



## PREVALENCE OF THEILERIOSIS AND ITS IMPACT ON HAEMOTOLOGICAL VALUES IN NATURALLY INFECTED BUFFALOES AT HYDERABAD

M. I. Memon<sup>1</sup>, N. Memon<sup>2</sup>, A. B. Kachiwal<sup>1\*</sup>, M. R. Memon<sup>1</sup> and B. Bhutto<sup>1</sup>

<sup>1</sup>Sindh Agriculture University, Tandojam, Pakistan

<sup>2</sup>University of Sindh Jamshoro, Pakistan

### ABSTRACT

The prevalence of *Theileria annulata* in buffaloes was determined through clinical findings and microscopic examination in peri-urban and urban areas of Hyderabad, Pakistan. Out of 2400 buffaloes evaluated during the study, 1845 (76.87%) were found infested with ticks. The overall prevalence of ticks was 970 (80.8%) in peri-urban and 875 (72.91%) in urban regions of Hyderabad. Ranking of predilection sites of tick infestation in buffaloes revealed that external genitalia, udder and perineum were most infested (54%), followed by dewlap (23%), inner thighs (10%) neck and back (5%), tail (3%), ears (2%), around eyes (1%), flanks (1%) and legs (1%). Out of these 1845 tick infested bovine samples, 1680 (91.05%) were found positive for *Theileria* species by Giemsa-stained method. The microscopic hematological examination revealed the prevalence of 70% *Theileria annulata* in the Hyderabad. The infected buffaloes showed clinical signs such as fever, anorexia, hair loss, open mouth with difficulty in breathing, lymph node enlargement, and protrusion of eyes, red skin and weakness. However, suspected buffaloes exhibited normal feed intake, urination and defecation during the survey. The occurrence of the parasitic infection was significantly higher ( $P<0.05$ ) in the peri-urban areas than in urban areas. Findings of the present study revealed that peri urban buffaloes were more susceptible to theileriosis in comparison to urban buffaloes. Twenty four hundred blood samples were randomly collected from urban and peri-urban regions of Hyderabad, Pakistan. Haematological studies revealed that *Theileria annulata* in buffaloes significantly produced effect ( $P<0.01$ ) on erythrocyte and leukocyte indices, whereas, platelet indices remained unaffected from *Theileria annulata* in buffaloes.

**Keywords:** buffalo, haematological, prevalence, *Theileria annulata*, tick

### INTRODUCTION

Livestock farming is one of the old and economic activities in the rural and urban areas of Pakistan indicating an increase of 2.7 percent from last year (Ahmad, 2014). This activity makes an important financial contribution to gross value addition of 55.9 percent and 11.8 percent to GDP during 2013-14 as compared

---

Corresponding author: kachiwal2003@gmail.com

to 55.5 percent and 11.9 percent from last year, respectively (Ahmad, 2014 and GoP, 2014). Theileriosis in farm animals caused huge health and production losses and therefore these remained socio-economic challenge and threat for the sustainable livestock sector (Swai *et al.*, 2007; Ali and Radwan, 2011; Durrani *et al.*, 2011).

Theileriosis also known as East Coast fever is an economically significant fatal tick-born protozoan disease of domesticated and wild animals. Theileriosis is widely distributed in Asia, North Africa, South Europe and Australia (Ali and Radwan, 2011). The disease in cattle and other animal species is caused by the protozoan parasite of *Theileria* species. *Theileria* species may overcome the immune system of the susceptible breed of the cattle and could lead to death. The pathology of the disease is related to intra-macrophage stage of the protozoa (schiznot) (Glass, 2001). The haemoprotozoan parasites such as *Anaplasma*, *Babesia* and *Theileria* are the highly important protozoa that cause infections in cattle and buffaloes in Pakistan. It has been reported that 27.69 and 16.12% of haemoparasites were prevalent in two livestock farms (Khan *et al.*, 2004). Generally, the clinical cases of theileriosis occur throughout the year Muhammad *et al.* (1999). However, there is an increase in clinical cases of the disease in cattle and buffaloes during the hot and humid weather conditions (Ashfaq *et al.*, 1983). Several studies reported the prevalence of *Theileria* parasites in the cattle and buffalo farms of Pakistan (Shahnawaz *et al.*, 2011).

The protozoa of this haemoparasitic disease are transmitted by the Ixodid ticks of the genus *Hyalomma* (Aktas *et al.*, 2004). The tick vectors such as *Hyalomma anatolicum*, *Hyalomma m. marginatum*, *Hyalomma a. excavatum* are well known for transmission of the *Theileria* species in tropical and sub-tropical regions (Viseras and Garcia-Fernandez, 1999). Generally the diagnosis of East coast fever is based on the detection of *Theileria* species using Giemsa-stained hematological and lymph-node smears and indirect immune-fluorescent antibody test (IFAT). The previous studies suggested that frequent cases of the bovine theileriosis, and wide distribution of vectors in the selected regions study is the major constraint in the livestock production. Therefore, this study was planned to identify the *theileria* species and prevalence regarding their relevance to tick infestation and farm setting in the local regions.

## **MATERIALS AND METHODS**

Twenty buffalo farms were selected for study of presence of *Theileria* species from 2013 through 2014 in urban and peri-urban areas of the Hyderabad, Pakistan. The study was designed to determine the occurrences of the protozoan parasites on the animal health and environmental conditions of the animal with respect to their farming status. During the survey study, the animal living conditions of bovine population, standardized physical examinations and the collection of biological samples were performed. Inclusion of the sampling animals was observed for tick infestation and physical conditions to enhance the representation of animals with carrier of the haemoprotozoan parasites. All clinically suspected buffaloes were clinically examined for fever, hemorrhages from mucosal membranes (conjunctival, nasal and oral) and enlargement of pre-scapular lymph nodes. The affected (2400) buffaloes were selected on the basis of clinical signs, appearance of ticks, history and presence of piroplasms in the

thin blood films. Clinically healthy tick-free buffaloes were used as control of similar age from 20 selected dairy farms.

### **Sample collection**

Twenty four hundred blood samples were randomly collected from buffaloes located in urban and peri-urban regions of Hyderabad, Pakistan from 2013 to 2014. Blood was collected from jugular vein and smears were prepared. A small drop of blood from the suspected animals was placed near one end of a clean slide and a second slide was used as the spreader, the blood was streaked into a thin film and allowed to dry. The thin smear prepared slides were air-dried and fixed with methanol and stained in 10% Giemsa stain. The slides were examined under oil immersion objective of microscope for piroplasms in red blood cells and infected leukocytes for schizonts of *T. annulata* (Soulsby, 1982).

### **Hematological studies**

Blood samples (5 ml) for haematological tests were collected from jugular vein in heparinized vacutainer tubes. The collected samples were then immediately placed on ice after the collection. Haematological examination of neutrophil granulocytes percentage (GR %), neutrophil granulocytes (NG %), hematocrit percentage (Hct %), hemoglobin (Hgb g/dL), lymphocytes percentage (LY %), lymphocytes (LY#), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), monocytes percentage (MO %), monocytes (MO#), mean platelet volume (MPV), platelets (Plt), Pct (%), platelet distribution width (PDW), red blood cells (RBC), red blood cell distribution width (RDW%) and white blood cells (WBC) were analyzed using Beckman Coulter AcT Diff Hematology Analyzer (Beckman Coulter, Tokyo, Japan) at postgraduate Central Scientific Laboratory, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam.

### **Statistical analysis**

The data was analyzed statistically using statistical software Minitab 15.

## **RESULTS**

The study was performed in order to determine the prevalence of *Theileria* species in buffaloes of peri-urban and urban areas of Hyderabad, Sindh, Pakistan. During the investigation, some of the animals showed tick infestation and clinical signs of Theileriosis. Hyalomma tick species were found prominent in the infected animals and distributed on external genitalia, ears, legs and udder (Figure 1 and Figure 2). Ranking of predilection sites of tick infestation in buffaloes indicated that external genitalia, udder and perineum were most infested (54%), followed by dewlap (23%), inner thighs (10%) neck and back (5%), tail (3%), ears (2%), around eyes (1%) flanks (1%) and legs (1%). Out of 2400 animals selected for the study, 1845 (76.87%) were found infested with ticks. The overall prevalence was 970 (80.8%) in peri-urban and 875 (72.91%) in urban regions. Out of these 1845 tick infested bovine samples, 1680 (91.05%) were found positive for *Theileria* species (Table 1).

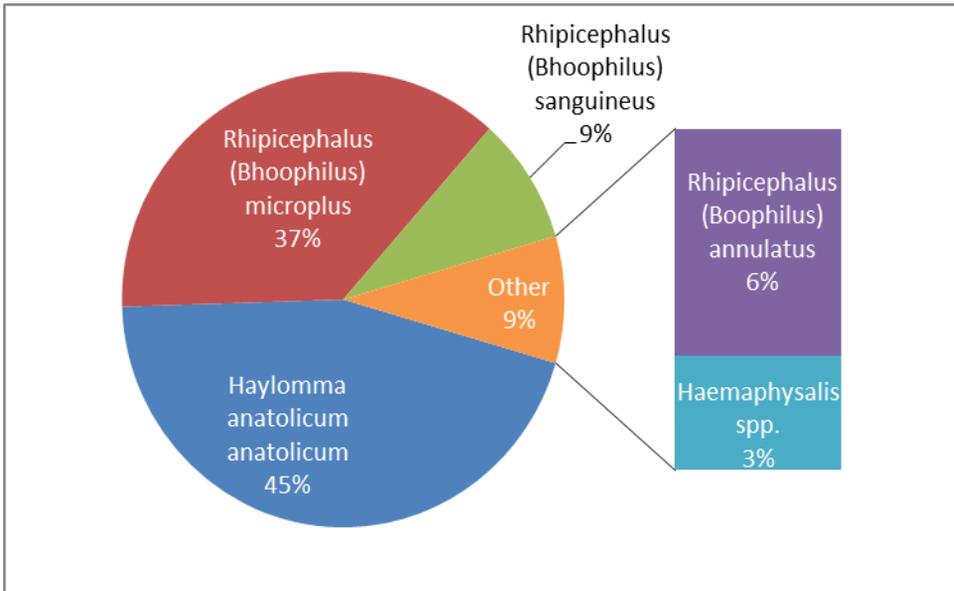
The infected animals showed clinical signs such as fever, anorexia, hair loss, open mouth with difficulty in breathing, red skin and weakness. However, suspected animals exhibited normal feed intake, urination and defecation during the survey.

The microscopic hematological studies revealed that total of clinically suspected 2400 blood samples examined, out of these 1680 (70%) were found positive for *Theileria annulata* (Table 1 and Figure 3). Geimsa's stained blood smears examination revealed 75% prevalence of the Theileriosis in the peri-urban and 64.9% prevalence was observed in urban area of the study. The occurrence of the parasitic infection of *Theileria* species were significantly higher ( $P \leq 0.01$ ) in the peri-urban areas than in urban areas of Hyderabad.

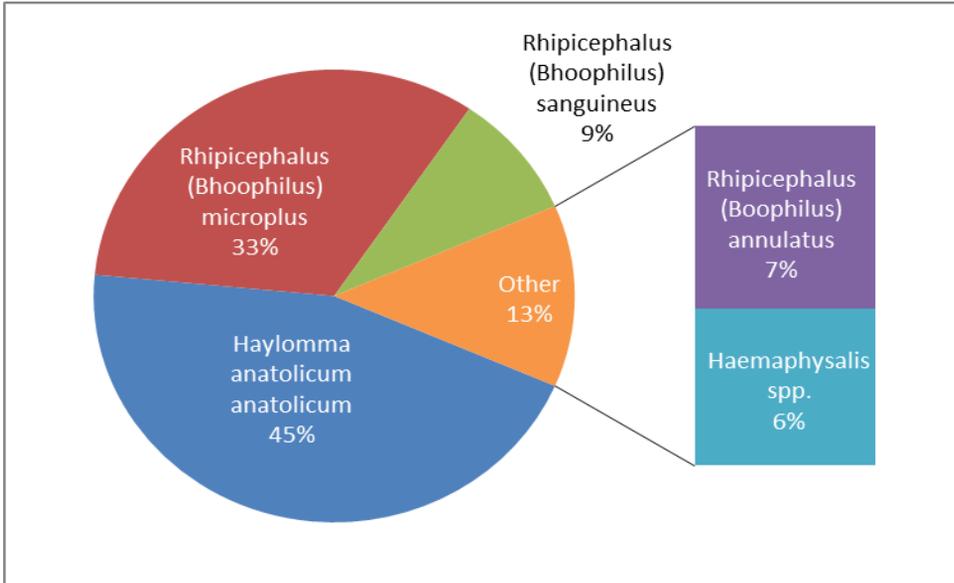
Our findings revealed that peri-urban buffaloes were more susceptible to Theileriosis in comparison to urban buffaloes. Haematological studies revealed the significant differences ( $P \leq 0.01$ ) between the *T. annulata* carrier and non-infected buffaloes. Haematological parameters such as lymphocyte percentage, lymphocyte volume, hematocrit percentage, MCV and RDW was significantly increased in *T. annulata* infected buffaloes, whereas, MCH and MCHC were significantly declined ( $P \leq 0.01$ ) in *Theileria annulata* infected buffaloes (Table 2). Whereas, other haematological parameters remained unaffected from *T. annulata* in buffaloes.

## DISCUSSION

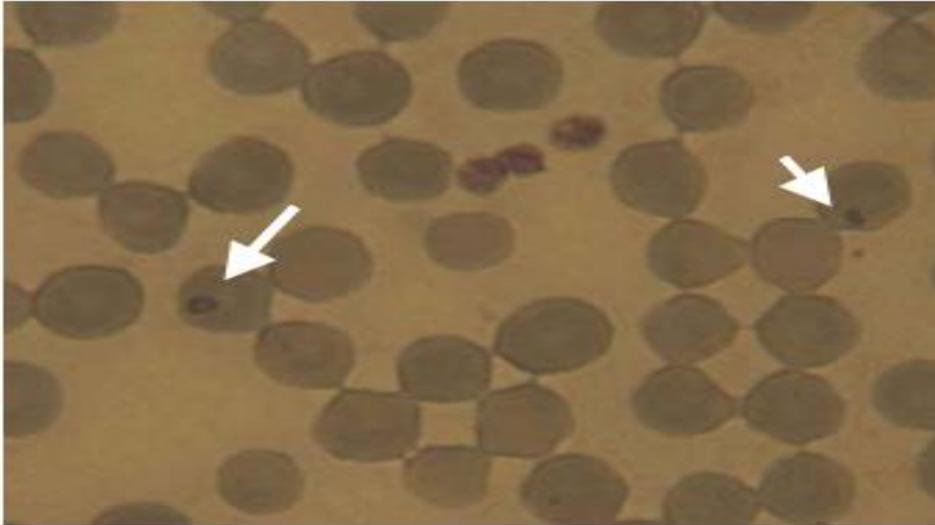
The study was designed and conducted to determine the presence of *Theileria* species and its relevance to tick infestation and farming setting in the peri-urban and urban regions of Hyderabad, Pakistan. In general, the occurrence of bovine Theileriosis has been largely attributed to the presence of tick vectors. In subclinical Theileriosis, bovine become chronic carrier of the piroplasma and thus source of infection for tick vectors reported by Altay *et al.* (2008). In present study, we isolated *Theileria annulata* at the highest prevalence in tick infested animals and the animals exhibited clinical signs. During the hot humid conditions, there was increase in tick vector population. Therefore, the cases of bovine theileriosis were prominent in hot climate regions where the high numbers tick vector populations have also been reported by other workers. In this study, the cases of the disease were relatively more prevalent in buffaloes reared in peri-urban farms. As the infrastructure and farm setting were different, locations greatly influence the tick vector population. Previously, it has been reported by Khan *et al.* (1996) that the distribution of the tick species such as *Boophilus microplus*, *Boophilus annulatus*, *Hyalomma aegyptium*, *Hyalomma anatolicum*, *Hyalomma marginatum marginatum* and *Rhipicephalus sanguineus* in the southern and northern regions of Pakistan. These tick species play significant role in transmission of the haemoprotozoan parasites in the farm animal species in tropical and subtropical South Asian countries as reported by Ghosh *et al.* (2007). The tick infestation evidence suggests that farming setting in peri-urban farms may be significant source of the infections in dairy buffalo.



**Figure 1.** Prevalence of tick species in peri-urban areas of Hyderabad



**Figure 2.** Prevalence of tick species in urban areas of Hyderabad



**Plate 3.** Giemsa staining method showing Piroplasmic forms of *Theileria annulata* in a microscopic field

Predilection sites for the infestation of tick specie vary with host like *Boophilus decoloratus* strongly like to occupy head, dewlap and back whereas, *Haylomma marginatum rafipes* opt external genitalia and tail reported by Tessema and Gashaw (2010). Predilection site of ticks was perineum in Nguni and non-descriptive cattle in South Africa as reported by Marufu *et al.* (2011). Ticks usually prefer thinner, short hair skin and highly blood supplied area like external genitalia and inguinal/groin region for infestation. This helps in easy penetration of mouth parts into richly vascular area for feeding. Atif *et al.* (2012) also reported that *Hyalomma anatolicum* and *Rhipicephalus (Boophilus) microplus* preferred to infest the sites of perineum, udder, external genitalia, dewlap and inner thighs in cattle.

**Table 1.** Prevalence of *Theileria annulata* in peri-urban and urban areas of Hyderabad

Sampling Site	No. of Samples	Tick infested	<i>T. annulata</i> +ve from tick infested	<i>T. annulatave</i> from overall samples	Prevalence of <i>T. annulata</i>
Peri-urban	1200	970/1200 (80.8%)	901/970 (92.88%)	299/901 (24.91%)	901/1200 (75%)
Urban	1200	875/1200 (72.91%)	779/875 (89.02%)	421/875 (35.08%)	779/1200 (64.9%)
Total	2400	1845/2400 (76.87%)	1680/1845 (91.05%)	720/1845 (30%)	1680/2400 (70%)

**Table 2.** Haematological studies on the normal and infected bovine samples (Mean values  $\pm$  SE)

Parameters	Non-infected buffaloes (Mean $\pm$ SE)	Infected buffaloes (Mean $\pm$ SE)
WBC ( $10^3$ /ul)	10.44 $\pm$ 1.1	5.61 $\pm$ 0.90
LY (%)	51.83 $\pm$ 2.3**	61.71 $\pm$ 2.56**
MO (%)	13.2 $\pm$ 2	14.5 $\pm$ 1.53
GR (%)	19.15 $\pm$ 3	20.38 $\pm$ 1.92
LY ( $10^3$ /ul)	2.84 $\pm$ 1.3**	7.33 $\pm$ 1.84**
MO ( $10^3$ /ul)	0.79 $\pm$ 0**	1.5 $\pm$ 0.21**
RBC ( $10^6$ /ul)	3.67 $\pm$ 0	3.65 $\pm$ 0.39
Hgb (g/dl)	7.17 $\pm$ 0	7.05 $\pm$ 0.37
Hct (%)	21 $\pm$ 0.2**	23.03 $\pm$ 1.23**
MCV (fL)	54.18 $\pm$ 1.02**	57.3 $\pm$ 1.5**
MCH (pg)	19.47 $\pm$ 1**	15.43 $\pm$ 0.43**
MCHC (g/dl)	35.36 $\pm$ 2**	24.61 $\pm$ 1.75**
RDW (%)	16.7 $\pm$ 1.5**	21.56 $\pm$ 1.71**
Plt ( $10^3$ /ul)	244 $\pm$ 21	233.53 $\pm$ 17.51
MPV (fL)	8 $\pm$ 1	7.42 $\pm$ 0.40
Pct (%)	0.16 $\pm$ 0	0.18 $\pm$ 0.21
PDW	17 $\pm$ 1	16.8 $\pm$ 0.35

Different superscripts on means in a row show significant differences at ( $P \leq 0.01$ )

During the clinical assessment, suspected animals showed the prominent signs such as fever, anorexia, hair loss, open mouth with difficulty in breathing, red skin and lethargy. However, there was no evidence of abortion and/or mortality in buffaloes during the investigation. The positive cases of the *Theileria* haemoparasites were detected in healthy buffaloes without showing clinical features by Mghirbi *et al.* (2008). The absence of the clinical features in positive cases could be in relation to seasonal variation and carriers of *Theileria* haemoparasites for many years as described by Mghirbi *et al.* (2008). Generally, the microscopic hematological examination is considered as the gold standard for detection of protozoan parasites such as *Theileria* and *Babesia* (Nayel *et al.*, 2012). In this study, the microscopic hematological examination revealed 70% prevalence of *Theileria annulata* in Hyderabad, Pakistan. Similar observations were also recorded by Durani and Kamal (2008). They reported the prevalence of 60% *T. annulata* in bovine in district Kasur, Pakistan. The incidence of this haemoprotozoan parasites in Pakistan were also observed in cattle about 44% by Durani *et al.* (2011), 19% by Shahnawaz *et al.* (1999) and 10.76% by Saleem *et al.* (2014). The reason of higher incidence in Hyderabad may be attributed to weather, age, breed, environment and type of animal housing. In this study, haematological profile revealed that *Theileria annulata* in buffaloes significantly produced effect ( $P < 0.01$ ) on erythrocyte and leukocyte indices, whereas, platelet indices remained unaffected from *Theileria annulata* in buffaloes. Similar findings were also reported by Durani *et al.* (2008) who reported that erythrocyte indices were significantly affected ( $P < 0.01$ ) in *Theileria annulata* infected buffaloes and produced macrocytic hypochromic anemia. Similar findings were also observed

by Hussein *et al.* (2008) and Qayyum *et al.* (2010) that the normocytic hypochromic anemia appears in cattle with theileriosis. Permanent blood sucking ticks also play a role to produce normocytic hypochromic anemia by causing persistent loss of blood in animals. Boulter and Hall (2000) reported that haemopoietic progenitors was suppressed by tumor necrosis factor- $\alpha$  (TNF-  $\alpha$ ) which implicated in the pathogenesis of anemia in bovine theileriosis. This could be attributed to the harmful effect on bone marrow by the toxic metabolites of *Theileria* spp. which interfere with the process of erythropoiesis.

Hussein *et al.* (2008) also observed a significant decrease ( $P < 0.001$ ) in total leucocytic count and neutrophils while the lymphocytes and monocytes indicated significant increase ( $P < 0.001$ ) in comparison with healthy control ones. Similar results were observed in *Theileria* infected cattle by Lamiaa (1997). Qayyum *et al.* (2010) also reported significantly reduced TEC, PCV, and TLC in *Theileria* infected buffaloes as compared to normal values. The decrease in total leukocyte count has been reported by Graham *et al.* (2001). Decrease in leukocyte is related to a destruction of leucocytes in lymphoid organs and infiltration of these cells in other organs (Clark *et al.*, 1986). Changes in Leucogram might be attributed to persistent harmful effects on the haemopoietic organs especially bone marrow of toxic metabolites of *Theileria* and their interference with the process of leucogenesis. Lymphocytes and monocytes increase is related with compensatory mechanism as target cells in response to their invasion with *Theileria* protozoan.

## CONCLUSION

Based on the findings of this study the prevalence of theileriosis was more in tick infested buffaloes and *Theileria annulata* significantly affected erythrocyte and leukocyte indices, whereas, platelet indices remained unaffected in *Theileria annulata* infected buffaloes.

## REFERENCES

- Ahmad, J. 2014. Economic Survey of Pakistan and Livestock Sector. <http://www.veterinaryhub.com/economic-survey-of-pakistan-livestock-sector-2013-2014>.
- Aktas, M., N. Dumanli and M. Angin. 2004. Cattle infestation by *Hyalomma* ticks and prevalence of *Theileria hyalomma* species in the east of Turkey. *Vet. Parasitol.*, 119: 1-8.
- Ali, A. E. F. and M. E. I. Radwan. 2011. Molecular detection of *Theileria annulata* in Egyptian buffaloes and biochemical changes associated with particular oxidative changes. *Adv. Life Sci.*, 1 (1): 6-10.
- Altay, K., M. F. Aydin, N. Dumanli and M. Aktas. 2008. Molecular detection of *Theileria* and *Babesia* infections in cattle. *Vet. Parasitol.*, 158: 295-301.
- Ashfaq, M., M. Ajmal and S. Ahmad, 1983. An outbreak of Theileriosis in crossbred neonate calves. *Pak. Vet. J.*, 3: 44-46.
- Atif, F. A., M. S. Khan, H. J. Iqbal, Z. Ali and S. Ullah, 2012. Prevalence of cattle tick infestation in three districts of the Punjab, Pakistan. *Pak. J. Sci.*, 64 (1): 49-53.
- Boulter, N. and R. Hall. 2000. Immunity and vaccine development in the bovine *Theileriosis*. *Adv. Parasitol.*, 44: 41-97.

- Clark, I. A., N. H. Hunt and W. B. Cowden. 1986. Oxygen-derived free radicals in the pathogenesis of parasitic disease. *Adv. Parasitol.*, 25: 1-44
- Durrani, A. Z. and N. Kamal. 2008. Identification of ticks and detection of blood protozoa in Friesian cattle by polymerase chain reaction test and estimation of blood parameters in district Kasur, Pakistan. *Trop. Anim. Health and Prod.*, 40 (6): 441-447.
- Durrani, A. Z., M. Younus, N. Kamal, N. Mehmood and A. R. Shakoori. 2011. Prevalence of ovine *Theileria* species in district Lahore, Pakistan. *Pak. J. Zool.*, 43 (1): 57-60.
- Ghosh, S., G. C. Bansal, S. C. Gupta, D. Ray, M. Q. Khan, H. Irshad, M. S. Zaman, U. Seitzer, Jabbar and S. Ahmed. 2007. Status of tick distribution in Bangladesh, India and Pakistan. *Parasitol. Res.*, 101 (2): 207-216.
- Glass, E. J. 2001. The balance between protective immunity and pathogenesis in tropical *Theileriosis*: what we need to know to design effective vaccines for the future. *Res. Vet. Sci.*, 70: 71-75.
- GoP. 2014. Pakistan Economic Survey. 2013-14. Economic Advisor's Wing, Finance Division, Government of Pakistan, Islamabad.
- Graham S. P., D. J. Brown, Z. Vatansver, D. Waddington, L. H. Taylor, A. K. Nichani, J. D. M. Campbell, R. E. Adamson, E. J. Glass and R. L. Spooner. 2001. Proinflammatory cytokine expression by *Theileria annulata* infected cell lines correlates with the pathology they cause *In vivo*. *Vaccine*, 19: 2932-2944.
- Hussein, A. H., N. A. E. S. Mohammed and H. K. Mohammed. 2007. *Theileriosis* and *babesiosis* in cattle: haemogram and some biochemical parameters. XIII. International Congress of ISAH Tartu, Estonia.
- Khan, M. Q., A. Zahoor, M. Jahangir, and M. A. Mirza, 2004. Prevalence of blood parasites in cattle and buffaloes. *Pak. Vet. J.*, 24 (4): 193-194.
- Khan, N. M., C. S. Hayat, Z. Iqbal, B. Hayat and A. Naseem. 1996. Prevalence of ticks on livestock in Faisalabad, Pak. *Vet. J.*, 13 (4):182-184.
- Lamiaa and M. Abo-EL-Hassan. 1997. Clinico-pathological study of *Theileriosis* in New Valley. M. V. Sci. Thesis, Department of Animal Pathology and Clinical Pathology, Faculty of Veterinary Medicine, Assiut University, Egypt.
- Mghirbi, M. Y., A. Hurtado, J. Brandika, K. Khelif, Z. Ketata and A. Bouattour. 2008. A molecular survey of *Theileria* and *Babesia* parasites in cattle, with a note on the distribution of ticks in Tunisia. *Parasit. Res.*, 103 (2): 435-442.
- Marufu, M. C., M. Chimonyo, C. Mapiye and K. Dzama. 2011. Ticks load in cattle raised on sweet and sour rangelands in the low-input farming areas of South Africa. *Trop. Anim. Health Prod.*, 43: 307-313.
- Muhammad, G., M. Saqib, M. Athar, M. Z. Khan and M. N. Asi. 1999. Clinico-epidemiological and therapeutic aspects of bovine theileriosis. *Pak. Vet. J.*, 19: 64-71.
- Nayel, M., K. M. El-Dakhly, M. Aboulaila, A. Elsify, H. Hassan, E. Ibrahim, A. Salama, and T. Yanai. 2012. The use of different diagnostic tools for *Babesia* and *Theileria* parasites in cattle in Menofia, Egypt. *Parasit. Res.*, 111: 1019-1024.

- Qayyum, M., U. Farooq, H. A. Samad and H. R. Chaudhry. 2010. Prevalence, clinicotherapeutic and prophylactic studies on theileriosis in district Sahiwal (Pakistan). *J. Anim. Pl. Sci*, 20 (4): 266-270.
- Saleem, M. I., A. Tariq, A. Shahzad and S. A. Mahfooz. 2014. Clinical, epidemiological and therapeutic studies on bovine tropical *Theileriosis* in Faisalabad, Pakistan. *Iraqi J. Vet. Sci.*, 28 (2): 87-93.
- Shahnawaz, S., M. Ali, M. A. Aslam, R. Fatima, Z. I. Chaudhry, M. U. Hassan, M. Ali and F. Iqbal. 2011. A study on the prevalence of a tick-transmitted pathogen, *Theileria annulata*, and hematological profile of cattle from Southern Punjab (Pakistan). *Parasitol. Res.*, 109: 1155-1160.
- Soulsby, E. J. L. 1982. Helminthes, arthropods and protozoa of domesticated animals, 3<sup>rd</sup> Ed. Bailliere Tindall and Cassell Ltd., London (UK) 65 p.
- Tessema, T. and A. Gashaw. 2010. Prevalence of ticks on local and crossbred cattle in and around Asella town, southeast Ethiopia. *Ethiop. Vet. J.*, 14: 79-89.
- Viseras, J. and P. Garcia-Fernandez. 1999. Studies on Theileriosis in Southern Spain. *Parassitologia*, 1: 111-115.

(Accepted January 04, 2016)