

Clinical, epidemiological and therapeutic studies on bovine tropical theileriosis in Faisalabad, Pakistan

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Abstract

While screening of selected herds of cattle (each having minimum 15 animals), 28 animals were found positive for tropical theileriosis (*Theileria annulata*) from a total of 260. The overall bi-monthly incidence rate was 10.8% from April-July, 2011 in Faisalabad. 89.3% and 10.71% in females and males respectively. To check breed wise prevalence, 4 out of all 28 positive animals were of Red Sindhi and Dhanni showing 14.2% prevalence, and rests of all were crossbred with 85.71% prevalence. The bi-monthly incidence during April-May was 28.57% while in June-July this was 71.42%. The suspected cattle were subjected to analysis by making thin blood and lymph smears. After confirmation, 28 animals were divided into two equal groups. The animals of group A were treated with extracts of *Peganum harmala harmala* at the dose rate of 7 mg/kg b.w intramuscularly while those of group B were treated with buparvaquone at the dose rate of 2.5mg/kg b.w intramuscularly (According to manufactured Comp.). Twelve animals were recovered after treatment with *P. harmala* (85.71%) and 2 could not be cured and died (14.28%) while in case of animals treated with buparvaquone, 13 cattle recovered (92.85%) and one animal died (7.14%). The obtained data were then subjected to Chi-Square Test for analysis of variance.

Keywords: Cattle; Theileriosis; *Peganum harmala*; buparvaquone

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دراسات سريرية وبائية وعلاجية لمرض ثايليريا الماشية في فيصل آباد، باكستان

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الخلاصة

عند فحص قطعان من الماشية (١٥ بقرة في الأقل بكل قطيع) وجد ٢٨ بقرة من أصل ٢٦٠ موجبة لمرض الثايليريا. كانت نسبة الحدوث لمرتين في الشهر ١٠،٨% من نيسان إلى تموز ٢٠١١ في فيصا آباد، وكانت ٨٩،٣% في الإناث و ١٠،٧١% في الذكور. تم العلاج بخلاصة نبات الحرمل ٧ ملغم/كغم، في العضل و بوبارفاكون ٢،٥ ملغم/كغم، في العضل. كانت نسبة الشفاء للعلاجين ٨٥،٧١% و ٩٢،٨٥%، على التوالي.

Introduction

Livestock sector is playing a pivotal role in the economy of Pakistan. It contributes 53.2% of the agricultural Gross domestic product (GDP) and 11.4% in

total GDP which is more than the total aggregate of crop section share i.e. 10.3%. According to Economic Survey of Pakistan total population of cattle is about 34.3. Total milk production is 36,299 thousand tons per year and its value is more than that of combined value of cash crops (1). This

industry of Pakistan is facing crisis due to monumental issues including unhygienic conditions and lack of proper tick control and, number of various tick borne diseases. Major hosts of this disease are cattle in (Zebu cattle) and water buffaloes (2,3). The climatic conditions of the tropical and subtropical countries are encouraging for the multiplication and growth of ticks that is a predisposing factor for the spread of various parasitic diseases. The ultimate consequence is a decline in the production of dairy animals (4). Weight loss, weakness, anorexia, pyrexia, conjunctival petechiae, enlargement of lymph nodes, anemia and cough are the most common symptoms. Exotic cattle, their crossbreds and young indigenous calves are highly susceptible while indigenous cattle are relatively resistant (5). Sporadic cases of this disease are seen throughout the year in Pakistan (6). However, outbreak in exotic and crossbred cattle occurs mostly during hot and humid months of the year (7). Tentative diagnosis of theileriosis in the field is mainly based on clinical sign, presence of vector and previous history of the disease. However, confirmation of the diagnosis depends on microscopic examination of Giemsa stained thin blood smear (8). The theileriosis is a devastating disease having impact on the small farmers which represent the majority of livestock owners in the endemic areas. Strategies currently used against theileriosis are very expensive and all have serious limitations in the sustainability and efficacy (9). The morbidity, mortality and prevalence of tropical theileriosis are considerably high in tropical areas (8). Hemato - biochemical changes are indication of severity of the disease and thought to be good tools for the diagnosis and prognosis for effective therapy. Various formulations have been used for the treatment of theileriosis. Among these, Buparavaquone has been considered as the drug of choice (10). Plant extract of *Peganum harmala* (harmal) has shown very good curative rate against tropical theileriosis and it is a natural drug which does not infiltrate in muscles of cattle (11). The pharmacological active ingredients of *P. harmala* are various alkaloids (12). These include β -carbolines, such as harmaline, harmine, harman, harmatol and quinazoline derivative such as vasicine as well as vasicinone. It has been cited in literature that alkaloid compounds found in *P. harmala* illustrate a diversity of antiprotozoal activity (12). Among these various alkaloids, harmaline (harmidine, C₁₃H₁₄N₂O) has been found to be the major active alkaloid (12). In present study, effort was made to evaluate the therapeutic effect of extract of *P. harmala* in comparison with buparvaquione. To estimate the bimonthly incidence rate of tropical theileriosis from April to July 2011 in the few selected herds in Faisalabad, Pakistan.

Materials and methods

The study area of this project was comprised of ten different dairy farms in the vicinity of district Faisalabad. For this purpose, ten dairy farms managing at least 15 cattle were investigated; Waseer Dairy Farm (244 R B Chammayana Waseeran Faisalabad), Riaz Dairy Farm (Factory Area Faizabad Faisalabad), Rana Dairy Farm (79 Ada near Baba Rangh Ali Ratna Faisalabad), Nasir Dairy Farm (Aziz Town Summundri Road Faisalabad), Livestock Farm (University of Agriculture, Faisalabad), Doggar Dairy Farm (Rahim Town Summundri Road Faisalabad), Shoukat Dairy Farm (Aziz Town Summundri Road Faisalabad), Hafiz Dairy Farm (Sadhar Bypass Road Faisalabad), Sappra Dairy Farm (Kukur Wala Abbas pur Road Faisalabad), Mian Dairy Farm, (248 R B Dalowal Faisalabad).

From the above dairy farms, 28 animals suspected for theileriosis out of 260 were further investigated and clinico-epidemiological data were collected on a Performa.

Clinical Examination

Following parameters were evaluated from theileriosis positive animals: Rectal temperature, Lymph node enlargement, Loss of appetite, Bulging of temporal fossa, Respiratory signs (tachypnoea, dyspnoea, pneumonia etc), Diarrhoea, Lacrimation, Haemoglobinuria, Anaemia (pale mucous membrane), Nervous signs (Convulsion, torticollis etc), Presence of ticks on the body of animals.

Collection of Blood

For the blood collection, marginal ear vein was prepared for disinfection with the help of methyl alcohol (5%). Then, the ear vein was punctured with the help of 23.5 gauge needle and then slides were prepared. Sterilized disposable syringes of 5 ml capacity with EDTA were used to collect blood from jugular vein for other slides and were stored in refrigerator at 4°C for 7 days.

Preparation of Slides

Thin blood smear on clean and dry glass slides was prepared from marginal ear vein and thin smear by aspirating swollen lymph nodes of animals. These smears were air dried and fixed in methyl alcohol (%) for 10 minutes and stained with working dilution of Giemsa stain (1:10) ratio with phosphate buffer solution having pH 6.8 and fixed for 30 minutes. The smear was washed with tap water to remove extra stain and was air dried (13) and Slides were examined under the oil immersion lens of a light microscopic (14). The parasite was identified by the characters described by Soulsby (15).

Therapeutic response of *P. harmala* and Buparvaquone Plan of Chemotherapy

Theileriosis positive animals based on the clinical signs and blood smear analysis were divided into two equal groups A and B. Fourteen animals of group A were treated with the crude extract of *Peganum Harmala* at-the dose rate of 7 mg/kg body weight intramuscularly for 3 days while other 14 animals of group B were treated with

Buparvaquone (Bupacin, Intracin Pharamaceuticals, Pvt. India) at-the dose rate of 2.5 mg/kg for once and Butalex was repeated after 48-72 hours. Drugs were injected along with antipyretics to lessen the fever and improve the efficacy of drug. Healthy animals of group C (n=10) that were kept to compare normal parameters with infected animals (Table 1).

Table 1: Description of treatment protocol for the therapy of natural infection with theileriosis in cattle

Groups	Treatment	Dose Rate	Dose Route	Repetition at post 1 st inj. (hours)
Group A	Water based Extract of <i>P. harmala</i>	7 mg/kg body weight	Intramuscular	24,48
Group B	Buparvaquone	2.5 mg/kg body weight	Intramuscular	48-72
Group C (Healthy)	No treatment (-ve Control)	N A	N A	N A

Extraction method of *Peganum harmala*

The seeds of *P. harmala* will be collected and were taxonomically identified by Dr. M. Mansoor, Botanist in Department of Botany, University of Agriculture, Faisalabad. Then extract of *P. harmala* was prepared from the seeds of plant according to the method described by Manske and Holmes (16). Crushed seeds of *P. harmala* were dissolved for three times, their weight of water containing 30 g of acetic acid per liter of water. The seeds swelled as they could absorb the liquid and form thick dough which was pressed after 3 days. The pressed seeds were again treated as above twice, their mass of dilute acetic acid and after maceration and then liquid was again pressed and concentrated extract was sterilized in ultraviolet light for 12 hours and dried below 70°C in an oven. Then, ten grams of the dried alkaloid was dissolved in 90 ml of distilled water and filtered with 0.2 micron meter syringe filter and then injected to infected animal at-the dose rate of 7 mg/kg intramuscularly.

Toxicity of *Peganum harmala*

According to literature it is safe up to 119 mg/kg in mice but we checked it by injecting in 10 rabbits, 5 cats, 5 dogs, 5 sheep and 5 cattle. We not found any local or systemic reaction in the said animals.

pH of extract of *Peganum harmala*

The pH of this solution was 4.89. Seeds of *Peganum harmala* crushed and treated with Acetic Acid under U V Light and placed in oven below 70°C.

Statistical Analysis

Data t, obtained were subjected to Chi-Square Test for analysis of variance (17).

Results

The planned study was conducted to determine the bi-month incidence rate of theileriosis in cattle in Faisalabad and to determine the comparative efficacy of Herbal medicine (Harmal) and with Buparvaquone in cattle. From these selected dairy farms animals were counted and sorted out. The animals that were suffering with moderate to severe degree of theileriosis. Out of 260 animals 28 cattle were positive for theileriosis and the incidence found was 10.76%. Incidence rate was determined by applying the following formula.

$$\text{Incidence} = \frac{\text{New cases occurring during a given time period}}{\text{Population at risk during the same time period}} \times 100$$

$$\text{Incidence} = 28/260 \times 100 = 10.76 \%$$

Therapeutic response of crude extract of *Peganum harmala* and Buparvaquone against bovine theileriosis

After screening the positive animals for theileriosis were divided into two equal groups of 14 animals in each group. Group C was kept as a negative control to compare the normal parameters with infected animals. So animals of group A were treated with extract of *peganum harmala* at-

the dose rate of 7 mg/kg body weight intramuscularly route with antipyretic to lessen the fever and to enhance the efficacy of drug. After three days, 12 animals recovered

(85.71%) and two animals were too severe and died (14.28%) (Table 2).

Table 2: Efficacy of *Peganum harmala* and buparvaquone for the treatment of Theileriosis in cattle

Group A (Rx with <i>P. harmala</i> at-the dose rate of 7mg/kg) (14 Animals)	Group B (Rx with Buparvaquone at-the dose rate of 2.5mg/kg) (14 animals)	Healthy Animal (Control Group) (14 Animals)
Lives 12 (85.71 %)	Lives 13 (92.85 %)	All Live
Died 2 (14.28 %)	Died 1 (7.14 %)	All Live

Mean = 1.11 ± 0.315; Chi-square value=17.28; P value=< 0.01.

Associated determinants

Prevalence was found highest in crossbred (24/28; 85.71%), in order followed by Red Sindhi (3/28; 10.71) and Dhanni (1/28; 3.57%). Female (25/28; 89.28%) has been found to be more burdened than male (3/28; 10.71). Among

the age groups, the animals less than four years have been found predominantly infected with theileriosis in order followed by 4-5 years (12/ 28; 39.3%) and more than five years (3.6%) (Table 3).

Table 3: Age, breed and sex wise Prevalence of Theileriosis in Cattle

Age wise			Breed wise			Sex wise		
Age	Frequency	Percentage	Breed	Frequency	Percentage	Sex	Frequency	Percentage
< 4	11	39.3	Cross Bred	23	82.10	Male	2	7.1
4-5	16	57.1	Red Sindhi	3	10.70	Female	26	92.9
> 5	1	3.6	Dahnni	2	7.10	-	-	-
Total	28	100.00	-	28	100.00	-	28	100.00

Age wise: Mean= 1.64 ± 0.6; Chi Square value= 12.5; P value=< 0.01, Breed wise: Mean= 1.25 ± 0.59; Chi Square value= 30.071; P value= < 0.01, Sex wise: Mean= 1.93 ± 0.262; Chi Square value= 20.51; P value= < 0.01.

Table 4: Frequency of Tick control in Diseased Animals

Control/Not Control	Frequency	Percentage
Control	8	28.6
Not Control	20	71.4
Total	28	100.00

Mean= 1.71 ± 0.46; Chi Square value= 5.14; P value = < 0.05.

Prevalence of individual symptom

Frequency of lacrimation

Twenty six animals out of 28 animals showed lacrimation of eye due to theileriosis (92.9%) while 2 animals did not show these signs (7.1%) (Table 5).

Frequency of conjunctiva of eye in diseased animals

It was observed during the present research that 20 animals out of 28 animals showed pale colour of conjunctiva (71.1%) that was highest from the others as 7 animals had shown redness of conjunctiva (25%) while only one animal had shown pink colour of conjunctiva that was 3.6% (Table 5).

Frequency of lymph nodes in diseased animals

Ten animals out of 28 animals had shown prescapular lymphnode enlargement that was 35.7% and 9 animals showed parotid lymphnode enlargement (32.1%) while 4 animals showed prefemoral lymphnode enlargement (14.3%). Only 1 animal had shown submaxillary lymphnode enlargement (3.6%) and only 4% animals did not show enlargement of lymphnode of (14.3%) (Table 5).

Frequency of nasal discharge in diseased animals

It was recorded that 13 animals out of 28 showed mucopurulent discharge (46.4%) and 10 animals showed watery nasal secretions (35.7%). Only 1 animal showed purulent secretion (3.69%) and 4 animals did not show any nasal secretion (Table 5).

Frequency of diarrhoea in diseased animals

Eight animals out of 28 showed pasty diarrhoea (28.6%) and only 1 animal showed bloody diarrhoea (3.6%) due to advanced stage of disease. Nineteen animals out of 28 animals did not show diarrhea (67.9%) (Table 5).

Table 5: Clinical aspects along with its frequency in diseased animals

General Appearance			Conjunctiva of Eye		
Appearance	Frequency	Percentage	Color	Frequency	Percentage
Dull	10	35.7	Pink	1	3.6
Depressed	12	42.9	Pale	20	71.1
Anemic	6	21.4	Redness	7	25.0
Total	28	100.00	Total	28	100.00
Mean=3.07 ± 1.12; Chi Square value= 2.00; P value= <0.004.			Mean=2.21 ± 0.49; Chi Square value= 20.214; P value = <0.01.		
Lymph-nodes Enlargement			Nasal Discharge		
Category	Frequency	Percentage	Category	Frequency	Percentage
No	4	14.3	No	4	14.3
Parotid	9	32.1	Watery	10	35.7
Submaxillary	1	3.6	Muco-purulent	13	46.4
Prefemoral	4	14.3	Purulent	1	3.69
Prescapular	10	35.7	-	-	-
Total	28	100.00	Total	28	100.00
Mean=3.25 ± 1.57; Chi Square value= 10.21; P value= <0.05.			Mean=2.39 ± 0.786; Chi Square value= 12.85; P value= <0.01.		
Diarrhea			Lacrimation of Eye		
Category	Frequency	Percentage	Category	Frequency	Percentage
No	19	67.9	Yes	26	92.9
Pasty	8	28.6	No	2	7.1
Bloody	1	3.6	-	-	-
Total	28	100.00	Total	28	100.00
Mean=1.36 ± 0.6; Chi Square value =17.64; P value = <0.01.			Mean=1.07 ± 0.262; Chi Square value= 20.57; P value = <0.01.		

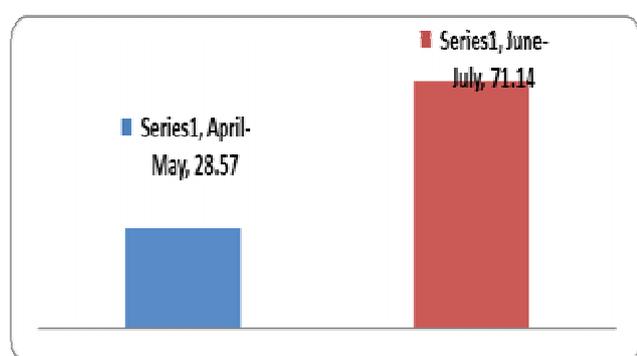


Figure 1: Bi-month wise prevalence of theileriosis in cattle showed in percentage.

Discussion

Haemoparasites like Babesia and Theileria are thought to be the important parasite of buffaloes and cattle in Pakistan (18). Theileriosis of cattle is serious problem in the improvement of the livestock industry in the major parts of the world. This disease caused by *T. annulata* which is serious protozoal disease of cattle and buffaloes in tropics

and sub-tropics. Environment of Pakistan is conducive for the maturation and growth of the ticks that make it susceptible to cause tick borne diseases which may be the explanation why Pakistan is endemic region of tropical theileriosis (18). Blood smear showed abnormal morphology of erythrocyte with presence of schizonts in infected cells. The infected RBCs revealed as echinocytes thorn like protrusions on the surface of the cells. The variations in the RBC morphology are mainly due to intravascular thrombi, erythrocytic oxidation (19). In the present study, the findings are in accordance with the results of Hashemi-Fesharki (19), who reported 88.7% recovery rate for the treatment of tropical theileriosis. Sayin et al., 2003 (20), reported that it occurs after the presence of ticks and goes to peak prevalence in the month of July. It is also reported that highest outbreak of theileriosis is in June to July by (6,10). Because in these months environmental temperature is very conducive for the multiplication and growth of ticks and that leads to theileriosis.

Degradation of piroplasms of theileria occurs after the administration of buparvaquone (Butalex) over one to four days (21). This may account for the time between 2 to 3 days between the administrations of the buparvaquone and comes back and return the body temperature to normal. The

findings of the present studies showed incidence of theileriosis in cattle as 10.76% which is in accordance with the findings of Sayin *et al.* (20), who noted prevalence of theileriosis as 11.1% in cattle. Similarly, prevalence of theileriosis was recorded 16.12% by Khan *et al.* (18), 16% in Holstein-friesian and 8% in Jersey cows by Zahid *et al.* (7) respectively in cattle. Our results of treatment with buparvaquone are in accordance with the finding of Muhammad *et al.* (10), who showed 93% curative rate of buparvaquone in tropical theileriosis. Likewise Zahid *et al.* (7) showed 100% curative rate and 81.73 % recovery rate showed by Qayyum *et al.* (22). In the present study mortality of the cattle could be due to advanced stage of theileriosis because the said drugs usually do not show good result in severe cases of the theileriosis. In the present study our work has shown the antitheilerial activity of Herbal medicine (*P. harmala*) extract on tropical theileriosis in cattle.

The activity of *P. harmala* has shown good results in theileriosis which open new ideas in the search for antitheilerial agents that are safest remedy from natural sources. Positive animals treated with *P. harmala* considered effective when body temperature be down to normal. Though, various researches rarely performed on the treatment of tropical theileriosis with the extract of *P. harmala* in cattle.

The findings of this study were approximately in accordance with the findings of other researcher (11), who showed the effectiveness of the total alkaloids of *P. harmala* for the treatment of tropical theileriosis as 93% curative rate. Similarly Mirzaiedehaghi (23) showed 65 % efficacy of *P. harmala* for the treatment of theileriosis in sheep, Muhammad (10) showed 78% curative rate in cattle and 100 % recovery rate was obtained by Derakhshanfar and Mirzaei (24).

A Sahiwal cattle are resistant as compared to exotic and cross bred cattle. In tropical countries, theileriosis is caused by *Theileria annulate*, *Theileria mutans*, *Theileria Lawrence* in bovine species (25). In Pakistan, theileriosis is caused by *Theileria annulata* (18). Outbreak of theileriosis in Pakistan has already been confirmed by (6,10) and the biological vector for its transmission is Hyalomma Ticks. In the present study 28 out of 260 cases were recorded positive from April 2011 to July 2011 which are the summer months in Pakistan.

Prevalence of theileriosis in cattle in ten selected dairy farms was 10.76%. The first symptom manifested by the Animal was fever above 105°F which coincide with the finding of Muhammad (10). A few days later over signs appeared including competence, cessation of rumination, streaked milk production and of lymph nodes enlargement and eye lids. Some of cattle which were positive of theileriosis but do not show any signs. The similar observation had been noted by (6) in Sahiwal cattle were

positive for Theileriosis without showing signs and symptoms. Soulsby (15) recommended the Giemsa, Leishnam and Wright stains for the identification of haemoparasite (*Theileria*) in red blood cells of the bovines. In the present study Giemsa stain was used.

Giemsa stain gave the best differentiator to demonstrate the theileria schizonts. While the other stains gave very poor result. *Theileria* is serious constraint for a developing country like Pakistan which is generating lot of money from livestock sector. But in theileriosis farmer has to face dual loss in term of decreased production, treatment cost because some time animal do not give response to treatment. Infected cattle showed significant decrease in red blood cell counts, total leukocyte counts, packed cell volume, hemoglobin concentration. Keeping in view the importance of theileriosis and its negative effect on livestock industry both in cattle and buffaloes and mostly in cross bred. So the following measures are recommended to safeguard the animals from theileriosis; a) when animal showing pyrexia, swollen eyes and lymph nodes should be brought to the hospital for treatment. The veterinarian should properly diagnosed the disease before treatment, b) the control of the vectors (Ticks) is the important factor to prevent and transmit of the disease. So proper method should be adopted to make farm free from ticks e.g. regular use of spray or ivermectin inj. etc for preventive measures.

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