Pakistan J. Zool., vol. 46(2), pp. 401-408, 2014.

Prevalence and Hematology of Tick Borne Hemoparasitic Diseases in Equines in and Around Lahore

Khadija Javed,[1] Muhammad Ijaz,[1*] Muhammad Muddassir Ali,[2] Iahtasham Khan,[3] Khalid Mehmood[4] and Sadaqat Ali[1]

[1]Department of Clinical Medicine and Surgery; University of Veterinary and Animal Sciences, Lahore 54000
[2]Institute of Biochemistry and Biotechnology; University of Veterinary and Animal Sciences, Lahore 54000
[3]Section of Epidemiology and Public Health, College of Veterinary and Animal Sciences, Jhang 35200
[4]Department of Medicine and Surgery; University College of Veterinary and Animal Sciences, Islamia University of Bahawalpur, Bahawalpur 63100

Abstract.- A total of 395 ticks infected equines (166 horses, 115 mules, 114 donkeys) were incorporated to study the prevalence and hematology of tick borne hemoparasitic diseases (TBHD) in equines from March 2012 to February 2013 in Lahore, Pakistan. In horses Theileriosis was the most prevalent (38/166; 22.89%) TBHD followed by Anaplasmosis (34/166; 20.48%), Babesiosis (32/166; 19.28%) and mixed infection (18/166; 10.84%). In mules, Babesiosis was the most prevalent (30/115; 26.09%) TBHD followed by mixed infection (25/115; 21.74%), Anaplasmosis (15/115; 13.04%) and Theileriosis (15/115; 13.04%). In donkeys, the most prevalent TBHD was Babesiosis (27/114; 23.68%) followed by Anaplasmosis (24/114; 21.05%), Theileriosis (21/114; 18.42%) and mixed infection (15/114; 13.16%). Statistical analysis revealed the significant difference ($P<0.05$) among the species of TBHDs. All the equines showed that due to tick infestation there was a remarkable increase in TLC values and slightly increased in TEC values than the values from the healthy equines while PCV remained in the normal range in horses and mules with a significant association ($p<0.05$) between them but values slightly increased in donkeys with significant difference in the values. There was an increase in Hb values in mules and donkeys but decrease in horses than the values from the healthy equines. According to the statistical analysis there was a significant difference ($p<0.05$) in Hb and TLC values of all equines than the normal values of equines.

Key words: Donkey, horse, mule, hematology, tick borne diseases, hemoparasitic diseases.

INTRODUCTION

According to the Agricultural Census Organization, the total equine population in Pakistan was 4.8 million in 2006. This has risen to 5.5 million (horses 0.4, asses 4.9 and mules 0.2) as per the census report in 2012-2013. All of them are reared mainly for the game and draught purposes and performing their roles expeditiously. Their participation in Pakistan economy as sources of inexhaustible draught Power is nearby equal to the other animals assisting the humanity (Anonymous, 2012-13).

In the rural areas where horses, mules and donkeys are raised for economical purpose, proficient parasitic control program is not readily available. Diseases caused by the parasites are more prevalent and are distributed worldwide including, Europe, Asian continent, South America, Africa and the reason of prevalence contemporizes with the presence of the tick as a vector (Kumar *et al.*, 2007). Ticks have been recognized as the important ectoparasites of equines and transmit various diseases such as equine Lyme borreliosis, piroplasmosis and equine granulocytic anaplasmosis are transmitted through ticks and considered as tick borne diseases (Sigg *et al.*, 2010). Infestations can get host susceptible to bacterial infections and screw-worm infestations by developing severe lesions. Lesions are present on ears, perineum, tail and groin areas in equal proportion (Labruna *et al.*, 2002). Due to long-term blood feeding scheme, ticks are able to regulate the immune system of their host (Jaworski *et al.*, 2001). Thus, ticks having harmful effects and influence the animals through loss of blood, general stress and irritation; depression of the immune system, function and transmission of opportunistic pathogens (Tolleson *et al.*, 2007).

* Corresponding author: mijaz@uvas.edu.pk
0030-9923/2014/0002-0401 $ 8.00/0

Theileriosis is also a tick-borne disease of domestic animals in the tropical and subtropica regions of the world (Rehman *et al.,* 2010). The one of the most significant tick borne disease of horses is equine piroplasmosis, which is caused by *Babesia caballi* and *Theileria equi* as *Theileria equi* infections are more prevalent than *Babesia caballi* infection and dominant in areas and environments where horses exhibit ticks, particularly the genera *Dermacentor*, *Hyalomma*, *Rhipicephalus* (Sigg *et al.*, 2010) and ticks of the *Ixodes* genus (Chang *et al.*, 2000). More than fifty percent of the species of the Ixodidae ticks belong to the genus *Amblyomma* (Nava *et al.,* 2009).

Diseases related to the tick-borne infections (chronic) can frequently result in abortion and decrease in weight gain (Teglas *et al.,* 2005). Clinical signs of Anaplasmosis comprise of several days of lethargy, decreased activity, hypophagia and mild stress, usually associated with pyrexia, edema of distal limb, ataxia, petechiation and thrombocytopenia (Madigan and Gribble, 1987).

Hematologic parameters were evaluated using a hematology Analyzer. Hematology is being used extensively in seeking the entropy about the status of disease and performance discomfort. Hematological data are provided for different breeds of horses, and these hematologic parameters are evaluated using a hematology Analyzer (Rubino *et al.*, 2006).

Ticks and tick borne diseases cause serious economic losses, being a threat to the equine industry worldwide. Keeping in view the importance of ticks and tick borne diseases the present study was designed.

MATERIALS AND METHODS

Source of samples

A total of 395 equines (n=166 horses, n=115 mules, n=114 donkeys) having tick infestations were examined. Ticks were collected during March 2012 to February 2013 from outdoor of University of Veterinary and Animal Sciences Lahore, Brooks's Hospital and randomly selected small holders farm in and around Lahore. In this study, animals of all ages and both sexes were included. The data related to all suspected equines was collected in a pre tested questionnaire.

Collection of blood samples

For tick borne hemoparasitic diseases, blood samples were collected from ear tip and jugular venipunture from the equines having tick infestation. Blood (5 ml) was drawn gently from the area of interest with the help of a sterilized syringe. The collected blood samples were transferred immediately into EDTA containing vacutainer and then mixed gently. Thin blood smears were prepared from the collected blood samples as described by Benjamin (1986).

Processing of blood smears

To detect tick borne hemoparasites in red blood cells one drop of cedar wood oil was placed on smear and was examined under the oil immersion lens (100X). Tick borne hemoparasitic diseases were identified by their morphological characteristics according to standard key. Prevalence of the disease was calculated as per formula described by Thrusfield (2002).

Hematological examination

Blood samples of 5 ml were collected directly from the jugular vein of 30 equines (horses=10; donkeys=10; mules=10) positive for tick and negative for tick borne hemoparasitic diseases into sterilized plastic bottles with anticoagulant coated with EDTA. Haemoglobin (Hb) values, erythrocyte sedimentation rate (ESR), total erythrocyte count (TEC), total leukocytes count (TLC) and packed cell volume (PCV) was calculated by using a hematology analyzer (Hasanpour *et al.*, 2008).

Statistical analysis

Data regarding the prevalence of disease was analyzed by using non-parametric, Chi-square test whereas data on hematology was analyzed by Student's *t*-test using statistical package for social science (SPSS). $P<0.05$ was considered significant.

RESULTS

Prevalence of tick borne hemoparasites

The overall prevalence in different species is shown in Table I. Out of 395 samples, 294 were declared positive (74.43%) for tick borne hemoparasitic diseases.

Table I.- Species-wise prevalence of tick-borne hemo-parasitic diseases in equine family (horses, mules, and donkeys) of Lahore district.

Specie	N	Overall TBHDs P (%)	Anaplasmosis P (%)	Babesiosis P (%)	Theileriosis P (%)	Mixed infection P (%)
Horses	166	122(73.49)	34(20.48)	32(19.28)	38(22.89)	18(10.84)
Mules	115	85(73.91)	15(13.04)	30(26.09)	15(13.04)	25(21.74)
Donkeys	114	87(76.32)	24(21.05)	27(23.68)	21(18.42)	15(13.16)

N, total number of animals examined; P, total number of positive animals; %, prevalence.

Table II.- Sex-wise prevalence of tick-borne hemo-parasitic diseases in equine of Lahore district.

Animal spp.	Sex	N	P	Anaplasmosis P (%)	Babesiosis P (%)	Theileriosis P (%)	Mixed infection P (%)
Horses	Male	62	47	13 (20.97)	12 (19.35)	15 (24.19)	07 (11.29)
	Female	104	75	21 (20.19)	20 (19.23)	23 (22.12)	11 (10.58)
	Total	166	122	34 (20.48)	32 (19.28)	38 (22.89)	18 (10.84)
Mules	Male	54	39	06 (11.11)	14 (25.93)	08 (14.81)	11 (20.37)
	Female	61	46	09 (14.75)	16 (26.23)	07 (11.48)	14 (22.95)
	Total	115	85	15 (13.04)	30 (26.09)	15 (13.04)	25 (21.74)
Donkeys	Male	38	27	07 (18.42)	08 (21.05)	08 (21.05)	04 (10.53)
	Female	76	60	17 (22.37)	19 (25.00)	13 (17.11)	11 (14.47)
	Total	114	87	24 (21.05)	27 (23.68)	21 (18.42)	15 (13.16)

N, total no. animals examined; P, total no. animals positive; %, prevalence, *****Significant (P<0.05)

Species-wise prevalence

In horses, theileriosis was the most prevalent (38/166; 22.89%) TBHD followed in order by anaplasmosis (34/166; 20.48%), babesiosis (32/166; 19.28%) and mixed infection (18/166; 10.84%). In mules, babesiosis was the most prevalent (30/115; 26.09%) TBHD followed by mixed infection (25/115; 21.74%), anaplasmosis (15/115; 13.04%) and theileriosis (15/115; 13.04%). In donkeys, the most prevalent TBHD was babesiosis (27/114; 23.68%) followed by anaplasmosis (24/114; 21.05%), theileriosis (21/114; 18.42%) and mixed infection (15/114; 13.16%). Statistical analysis revealed the significant difference among the species of TBHDs (P<0.05) as shown in Table I.

Sex-wise prevalence

The prevalence of theileriosis was higher (24.19%) in male than female horses (22.12%). Second highest prevalence was registered in anaplasmosis in male (20.97%) than female horses (20.19%). Prevalence of babesiosis was higher (19.35%) in male than female (19.23%). Mixed infection was more frequently diagnosed in male (11.29%) than female (10.58%) horses.

In mules, babesiosis recorded the highest prevalence (25.93%) in males which was being followed by mixed infection (20.37%), theileriosis (14.81%) and anaplasmosis (11.11%). In females, babesiosis had the highest prevalence (26.23%) and the lowest was of theileriosis (11.48%).

In donkeys, the highest prevalence was identified in theileriosis (21.05%) and babesiosis (21.05%) and the lowest was in mixed infection (10.53%) in males. In females, out of 60 positive samples, the highest prevalence was recorded by babesiosis (25%), followed by anaplasmosis (22.37%), theileriosis (17.11%) and mixed infection (14.47%). Statistical analysis revealed that there was a significant association (P<0.05) of TBHDs between male and female equines as mentioned in Table II.

Age-wise prevalence

The highest prevalence of theileriosis (23.18%) in horses was recorded at the age range between 1 and 5 years, and the lowest in mixed infections. The prevalence of theileriosis in horses in >5 years age was 22.68%, babesiosis was 21.64%, anaplasmosis was 20.61% and of mixed infection was 9.27%.

In mules, the highest prevalence of theileriosis (29.78%) was observed at 1-5 years of age and the lowest was of mixed infection and anaplasmosis (12.76%). The highest prevalence of babesiosis (29.41%) was observed above 5 years of age and lowest in theileriosis (1.47%).

In donkeys, the age range between 1-5 years was the most susceptible to theileriosis (30.23%) while babesiosis was considered as the most prevalent TBHD (26.70%) above 5 years of age. Statistical analysis showed significant difference ($P<0.05$) of TBHDs between 1-5 years and above 5 years of age as shown in Table III.

Season-wise prevalence

In horses, the highest prevalence of anaplasmosis (33.33%) was recorded during the spring, babesiosis was in the summer (35.71%), theileriosis was in autumn (51.85%) and mixed infection was in winter (15.38%). Out of 85 positive samples in mules, the highest prevalence of anaplasmosis was in autumn (100%), babesiosis (37.50) and theileriosis (25%) in winter, and mixed infection was in spring (33.33%). Out of 87 positive samples in donkeys the highest prevalence of anaplasmosis was in winter (29.41%), babesiosis was in autumn (50%), theileriosis was in (37.50%) spring season and mixed infection was in summer (22.22%). Statistical analysis revealed significant differences ($P<0.05$) of anaplasmosis, theileriosis, babesiosis and mixed infection in different seasons in all equine species, as shown in Table IV.

Hematological values of equines having tick infestation

All the equines showed that due to tick infestation there was a remarkable increase in TLC values and slightly increased in TEC values than the values from the healthy equines while PCV remained in the normal range in horses and mules with a significant association between them but values slightly increased in donkeys with significant difference in the values ($p<0.05$). There was an increase in Hb values in mules and donkeys but decrease in horses than the values from the healthy equines.

According to the statistical analysis there was a significant difference in Hb and TLC values of all equines than the normal values of equines ($p<0.05$). The value (Mean± SD) of blood parameters in equines having ticks infestation are presented in Table V.

DISCUSSION

Prevalence of TBHDs in equines

The overall prevalence (74.43%) of tick borne hemoparasitic diseases in the present study is congruent to other studies like Garba *et al.* (2011) reported overall equines prevalence of theileriosis 80.4% in Niger state. Rubino *et al.* (2006) reported 34.9% prevalence of hemoparasites in horses. Nagore *et al.* (2004) observed 37.5% prevalence of theileriosis and 20.81% of babesiosis in horses through blood smears examination between August and November 2002 in Northern Spain. Alanazi *et al.* (2012) recorded the highest seroprevalence of *T. equi* 16.5% and *B. caballi* 8.8% in horses of different districts of the central province of Saudi Arabia. Qablan *et al.* (2013) reported that out of 288 animals examined, the piroplasmid DNA was detected in 78 (27.10%). Ribeiro *et al.* (2013) reported that twenty-nine horses were seropositive to *T. equi* (17.9 %) and 21 to *A. phagocytophilum* (13.0 %). Ros-Garcia *et al.* (2013) studied 12.5% of piroplasm species in horses in Tunisia. Al-saad *et al.* (2010) observed 78.3% prevalence of babesiosis in naturally infected draught horses in southern Iraq through blood smear method and confirmed by cELISA. Mekibib *et al.* (2010) reported *Babesia equi* up to 71.43% in donkeys during November 2008 to march 2009 in Ethopia. Shehzad *et al.* (2003) described that out of positive animals 22 (5.06%) were positive for *Babesia* sp. of which 6 (27.27%) were positive with mixed infection of *B. equi*. Teglas *et al.* (2005) reported that the prevalence of equine babesiosis was 67% in Coaba village, Guatemala, which is similar to our result

Table III.- Age-wise prevalence of tick-borne hemo-parasitic diseases in equines of Lahore district.

Animal spp.	Age	N	P	Anaplasmosis P (%)	Babesiosis P (%)	Theileriosis P (%)	Mixed infection P (%)
Horses	≤ 5years	69	50	14(20.28)	11(15.94)	16(23.18)	09(13.04)
	>5years	97	72	20(20.61)	21(21.64)	22(22.68)	09(9.27)
	Total	166	122	34(20.48)	32(19.27)	38(22.89)	18(10.84)
Mules	≤ 5years	47	36	06(12.76)	10(21.27)	14(29.78)	06(12.76)
	>5years	68	49	09(13.23)	20(29.41)	01(1.47)	19(27.94)
	Total	115	85	15(13.04)	30(26.08)	15(13.04)	25(21.73)
Donkeys	≤ 5years	43	37	07(16.27)	08(18.60)	13(30.23)	09(20.93)
	>5years	71	50	17(23.94)	19(26.70)	08(11.26)	06(8.45)
	Total	114	87	24(21.05)	27(23.68)	21(18.42)	15(13.15)

Table IV.- Season-wise prevalence of tick-borne hemo-parasitic diseases in equines of Lahore district

Animal spp.	Season	N	P	Anaplasmosis P (%)	Babesiosis P (%)	Theileriosis P (%)	Mixed infection P (%)
Horses	Spring	30	20	10 (33.33)	06 (20.00)	00	04 (13.33)
	Summer	56	40	08 (14.29)	20 (35.71)	04 (07.14)	08 (14.29)
	Autumn	54	44	12 (22.22)	02 (03.70)	28 (51.85)	02 (03.70)
	Winter	26	18	04 (15.38)	04 (15.38)	06 (23.08)	04 (15.38)
	Total	166	122	34 (20.48)	32 (19.28)	38 (22.89)	18 (10.84)
Mules	Spring	45	30	05 (11.11)	10 (22.22)	00	15 (33.33)
	Summer	25	20	05 (20.00)	05 (20.00)	05 (20.00)	05 (20.00)
	Autumn	05	05	05 (100)	00	00	00
	Winter	40	30	00	15 (37.50)	10 (25.00)	05 (12.50)
	Total	115	85	15 (13.04)	30 (26.09)	15 (13.04)	25 (21.74)
Donkeys	Spring	24	15	03 (12.50)	03 (12.50)	09 (37.50)	00
	Summer	27	21	03 (11.11)	06 (22.22)	06 (22.22)	06 (22.22)
	Autumn	12	12	03 (25.00)	06 (50.00)	03 (25.00)	00
	Winter	51	39	15 (29.41)	12 (23.53)	03 (05.88)	09 (17.65)
	Total	114	87	24 (21.05)	27 (23.68)	21 (18.42)	15 (13.16)

N = total no. animals examined, P = total no. animals positive, % = prevalence, ***** Significant (P<0.05)
Spring (March to April), Summer (May to August), Autumn (September to October), Winter (November to February)

Table V.- Hematological values of equines having tick infestation ± SD

Blood parametrs	Healthy equines			Affected equines		
	Horses(n=10)	Mules(n=10)	Donkey(n=10)	Horses(n=10)	Mules(n=10)	Donkey(n=10)
PCV %	30.314±2.371	31.458±4.258	31.24±3.85	30.96±12.50	31.72±0.95	41.26±1.30
Hb g/L	12.473±1.714	11.9±0.831	11.696±0.929	11.07±0.69	14.3±0.547	12.92±0.758
TEC× 10^{12}/µL	7.721±1.09	8.03±1.132	7.731±1.289	8.31±0.694	9.27±0.444	10.01±0.795
TLC× 10^{9}/L	7.938±1.876	8.075±1.526	8.336±2.13	20.08±1.055	50.08±0.778	46.63±0.831

PCV (%) = H 0.8639, M 0.8874, D 0.0000 *****Significant (P<0.05); Hb(g/L)= H 0.0000, M 0.0000, D 0.005 *****Significant (P<0.05); TEC×10^{12}/µL= H 0.18, M 0.007, D 0.0003 *****Significant (P<0.05); TLC× 10^{9}/L= H 0.0000, M 0.0000, D 0.0000 *Significant (P<0.05), H = horse, M = mule, D = donkey

i.e., 69%. Teglas also observed the anaplasmosis 13% in horses. During the spring and early summer Passamonti *et al.* (2010) observed 17.03% prevalence of anaplasmosis, 60.86% of babesiosis and 56.52% of theileriosis in equines of central Italy. In the present study the prevalence of theileriosis was 22.89% which coincides with the study of Sigg *et al.* (2010) who reported 27.3% in Spain and 25% in Portugal. Salib *et al.* (2013) reported that the prevalence of theileriosis was 41.61% in horses in Giza, Egypt. The prevalence of *T. equi* was higher in males than female horses. The highest prevalence was among age group ranged from 5 to 10 years as 22.81%. Sigg *et al.* (2010) also supported the present study which reveals that prevalence of theileriosis was higher in male horses than females at 6.2% in stallion and 4.4% in mares. Heuchert *et al.* (1999) reported that the prevalence of babesiosis was 38.1% in female horses in Brazil in 1998. According to Machado *et al.* (2012) the prevalence of theileriosis was 31.81% and babesiosis was 20.45% in donkeys in Brazil. Chahan *et al.* (2006) found 9.6 and 38.7% prevalence of theileriosis and babesiosis, respectively among donkeys from China. Kouam *et al.* (2010) detected theileriosis (76.9%) and babesiosis (38.5%) among 13 donkeys from mainland Greece. The report of Teglas *et al.* (2005) on the prevalence of babesiosis in horses (93%) is less associated with our findings (19.28%). In accordance to present study, Salib *et al.* (2013) reported that the prevalence of theleriosis in Egypt was highest (25.81%) in July and the disease was more prevalent in summer than winter but Seo *et al.* (2011) observed 2% theleriosis prevalence during winter and summer season which was less correlates with our study. These low values of prevalence might be due to good management practices followed by their owners, carrier stage of infection or season of low tick density.

Hematological changes by tick infestation in equines

Rashid *et al.* (2009) and Rubino *et al.* (2006) reported a decrease in TEC and hemoglobin but slight increase in TLC value. These findings were concurring with the finding of the current study. In the present study, Hb values slightly increased in infected donkeys which correlate with the findings of Kumar *et al.* (2007) who first reported the decrease in Hb values of ticks infected donkeys from day 9 onward and then increased gradually after 38 days of ticks infestation. Hb values decreased in horses than the values from the healthy equines. Results of Zobba *et al.* (2008) about Hb values was in lined with current study. Shehzad *et al.* (2003) described that the hematology of hemoparsitic infected equines with a significant decrease ($P<0.05$) in the total erythrocyte count, total leukocyte count, haemoglobin estimation, packed cell volume, neutrophils and basophils in the infected horses as compared with healthy horses.

REFERENCES

ALANAZI, A.D., ALYOUSIF, M.S. AND HASSIEB, M.M., 2012. Seroprevalence study on *Theileria equi* and *Babesia caballi* antibodies in horses from central province of Saudi Arabia. *J. Parasitol.*, **98**: 1015-1017.

AL-SAAD, K.M., AL-SAAD, E.A. AND AL-DERAWIE, H.A., 2010. Clinical and diagnostic study of equine babesiosis in drought horses in some areas of Basrah Province. *Res. J. Anim. Sci.*, **4**: 16-22.

ANONYMOUS., 2012-2013. *Pakistan economic survey*, Planning and Development Division, Govt. of Pakistan, Islamabad, pp: 29.

BENJAMIN, M.M., 1986. *Outline of veterinary clinical pathology.* 3rd Ed. The Iowa State Uni. Press, Ames, Iowa,USA, pp. 7-8; 29-30.

CHAHAN, B., ZHANG, S., SEO, J.Y., NAKAMURA, C., ZHANG, G., BANNAI, H., JIAN, Z., INOKUMA, H., TUCHIYA, K., SATO, Y., KABEYA, H., MARUYAMA, S., MIKAMI, T. AND XUAN, X., 2006. Sero-epidemiological evidence for the possible presence of *Babesia (Theileria) equi* and *Babesia caballi* infections in donkeys in western Xinjiang, China. *J. Vet. Med. Sci.*, **68**: 753-755.

CHANG, Y., MCDONOUGH, S.P., CHANG, C.F., SHIN, K.S., YEN, W. AND DIVERS, T., 2000. Human granulocytic ehrlichiosis agent infection in a pony vaccinated with a *Borrelia burgdorferi* recombinant OspA vaccine and challenged by exposure to naturally infected ticks. *Clin. Diagn. Lab. Immunol.*, **7**: 68-71.

GARBA, U.M., SACKEY, A.K.B., TEKDEK, L.B., AGBEDE, R.I.S. AND BISALLA, M., 2011. Clinical manifestations and prevalence of piroplasmosis in Nigerian Royal Horses. *J. Vet. Adv.*, **1**: 11-15.

HASANPOUR, A., MOGHADDAM, G.A. AND

NEMATOLLAHI, A., 2008. Biochemical, hematological, and electocardiographic changes in buffalo naturally infected with *Theileria annulata*. *Korean J. Parasitol.,* **46**: 223-227.

HEUCHERT, C.M.S., GIULLI, V.D.J., ATHAIDE, D.F., BOSE, R. AND FRIEDHOFF, K.T., 1999. Sero-epidemiologic studies on *Babesia equi* and *Babesia caballi* infections in Brazil. *Vet. Parasitol.,* **85**: 1-11.

JAWORSKI, D.C., JASINSKAS, A., METZ, C.N., BUCALA, R. AND BARBOUR, A.G., 2001. Identification and characterization of a homologue, of thepro-inflammatory cytokine macrophage migration inhibitory factor in the tick, *Amblyomma americanum. Insect mol. Biol.,* **10**: 323-331.

KOUAM, M.K., KANTZOURA, V., GAJADHAR, A.A., THEIS, J.H., PAPADOPOULOS, E. AND THEODOROPOULOS, G., 2010. Seroprevalence of equine piroplasms and host-related factors associated with infection in Greece. *Vet. Parasitol.,* **169**: 273–278.

KUMAR, S., MALHOTRA, D.V., SANGWAN, A.K., GOEL, P. AND KUMAR, A., 2007. Infectivity rate and transmission potential of *Hyalomma anatolicum anatolicum* ticks for *Babesia equi* infection. *Vet. Parasitol.,* **144**: 338-343.

LABRUNA, M.B., KASAI, N., FERREIRA, F., FACCINI, J.L.H. AND GENNARI, S.M., 2002. Seasonal dynamics of ticks (Acari: Ixodidae) on horses in the state of Sao Paulo Brazil. *Vet. Parasitol.,* **105**: 65-77.

MACHADO, R.Z., TOLEDO, C.Z.P., TEIXEIRA, M.C.A., ANDRÉ, M.R., FRESCHI, C.R. AND SAMPAIO, P.H., 2012. Molecular and serological detection of *Theileria equi* and *Babesia caballi* in donkeys (*Equus asinus*) in Brazil. *Vet. Parasitol.,* **186**: 461-465.

MADIGAN, J.E. AND GRIBBLE, D.H., 1987. Equine ehrlichiosis in northern California: 49 cases (1968–1981). *J. Am. Vet. Med. Assoc.,* **190**: 445-448.

MEKIBIB, B., MANEGEREW, M., TADESSE, A., ABUNA, F., MEGERSA, B., REGASSA, A., MEKURIA, S. AND ABEBE, R., 2010. Prevalence of haemoparasites and associated risk factors in working donkeys in Adigudem and Kwiha districts of Tigray region, Northern Ethiopia. *J. Anim. Vet. Adv.,* **9**: 2249-2255.

NAGORE, D., SANMARTIN, J.G., PEREZ, A.L.G., JUSTE, R.A. AND HURTADO, A., 2004. Detection and identification of equine Theileria and Babesia species by reverse line blotting, epidemiological survey and phylogenetic analysis. *Vet. Parasitol.,* **123**: 41–54.

NAVA, S., MANGOLD, A.J., MASTROPAOLO, M., VENZAL, J.M., OSCHEROV, E.B. AND GUGLIELMONE, A.A., 2009. *Amblyomma boeroin* sp. (Acari: Ixodidae), a parasite of the Chacoan peccary *Catagonus wagneri* (Rusconi) (Artiodactyla: Tayassuidae) in Argentina. *Syst. Parasitol.,* **73**: 161-174.

PASSAMONTI, F., FABRIZIA, V., KATIA, C., STEFANO, C., GIACOMO, C., LUISA, M.M., DANIELA, P.F., ANDREA, V.S. AND MAURO, C., 2010. *Anaplasma phagocytophilum* in horses and ticks: A preliminary survey of Central Italy. *Comp. Immunol. Microbiol. Infect. Dis.,* **33**: 73-83.

QABLAN, M.A., OBORNIK, M., PETRZELKOVA, K.J., SLOBODA, M., SHUDIEFAT, M.F., HORIN, P., LUKES J. AND MODRY, D., 2013. Infections by *Babesia caballi* and *Theileria equi* in Jordani an equids: epidemiology and genetic diversity. *Parasitology,* **140**: 1096-1103.

RASHID, A., MUBARAK, A. AND HUSSAIN, A., 2009. Babesiosis in equines in Pakistan: a clinical report. *Vet. Ital.,* **45**: 391-395.

REHMAN, Z., KHAN, MS., AVAIS, M., ALEEM, M., SHABBIR, M.Z. AND KHAN, J.A., 2010. Prevalence of theileriosis in sheep in Okara district, Pakistan. *Pakistan J. Zool.,* **42**(5): 639-643.

RIBEIRO, A.J., CARDOSO, L., MAIA, J.M., COUTINHO, T. AND COTOVIO, M., 2013. Prevalence of *Theileria equi*, *Babesia caballi*, and *Anaplasma phagocytophilum* in horses from the north of Portugal. *Parasit. Res.,* **112**: 2611-2617.

ROS-GARCIA, A., YOUMNA, M.G., ANA, H. AND ALI, B., 2013. Prevalence and genetic diversity of piroplasm species in horses and ticks from Tunisia. *Infect. Genet. Evol.,* **17**: 33-37.

RUBINO, G., CITO, A.M., LACINIO, R., BRAMANTE, G., CAROLI, A., PIERAGOSTINI, E. AND PETAZZI, F., 2006. Hematology and some blood chemical parameters as a function of tick-born disease (TBD) signs in horses. *J. Equine Vet. Sci.,* **26**: 475-480.

SALIB, F.A., YOUSSEF, R.R., RIZK, L.G. AND SAID, S.F., 2013. Epidemiology, diagnosis and therapy of *Theileria equi* infection in Giza, Egypt. *Vet. World,* **6**: 76-82.

SEO, M.G., YUNA, S.H., CHOIA, S.K., CHOA, G.J., PARKB, Y.S., KWONA, O.D., CHOB, K.H., TAE-HWAN, KIMA, K.S.J., PARKA, S.J., KWONA, Y.S. AND KWAK, D., 2011. Seroprevalence of equine piroplasms in the Republic of Korea. *Vet. Parasitol.,* **179**: 224–226.

SHEHZAD, W., ASLAM, A., ASHRAF, K. AND HASHMI, H.A., 2003. Haemoparasites of equine and their effect on blood parameters during winter season. FAO., 54(3-4):

SIGG, L., GERBER, V., GOTTSTEIN, B., DOHERR, M.G. AND FREY, C.F., 2010. Seroprevalence of *Babesia caballi* and *Theileria equi* in the Swiss horse population. *Int. J. Parasitol.,* **59**: 313-317.

TEGLAS, M., MATERN, E., LEIN, S., FOLEY, P., MAHAN, S.M. AND FOLEY, J., 2005. Ticks and tick-borne disease in Guatemalan cattle and horses. *Vet. Parasitol.,* **131**: 119-127.

THRUSFIELD, M., 2002. *Veterinary epidemiology*. 2nd Ed.

Blackwell publisher London.

TOLLESON, D.R., TEEL, P.D., STUTH, J.W., STUTH, O.F.X., JR, T.H.W. AND CARSTENS, G.E., 2007. Fecal NIRS: Detection of tick infestations in cattle and horses. *Vet. Parasitol.*, **144**: 146-152.

ZOBBA, R., ARDU, M., NICCOLINI, S., CHESSA, B., MANNA, L., COCCO, R. AND PARPAGLIA, M.L.P., 2008. Clinical and laboratory findings in equine piroplasmosis. *J. Equine Vet. Sci.*, **28**: 301-08.

(Received 27 December 2013, revised 2 February 2014