sheep.

MATERIALS AND METHODS

Infection rate of theileriosis in sheep

A total of 400 Lohi sheep in and around the Livestock Production Research Institute, Bahadurnagar, district Okara, Pakistan, were tested to find out the infection rate of theileriosis in sheep.

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The presence of the disease was confirmed on the basis of Giemsa stained blood smears, clinical signs and lymph node biopsy smear examination as described by Razmi (2006), El-Azazy *et al.* (2001) and Chen *et al.* (2000), respectively.

Efficacy of oxytetracycline and buparvaquone

In this part of the experiment, oxytetracycline and buparvaquone were evaluated as theileriocidal agents. Thirty sheep naturally infected with theilerial parasite were randomly divided into 3 groups (A, B, and C) each comprising 10 animals. Another group (group D) was comprised of 10 healthy sheep. All animals were kept in experimental sheds of Livestock Production Research Institute, Bahadurnagar. The four groups were kept in four different isolation rooms. The animals were maintained under standard conditions of health and management and were given balanced ration. The animals in group A were given oxytetracycline (Rimoxyn, PDH Pharma Pakistan) @ 10mg/kg body weight intramuscuarly for five days. The animals in group B were given Buparvaquone (Butalex, ICI Pakistan) @ 2.5mg/kg body weight IM once. The animals in groups C and D served as positive and negative controls, respectively.

Blood smears from all animals were made on day 0 (pre-medication), and at 7 and 10 days (post-medication). The smears were examined for the presence of theilerial parasite. The efficacy of the drugs was calculated on the basis of reversal of clinical signs and disappearance of theileria from blood smears.

Hematological studies

To see the effect of theileria on various blood parameters 5ml blood was collected from the animals of all groups at day 0 (pre-medication), and then at day 7 and 10 (post-medication). The blood was analyzed for hemoglobin content (g/dl) according to Sahli's method (Wintrobe, 1929), total leukocytic count (TLC) (1000/mm^3) according to Benjamin (1986) and differential leukocytic count (DLC) on blood films stained by Giemse's method (Schalm, 1975).

Statistical analysis

The data thus obtained were analyzed statistically by using Z-test and one side analysis of variance (ANOVA) (Zar, 2002). The diagnostic test used for ovine theileriosis also compared in terms of specificity and sensitivity using Kappa statistics.

RESULTS AND DISCUSSION

Infection rate

Out of total sheep examined (n = 200), 104 and 296 were lambs (< than 6 month of age) and adults (> than 6 month of age), respectively, while 296 were females and 104 were male. Out of 104 lambs and 296 adults, 18 (17.3%) and 48 (16.2%) were found to be positive for the presence of theileriosis, respectively. Moreover, 49 (16.5%) and 17 (16.3%) female and male sheep were found to be positive for the natural infection with *Theirleria annulata* (Table I).

Table I.- Infection rate of theileriosis in L.P.R.I. Bahadurnagar in different age and sex groups*.

Groups		No. of sheep	DSM**	
			Positive	Negative
Age	Lambs***	104	18 (17.3)	96 (82.7)
	Adults	296	48 (16.2)	248 (83.8)
Sex	Female	296	49 (16.5)	247 (83.5)
	Male	104	17 (16.3)	97 (83.7)

*(n = 400)
** Direct smear method
*** Up to 5 months of age

Lymphnode biopsy (LNB) showed 65.152 (95%CI; 53.656-76.647) sensitivity and 100% specificity by taking direct smear method (DSM) as gold standard. Similar trend was seen in case of negative predictive values (NPV) and positive predictive values (PPV). Agreement between LNB and DSM was found to be good (0.757; 95%CI; 0.662-0.852). Similarly, by taking LNB as gold standard showed 100% sensitivity and 94.659 (95%CI; 92.432-96.957), while similar trend was observed in NPV and PPV. Agreement between the two tests performed *i.e.*, LNB and DSM was 0.672 (95%CI; 0.580-0.765).

Out of 400 sheep 16.5% (66) were found positive for theileriosis by Giemsa stained blood smear examination. Out of these 66 sheep 65.1% (43) were exhibiting clinical signs. These clinically positive animals were found positive in giemsa stained LNB smears. This study is in accordance with the study of Guo *et al.* (2002) who reported 17.12% infection rate in Gansu province of China. The results of current study also resemble with Altay *et al.* (2005) who examined 124 sheep out of which 24 were found infected by using microscopical examination of blood smears. The finding of present study also resemble with Tageldin *et al.* (2005) who reported 17% prevalence in Sultanate of Oman.

The findings of present study are not in agreement with Alyasino and Greiner (1999) who reported a seroprevalence of 59.9%, Papadopoulos *et al.* (1996) who reported 24.6% in Macedonia region of Greece and Jianxung and Hong (1997) who reported 78–85% infection.

Distribution of positive individuals on the basis of age groups was recorded as 17.3% in lambs and 16.2% in adults that is not in agreement with Jianxung and Hong (1997) who reported 78-85% in lambs and 9% in adult animals. While the distribution on the basis of sexes was recorded as 15.8% in male and 16.6% in females (Table I).

Clinical signs observed were pyrexia, emaciation, dyspnoea and swelling of the superficial lymph nodes, which are in accordance with study of Hooshmand and Hawa (1973) who reported the same clinical findings.

The agreement and validity of DSM and LNBS by taking DSM and LNBS as gold standard consecutively were checked at the confidence interval of 95% (Table II). The Kappa values of DSM and lymph node biopsy methods while taking them as gold standard are 0.757 (CI 95%; 0.662-0.852) and 0.672 (CI 95%; 0.580-0.765). The results of Kappa statistics shows that DSM is more specific and LNB smear method is more sensitive (Table II).

Drug trials

Animals of group A were treated with Rimoxyn at the dose rate of 10mg/kg of body weight for five days showed 30% efficacy at day 10 (post-medication) and animals of group B were treated with Butalex at the dose rate of 2.5mg/kg once showed marked high efficacy than group A *i.e.* 90% at 10^{th} day (post-medication), While group C was kept as infected control and group D served as healthy control.

The results of drug trials are in line with Singh *et al.* (1993) and Radostits *et al.* (2007). The study of McHardy (1984) also supports the results of group A, but he reported that Buparvaquone at the rate of 20mg/kg body weight is the most effective which is eight times greater than used in this experiment, same dose of Buparvaquone was used by Gill *et al.* (1984) and reported 100% efficacy.

Hematological studies

Table III shows haemoglobin concentration, and total leukocyte count (TLC) of different groups of sheep. A significant difference ($p<0.05$) was observed between the average Hb values of groups. A mild to severe anemia in the infected animal was observed which is in agreement with the Hooshmand and Hawa (1973). This anemia markedly subsides in the group B (treated with butalex) as compared to group A (treated with rimoxyn).

The results of TLC are in line with Osman and Al-Gaabary (2007) who reported a marked difference in TLC of healthy and diseased animals. It was observed that a significant difference ($P<0.05$) was present among all the groups.

Differential leukocytic count (DLC)

In this part of hematological study five different types of leukocytes were observed *i.e.* neutrophils, lymphocytes, monocytes, eosinophils and basophils. The average (%) values are given in Table IV.

In group A, insignificant difference observed in the percentage values of neutrophils, monocytes and basophiles while significant differences were in percentage values of lymphocytes ($P<0.003$) and eosinophils ($P<0.006$). In group B, insignificant difference was observed in the percentage values of neutrophils, monocytes and basophiles while significant differences were in percentage values of lymphocytes ($P<0.001$) and eosinophils ($P<0.001$). In groups C and D, insignificant difference was

Table II.- Agreement and validity of direct smear method (DSM) and lymph node biopsy (LNB) by taking DSM and LNB as gold standard consecutively.

		Gold standard (DSM for LNB and LNB for DSM)		Kappa	Sensitivity	Specificity	PPV*	NPV**
		+	-					
LNB	+	43	0	0.757 (CI 95%; 0.662-0.852)	65.152 (CI 95%; 53.656-76.647)	100 (CI 95%; 100.0-10.0)	100 (CI 95%; 100.0-100.0)	93.557 (CI 95%; 91.011-96.144)
	-	23	334					
DSM	+	23	20	0.672 (CI 95%; 0.580-0.765)	100 (CI 95%; 100.0-100.0)	94.659(CI 95%; 92.432-96.957)	53.488 (CI 95%; 38.580-68.397)	100 (CI 95%; 100.0-100.0)
	-	0	357					

*PPV, Positive predictive value; **NPV, Negative predictive value.

Table III.- Hemoglobin estimation in sheep of different groups of sheep at 0, 7th and 10th day* of haematological parameters.

Groups	Hemoglobin concentration on day			Total leucocytic count on day		
	0	7	10	0	7	10
A = Oxytetracycline @ 10 mg/kg body wt.	7.20 ± 0.397	8.02 ± 0.543	9.13 ± 0.429	12.86 ± 1.468	12.20 ± 1.064	11.64 ± 0.758
B = Buparvaquone @ 2.5 mg/kg body wt.	7.13 ± 0.489	8.15 ± 0.544	9.80 ± 0.864	12.93 ± 1.462	12.36 ± 1.10	11.99 ± 0.869
C = Infected (Control)	6.84 ± 0.426	6.60 ± 0.374	6.54 ± 0.424	14.02 ± 1.059	14.30 ± 0.822	14.43 ± 0.758
D = Health (Control)	9.63 ± 0.549	9.89 ± 0.514	10.41 ± 0.969	8.03 ± 0.935	8.11 ± 0.839	8.30 ± 1.045

* Data shown is mean (%) ± S.D (n= 10)
Significant difference (P<0.05) found between the groups.

Table IV.- Average percentage of DLC in sheep of groups A & B at day 0, 7, and 10*.

Type of leukocytes	Group A = Oxytetracycline @ 10 mg/kg body wt. on day			Group B = Buparvaquone @ 2.5 mg/kg body wt. on day		
	0	7	10	0	7	10
Neutrophiles	37.9	36.0	35.6	32.4	37.7	35.3
Lymphocytes [a]	47.5	50.4	52.5	49.2	50.2	54.1
Monocytes	2.4	2.3	1.7	2.0	1.4	1.5
Eosinophils [a]	11.5	10.2	9.3	11.9	9.9	8.1
Basophils	0.7	1.4	0.8	0.8	0.7	0.9

*Data shown is mean (%) (n= 10)
Significant difference (P<0.05) found between days

observed in the percentage values of all five types of leukocytes *i.e.*, neutrophils, lymphocytes, monocytes, eosinophils and basophiles. The results of present study are also in line with Osman and Al-Gaabary (2007) who reported variation in the different types of leukocytes in healthy and diseased animals.

CONCLUSION

The results of present study reflects that ovine theileriosis is an important tick born problem in Pakistan. Adverse effects of this disease on health and production of the animals needs further investigation on molecular epidemiology of the

organism in the areas where sheep is densely populated.

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