PREVALENCE OF THEILERIOSIS IN BUFFALOES AND DETECTION THROUGH BLOOD SMEAR EXAMINATION AND POLYMERASE CHAIN REACTION TEST IN DISTRICT LAHORE

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ABSTRACT

Blood samples (336) were collected record prevalence of Theileriosis in buffaloes from twenty one villages of District Lahore. High fever, lacrimation, swelling of sub mandibular and sub scapular lymph nodes, weakness, increased respiration & pulse, corneal opacity, anorexia, loss of condition, pale conjunctiva, anemia, in-coordination and rough hair coat were observed in buffaloes suffering from theileriosis during summer months. Packed cell volume, haemoglobin concentration, total erythrocytic count, and blood smear examination showed presence of macrocytic hypochromic anemia in buffaloes infected with the disease. Based on microscopic examination 39.9 (134/336) prevalence was recorded as compared to 53.3% (179/336) with polymerase chain reaction (PCR) test.

Key words: Theileria, buffaloes, PCR test and blood smear examination.

INTRODUCTION

Ticks comprise a burning veterinary problem because they transmit diseases e.g. Theileriosis, babesiosis, anaplasmosis and trypanosomiasis, induce paralysis or toxicosis and cause physical damage to livestock (Rajput *et al.* 2005). Certain Ixodid ticks such as *Hyalomma anatolicum anatolicum, H. m. marginatum*, and *H. a. excavatum*, known to transmit *Theileria annulata*, are found in large numbers in the Mediterranean region, especially in semi-arid areas. Bovine theileriosis is caused by the protozoan parasite *Theileria annulata* and *Theileria parva*. The disease is considered one of the most destructive obstacles to livestock production (Ogre, 1999). Bovine *Theileria* species are intracellular parasites that cause severe and mild infections in their hosts. *T. annulata* is widely distributed in many areas of the world, extending from southern Europe to southern Asia (Brown, 1990). Clinical signs of the infected buffaloes include, pyrexia (40.5–41.5 °C), anorexia, enlargement of superficial lymph nodes (parotid, prescapular and prefemoral), slight nasal and ocular discharges with congestion of conjunctiva and salivation. Constipation was recorded in some cases later turning to tarry diarrhoea, with pale mucous membranes, milky infiltration of the cornea and respiratory distress in the form of dyspnoea, coughing and evidence of pulmonary oedema and nervous manifestations in the form of hyperesthesia, head pressing, convulsions, tremors and paddling prior to death. (Sutherland *et al*. 2007). The diagnosis of theileriosis in acute cases is mainly based on clinical findings and microscopic examination of Giemsa's stained thin blood smears. In long standing carrier animals blood smears are negative on microscopy (Aktas *et al.* 2006). The advent of the PCR coupled with the specificity of deoxyribonucleic acid (DNA) hybridization had led to the development of specific and sensitive molecular diagnostic tests to detect and characterize the organisms that cause theileriosis (Collins *et al.* 2002).

Keeping in view the economic importance of theileriosis, the present study was designed to determine the prevalence of disease in buffaloes in district Lahore by blood smear examination and PCR.

MATERIALS AND METHODS

Blood samples were collected from buffaloes infested with ticks from 21 villages of District Lahore during months of May, June, July and August of 2007. Eighty four samples were collected in each month, four samples from each village.

Clinical Manifestations Recorded: The clinical manifestation of high temperature, lacrimation, swelling of sub mandibular and sub scapular lymph nodes, weakness, increased respiration and pulse , corneal opacity, anorexia, loss of condition, pale conjunctiva, anemia, with in-coordination and rough hair coat were recorded during months of summer season (May, June, July and August).

Collection of blood samples: Blood smears were prepared from blood taken from jugular vein. The dried smears were stained by Giemsa's staining technique described by Benjamin (1978). For polymerase chain reaction test, 3 ml from each, blood sample were

collected in EDTA coated vacutainers. Estimation of blood parameters of diseased animals was done by hematology analyzer at clinical laboratory Deptt. of Pathology University of Veterinary and Animal Sciences, Lahore.

Polymerase Chain Reaction Test

Analysis of Samples: Total DNA was extracted from samples with the help of DNA extraction kit according to prescribed method (PUREGENE ® USA, GENTRA) Analysis of extracted DNA was made by spectrophotometer and agarose gel electrophoresis based analysis. The Primers used in the present study were N516 (F) GTAACCTTTAAAAACGT, N517(R) GTTACGAACATGGGTTT, 989 (F) AGTTTCTGACCTATCAG, 990 (R) TTGCCTTAAACTTCCTTG. The primers were previously described by Allsopp *et al* (1993) polymerase chain reaction (PCR) test was performed after Christine, (1995) with two concentration of $MgCl_2$, 5ul & 6ul for *Theileria annulata*. Amplification was done with the help of thermal cycler set for 30 cycles starting with temperature 94°C for 5 min. for denaturing. Temperature was lowered for several minutes to allow both forward and backward (right or left) primers to anneal with the complementary sequences. At this stage three conditions 50 °C, 55 °C and 60 °C for 30 seconds were checked for each primer set. For extension, temperature was raised to 72 °C for 45 seconds and completed with 72 °C for 7 min. Analysis of amplified product by electrophoresis was done with a 1% agarose gel. The results were photographed.

Infection percentage was calculated as described by Thrusfield, (1986) according to following formula;

$$\frac{\text{No. of cases present in population at a specific time}}{\text{Total population at a specific time in same area}} \times 100$$

Statistical analysis: Statistical Analysis was done by applying T. test of proportion against Blood smear and PCR test (Zar, 2004).

RESULTS

Blood smear examination: The blood films contained *Theileria* piroplasms; including cocci, rod, and stick, comma, fusiform, racquet-shaped, signet ring, and pear-shaped forms with diameter of 0.5-1.5 micrometer. Abnormalities in erythrocyte structure were also observed in both *Theileria* and *Babesia* positive smears. (Figure 1)

The highest prevalence 44% (37/84) of the disease was recorded in June and lowest prevalence 36% (30/84) was recorded in the month of August whereas prevalence of 47% (37/84) and 33% (32/84) was observed during the months of May and July, respectively by blood smear examination. Village wise prevalence was recorded and is summarized in table 1.

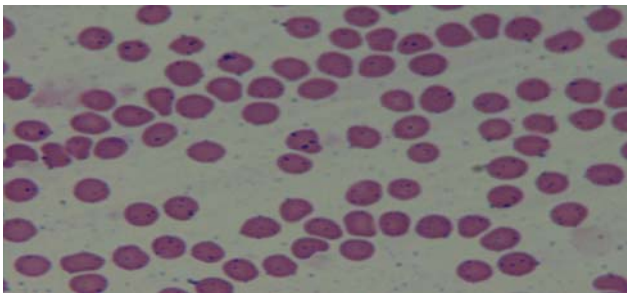

Figure 1: Blood Smear showing abnormalities in erythrocytes with Theileria parasites.

Polymerase chain reaction test: Data recorded on ten DNA extracted samples by spectrophotometeric analysis showed the ratio between 260nm and 280 nm in the range of 1.6-2.2. It was found out that 6ul of MgCl2 gave successful results than 5ul concentration. It was found out that primers set A anneal at Tm 55 °C while Primer set B anneal at temperature 60 °C. The 721-bp fragment was generated in all samples tested with N516/N517. For primer set 989/990 the expected 1098 bp fragment of DNA was amplified except in samples taken before infection (Figure 2). The over all prevalence 53.3% (179/336) was recorded for *Theileria annulata*, during summer seasons based on PCR test. The maximum prevalence was recorded in the month of June 58.33% (49/84), followed by May 53.6% (45/84), followed by July 52.4% (44/84) and the minimum 48.8% (41/84) was recorded during the month of August. Village wise prevalence is summarized in Table 1.

L1 L2 L3 L4 L5 L6 L7 L8 L9 L10 L11 L12

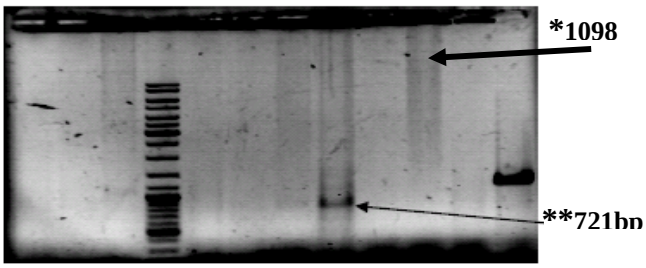

Figure 2: **Amplified DNA by Primer Specific for Theileria**

L1, L2, L3, L5, L6, L7, L9, L10, L11 are negative L4 is ladder mix and L8 indicating Theileria annulata and L12 theileria specific.

Comparison of Blood Smear and PCR Test: The efficacy of microscopic examination revealed during present study was 39.9% (134/336), while efficacy of PCR test was 53.3% (179/336). The samples positive for blood smear were also positive by PCR test, while out of 179 samples, 44 samples positive by PCR test were not detected by microscopic examination. Therefore, PCR

test showed high sensitivity as compared to microscopic examination. Statistical analysis showed significant difference in both diagnostic methods.

Table 1: Village wise prevalence of theileriosis in buffaloes:

Name of Village	Blood Smear positive out of 16	Blood Smear %	PCR positive out of 16	PCR% positive
Manga	8	50.0	11	68.8
Ottar Halloki	8	50.0	11	68.8
Jia Bagga	7	43.8	11	68.8
Ghawandi	7	43.8	10	62.5
Khushal Kalan	7	43.8	10	62.5
Karbath	7	43.8	10	62.5
Jhadoo	7	43.8	10	62.5
Khana	7	43.8	9	56.3
Khananpur	7	43.8	9	56.3
Panghali	7	43.8	9	56.3
Chak boota	7	43.8	9	56.3
Rakh Dera Chaal	7	43.8	9	56.3
Wagha	7	43.8	9	56.3
Kingrey	7	43.8	9	56.3
Mangha	7	43.8	9	56.3
Mangha Hithar	6	37.5	9	56.3
Burki	6	37.5	9	56.3
Ladekey	6	37.5	8	50.0
Bhaseen	6	37.5	7	43.7
Baoowali	5	31.2	6	37.5
Jallopind	5	31.2	6	37.5

Blood Parameters: The blood parameters PCV, Hb concentration and TEC showed presence of macrocytic hypochromic anemia in diseased animals (Table 2)

TABLE 2: Comparison on Normal Values of PCV, TEC, and Hb concentration With Present Findings in Buffaloes

Hematological Parameters	Normal*		Diseased (Hemoparasites infected)	
	Range	Mean	Range	Mean
PCV (l/l)	0.24-0.46	0.35	0.10-0.20	0.15
Total Erythrocyte count(X10 ^{12}/l)	5-10	7.5	2-4	3.00
Hemoglobin (gm/l)	80-150	115	29-100	64.50

*Normal values, Dorner and Hoffman, (1978).

DISCUSSION

Lalchandani (2001) studied the prevelance of blood protozoa in Kundhi buffaloes and reported 39.21 percent (197/500) animals infected with different blood protozoa. According to his study, light intensity infection (58.82 percent) theileriosis was prevalent in buffaloes, which is similar to the present study (PCR test). Oliveira *et al.*, (1995) detected Theileria annulata through PCR test in blood samples of cattle and reported higher efficacy of PCR test (75%) compared to blood smear examination (22%). Young *et al*. (1978) studied the incidence of theilerial parasites in East African buffaloes (Syncerus caffer). A total of 245 buffaloes from 13 areas of East Africa were examined for theilerial infections out of which 97.1 percent had piroplasms in their erythrocytes. The results revealed the persistence of anemia and an enhanced inflammatory response. Abnormalaties in erythrocytes were also present. The epidemiological work on theileriosis in buffaloes in district Lahore had never been reported before. The reason of higher incidence in district Lahore may be attributed to that the animals are mainly housed in animal sheds with brick soling flours which had the crevices between bricks to be the breeding sites for ticks obtained from carrier animals.

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REFERENCES

Ahmad, J.S. and H. Mehlhorn (1999). Review: the cellular basis of immunity and immunopathogenesis of tropical theileriosis. Parasitol. Res., 85: 539-549.

Aktas, M., K. Altay and N. Dumanli (2006). A molecular survey of bovine *Theileria* parasites among apparently healthy cattle and with a note on the distribution of ticks in eastern Turkey, Vet. Parasitol., 138 (3-4): 179 –185.

Allsopp, B. A., H. A. Baylis, M. T .E. P. Cavalier-Smith, R.P.Bishop (1993). Discrimination of six species of *Theileria* using oligonucleotide probes which detect small subunit ribosomal RNA sequences. Parasitology, 107(2): 157-165

Benjamin, M. (1978). Outline of Veterinary Clinical Pathology. Third Ed. The Iowa State University, Press, Ames, Iowa, U.S.A. pp: 51-53.

Brown, C., G. C. Stagg, D. A. Purnell, R. E. Kanhai, and G. K. Paynie (1990). Infection and transformation of bovine Iymphoid cells in vitro by infective particles of Theileria annulata. Nature, 245: 101-103.

Christine, O (1995). Detection of Theileria annulata in blood samples of carrier cattle by PCR test. J.Clinical Microbiology, 77: 266-269**.**

Collins. N. E., M. T. Allsopp and B. A. Allsopp (2002) Molecular diagnosis of theileriosis and heart water in bovines in Africa. J. Vet. Med., 50(6):. 309-310.

Dorner, J. L and W.E. Hoffman (1978).Normal haematological parameters. J. Am. Anim. Hosp. Assoc. 14: 219.

Lalchandani. C. L (2001) Efficacy of various drugs against blood protozoa in Kundhi Buffaloes. Parasitologia, 32: 165-176.

Ogre, P.B. (1999). Assessment of natural Ixodid tick infestation. J. Vet. Med. 46: 405-419.

Oliveira. C. D, M.V Weide, M.A Habela, P.Jacquiet, and F. Jongejan (1995). Detection of Theileria annulata in blood samples of carrier cattle by PCR test. J.Clin. Microbiol; 33(10): 2665–2669

Rajput. Z. I, S.H. Hu, A.G. Arijo, M.Habib, and M.Khalid (2005). Comparative study of *Anaplasma* parasites in tick carrying buffaloes and cattle. J.Zhejiang University, 45: 90-91.

Thrusfield, M.V (1986) Describing disease occurrence: Veterinary Epidemiology 1st Edition: Butterworth & CO.Ltd pp 191-197.

Young, A. S, C. G. Brown, M. J. Burridge, J. G. Grootenhuis, G. K. Kanhai, R.E. Purnell, D. A. Stagg (1978) the incidence of theilerial parasites in East African buffaloes (Syncerus caffer). Tropenmed Parasitol. ; 29(3):281-8.

Zar. H. J (2004) Biostatistical Analysis Fourth edition. Published by person education (Singapore) Pte.Ltd.Indian Branch, 482, F.I.E, Patpargany, Delhi, 110092 India, Pp.137- 160.