

EFFECT OF *BABESIA BIGEMINA* AND *THEILERIA ANNULATA* ON WEIGHT GAIN, MILK YIELD AND ESTROUS CYCLE IN EXPERIMENTALLY INFECTED *BOS TAURUS* AND *BUBALUS BUBALIS*

A.W. Manzoor¹, S. Ali¹, S. Ahmad¹, *S. Ali², R. Rafique², H. M. Waqar¹, R. Akram³, R. Rafique⁴, M. Shaukat⁵, H. Shaukat⁶, A. Abbas¹ and S. G. M. Din⁷

¹ Veterinary Research Institutes, ² Foot and Mouth Disease Research Center, Zarrar Shaheed Road, Lahore Cantt. 54810, Pakistan

³ University of Veterinary and Animal Sciences, Lahore-54000, Pakistan

⁴ PMAS Arid Agriculture University, Rawalpindi-46000, Pakistan

⁵ School of Biological Sciences, University of the Punjab, Quaid-i-Azam Campus Lahore-54590, Pakistan

⁶ Department of Zoology, University of the Punjab, Lahore-54590, Pakistan

⁷ Department of Veterinary Medicine, Guangdong Ocean University, Zhanjiang-524088, China

Corresponding author's E-mail: dshat1963@gmail.com

ABSTRACT: In Pakistan, buffaloes and cattle are the main source for the milk and meat production ultimately having a great economic impact on the socio-economic living of the people. It is believed that blood protozoan parasitic diseases like Babesiosis and Theileriosis caused by tick-borne blood parasite greatly reduce the productivity of the animals. Hence, the study was designed to assess the effect of *Babesia bigemina* and *Theileria annulata* infection on the body weight, estrous cycle and milk production in cattle and buffaloes. Two hundred animals comprising of *Bubalus bubalis* and *Bos taurus* (Holstein Friesian, Jersey and Sahiwal) adults and calves were selected for this study. A significant decrease was observed in weight of the affected animals. Delayed maturity was also seen in calves affected with Theileriosis as compared with the control group of calves as the animals gained weight at slower rate after recovery from the disease. Production of milk in control group of *Bos taurus* and *Bubalis bubalis* was $14.6 \pm 0.23\text{Kg}$, $13.3 \pm 0.2\text{Kg}$, $11.5 \pm 0.4\text{Kg}$ and $12.0 \pm 0.31\text{Kg}$ whereas milk production in Babesia affected groups declined to $4.7 \pm 0.02\text{Kg}$, $6.1 \pm 0.5\text{Kg}$, $4.3 \pm 0.3\text{Kg}$ and $4.3 \pm 0.24\text{Kg}$ respectively. Milk production in control groups of animals of *Bos* was $13.3 \pm 0.2\text{Kg}$, $14.6 \pm 0.23\text{Kg}$ and $11.5 \pm 0.4\text{Kg}$ whereas milk yield in Theileriosis affected groups decreased to $5.6 \pm 0.37\text{Kg}$, $4.9 \pm 0.41\text{Kg}$ and $4.7 \pm 0.43\text{Kg}$ respectively. Animals of *B. bubalis* and *Bos. taurus* species infected experimentally with *Babesia bigemina* and *Theileria annulata* showed irregular and prolonged estrous cycle whereas healthy animals showed normal estrous cycle. The duration of estrous cycle observed in Babesiosis affected animals was 76.0 ± 4.4 days, 65.0 ± 3.29 days, 58 ± 5.2 days and 47.0 ± 4.2 days. The duration of estrous cycle in Theileriosis affected *Bos* species was 63.0 ± 8.57 , 68.0 ± 10.0 and 31.0 ± 5.7 days respectively.

Keywords: Babesiosis, Theileriosis, *Bubalus bubalis*, *Bos taurus*, Milk production, Estrous cycle, Body weight

(Received 11.11.2020

Accepted 29.11.2020)

INTRODUCTION

In developing countries like Pakistan there is scarcity of well-planned and structured farming for keeping the animals. The demand for milk and meat is met by individual efforts mostly. In such circumstances it is the dire need of time to make arrangements for the awareness of farmers regarding modern animal husbandry practices and effective disease prevention and control for profitable farming. Along with other diseases tick-borne diseases are a major source of economic losses to the farmers.

Economic losses due to tick-borne diseases in terms of less milk yield, reduced weight gain, disapproval of carcass and mortality have been observed globally in

cattle (Sharma *et al.*, 2000; Raza *et al.*, 2007). Ticks belong to the order Acarina of Phylum Arthropoda and super family Ixodidae about 896 species of ticks have been known. There are 702 species of hard ticks which have scutum on the dorsum of their bodies and belong to the family Ixodidae (Guglielmone *et al.*, 2010). The regions having moderate climate, less rainfall and heat harbor ticks more frequently, restricting the development of the livestock industry (Rajput *et al.*, 2006). The ticks which do not have dorsal scutum are soft ticks and belong to the family Argasidae. Mostly pathogens of poultry are spread by soft ticks (Ghosh *et al.*, 2007).

Hard ticks serve as vectors for pathogens of numerous diseases thus causing heavy losses and are of medical and veterinary importance. The pathogens of

various diseases of animals like Babesiosis, Theileriosis, Crimean-Congo Haemorrhagic Fever (CCHF), Anaplasmosis and Toxoplasmosis are transmitted by these ticks (Heyman *et al.*, 2010). Theileriosis and Babesiosis have harmful effects on the development of livestock across the globe. The diseases transmitted by ticks to human beings and livestock accede in number to various diseases transmitted by other arthropods in developing countries like Pakistan and India (Muhammad *et al.*, 2008). To enhance meat and milk production, hybrid breeds have been developed by crossing exotic breeds with local ones. These breeds due to their less resistance to tick infestation are more prone to various tick born infections (Ahmed *et al.*, 2012).

In Pakistan the prevalent species of ticks belong to the genus *Rhipicephalus* and *Hyalomma* are hard ticks of family Ixodidae. *Rhipicephalus* and *Hyalomma* transmit *Babesia bigemina* (*B. bigemina*) and *Theileria annulata* (*T. annulata*) respectively. The environmental circumstances in Pakistan are favorable for the rapid and vibrant propagation of ticks. The presence of meager hygienic conditions also helps in ticks survival thus posing a threat for livestock development (Durrani *et al.* 2008). Tick infestation can lead to lethal consequences in the form of mild, sub lethal and acute infections resulting to death if not treated effectively. The animals recovered from sub lethal infections cannot regain their normal production level and suffer from reproductive disorders and weight loss (L'Hostis and Seegers, 2002). In several parts of the world haemoparasitic infections are major public health, veterinary and socio-economic problems as well as they impose a burden on the healthcare infrastructure of both animals and their handlers. Due to great economic impact these infections are of worldwide significance (Mohammed and Elshahawy, 2017).

Keeping in view the hazards caused by ticks and tick borne diseases this experiment was framed to study the effects of *B. bigemina* and *T. annulata* on milk production estrous cycle and body weight in *B. bubalis* and *Bos taurus* species.

MATERIALS AND METHODS

The calves and adults of *Bos species* (Holstein Friesian, Jersey and Sahiwal) and *B. bubalis* were selected for this study. Various Government Livestock Experimental Stations situated at Qadirabad (Sahiwal), Bhunikey (Kasur), Bahadarnagar (Okara), different civil veterinary hospitals, private livestock farms and private clinics of Gujranwala, Okara, Lahore, Faisalabad, Sahiwal and Kasur districts were chosen to study tick infestation and epidemics of Babesiosis and Theileriosis.

After visiting various above said veterinary facilities ticks were picked from infested animals and characterized morphologically using a stereomicroscope according to the keys described by Estrada-Pena *et al.*,

2004; Mc Carthy, 1967. The morphologically characterized tick species from the collected samples comprised of *Hyalomma anatolicum anatolicum* (*H. a. anatolicum*) and *Rhipicephalus microplus* (*R. microplus*) total of 200 animals included calves and adults of *Bos* (exotic, cross bred and indigenous) and *B. bubalis* were selected for experimental infestation with ticks to study the effects of *B. bigemina* and *T. annulata* on weight gain, milk production and estrous cycle. The selected animals were infested with ticks as described below;

(I) Infestation with Ticks

(a) **Babesiosis:** To induce the disease experimentally 10 males and 10 females of *R. microplus* ticks recovered from the infected area were placed in a linen ear carrier. Later on linen ear carriers were attached with the ears of *B. bubalis* (4 calves and 4 adults) and *Bos taurus* (4 calves and 4 adults) to infect these animals with *B. bigemina*.

(b) **Theileriosis:** For induction of disease in experimental animals 10 males and 10 females of *Hyalomma anatolicum* ticks recovered from the infected animals were placed in a linen ear carrier and attached to the ear of four *Bos taurus* calves and adults.

(II) Inoculation of Blood

(a) **Babesiosis:** The experimentally infected animals were checked for the infection 13 days post infestation. In acute stage of the disease 10ml blood was collected from each species of *Bos* and *B. bubalis* and injected into four healthy calves and adults of *B. bubalis* and *Bos* species each intravenously. Clinical signs and symptoms were recorded in the animals injected with infected blood.

(b) **Theileriosis:** Subsequent upon the confirmation of Theileriosis in the animals infested with ticks 10ml blood was collected from these animals. Four healthy calves and adults of all the three breeds of *Bos taurus* were injected intravenously. Clinical signs and symptoms of disease were also recorded. The animals of control group were injected with the blood collected from healthy animals having no tick infestation.

Experimental Study: The diseased animals were kept under observation up to two weeks to study the effects of the disease on their weight gain, estrous cycle duration and milk yield. Blood smears were prepared from the infected animals and stained with Giemsa stain. Stained smears were examined microscopically under oil emulsion lens to check the presence of Babesia and Theileria species. Ticks collected from animals negative for *B. bigemina* and *T. annulata* were also infested on the animals of control group.

(1). **Body Weight:** Four adult animals of *B. bubalis* and four adult animals of *Bos taurus* (exotic, indigenous and cross bred) experimentally infected with *B. bigemina*

were weighed at Khalid Dairy Farm Barki, Cattle Colony Harbanspura and Syed Shahid Farm Badian Road, Lahore. Adult and calves of *Bos*, four of each species (6-9 months old) infected with *T. annulata* were weighed at Livestock Experimental Station (LES) Bahadarnagar District Okara, LES Qadirabad, District Sahiwal and LES Bhunikey, District Kasur. Body weight of healthy animals of same species having same feeding and management regime was also taken as control. After the onset of symptoms the diseased animals were weighed at 24 hours, 48 hours and 72 hours interval. The diseased animals were treated 72 hours after the appearance of symptoms with injection Imizol® (Imidocarb Dipropionate) (ICI) 120 mg/100 Kg body weight and Butalex® (Buparvaquone) (ICI) 2.5mg/ Kg b/w, intramuscularly for Babesiosis and Theileriosis respectively. Treatment time was considered as day zero, after that animals recovered gradually. Both the diseased and control groups animals were weighed at day 0, 30, 60, 90 and 120 post treatment.

(2) **Milk Yield:** Milk production of four animals of each exotic, cross bred and indigenous breeds of *B. taurus* and four animals of *B. bubalis* infected with Babesiosis was recorded at day 0, 30, 60, 90 and 120 post infection. Milk yield of cross bred, exotic and indigenous *Bos species*, infected with Theileriosis was also noted at day 0, 30, 60, 90 and 120 days post infection. Milk production of healthy *B. bubalis* and *Bos. taurus*, reared in the same environmental and management conditions was also recorded as control. The study was conducted at LES Bhunikey, LES Qadirabad and LES Bahadarnagar.

(3) **Estrous Cycle:** Inter-estrous period of four animals of each cross bred, indigenous and exotic *B. taurus* and four animals of *B. bubalis* infected with *B. bigemina* was documented. Infected animals kept under observation for 120 days. Likewise inter-estrous period of exotic, cross bred and indigenous *Bos species* infected with Theileria was also recorded. The control group consisted of healthy animals, four of each species. These observations were made at LES Bahadarnagar, LES Qadirabad and LES Bhunikey.

RESULTS

1- Body Weight

a- **In Babesiosis affected Animals:** Average body weight of healthy *B. bubalis* (3 years old), *Bos taurus* (crossbred, indigenous and exotic) was found to be 430.75 ± 2.25 Kg, 286.5 ± 3.12 Kg, 334.0 ± 2.3 Kg and 328.5 ± 1.4 Kg. The body weight of the diseased animals was recorded at 24, 48 and 72 hours. The body weight of diseased *B. bubalis* animals decreased to 427.0 ± 2.02 Kg, 424.7 ± 1.7 Kg and 395.2 ± 3.05 Kg at 24, 48 and 72 hours interval, respectively. The body weight of diseased cross bred animals decreased to 282.0 ± 2.9 Kg, $280.0 \pm$

3.3 Kg and 251.0 ± 4.2 Kg after 24, 48 and 72 hours interval respectively. The body weight of diseased indigenous animals decreased to 330.0 ± 2.6 Kg, 328.0 ± 2.5 Kg and 308 ± 1.1 Kg after 24, 48 and 72 hours interval. The body weights of the diseased exotic animals decreased to 325.0 ± 1.6 Kg, 323 ± 1.7 Kg, 302 ± 2.0 Kg after 24, 48 and 72 hours interval respectively (Table-1).

b- **Treated Animals:** After treatment with Imizol (Imidocarb Dipropionate) the body weights of *B. bubalis* and *B. taurus* indigenous, cross bred and exotic breeds gradually increased to 416.0 ± 1.25 Kg, 323.0 ± 1.22 Kg, 269.5 ± 4.76 Kg and 319.5 ± 1.25 Kg respectively in 120 days (Table-2). Statistical analysis revealed that up to 48 hours the decrease in weight was non-significant in diseased animals while after 72 hours a significant decrease was observed. It was also observed that the gain in weight by the control group was not equal to the weight gain in disease affected group when analyzed statistically.

c- In Theileriosis Affected Animals

Bos. taurus Adults and Calves: Average body weight of healthy 2.5 to 3 years old adults of *B. taurus* (exotic, crossbred and indigenous) was observed 328.0 ± 1.4 Kg, 286.5 ± 3.12 Kg and 334.0 ± 2.3 Kg while the body weight decreased to 303.0 ± 2.8 Kg, 253.0 ± 6.2 Kg and 307.0 ± 1.37 Kg at 24, 48 and 72 hours respectively, after the onset of the clinical signs including fever, dyspnea and dullness (Table-3). Average body weight of, healthy 6-7 months old *B. taurus* calves (exotic, cross bred and indigenous) was found to be 79.0 ± 1.4 Kg, 69.0 ± 4.3 Kg and 72.0 ± 1.3 Kg while the body weight of diseased animals decreased and was observed as 64.0 ± 2.6 Kg, 55.0 ± 1.7 Kg and 55.0 ± 2.36 Kg, respectively at 24, 48 and 72 hours intervals (Table-4).

d- Treated Animals:

Bos. taurus Adults and Calves

A gradual improvement was observed in Theileriosis affected animals after treatment with injection Butalex (Buparvaquone). After recovery from the disease a gradual increase in body weight of exotic, cross bred and indigenous breeds was observed in 120 days and recorded as 322.2 ± 1.64 Kg, 271.0 ± 4.3 Kg and 321.0 ± 1.28 Kg in respective species but not as equal to control group (Table-5). Similarly treated calves were recovered from the disease and their body weight increased to 76.0 ± 2.3 Kg, 71.0 ± 3.0 and 71.0 ± 1.49 Kg, respectively. The calves in recovered group were not able to attain weight equal to calves of control group animals (Table-6).

2- Milk Yield

a- In Babesiosis Affected Animals

Milk production in control group of *B. bubalis* and *B. taurus* (exotic, crossbred and indigenous) was observed as 12.1 ± 0.31 L, 14.6 ± 0.23 L, 13.3 ± 0.2 L and

11.5 ± 0.45L while milk yield in diseased groups declined abruptly to 4.7 ± 0.2L, 6.1±0.4L, 4.3±0.3L and 4.3±0.24L respectively. After recovery from disease gradual increase in milk yield was observed to be 8.5 ± 0.24L, 9.8 ± 0.2L, 8.7 ± 0.25L and 10.1 ± 0.23L in each group respectively when measured at 120th day (Table-7).

b- In Theileriosis Affected Animals

Milk production in exotic, cross bred and indigenous *Bos taurus* control group animals was recorded as 14.6 ± 0.23L, 13.4 ± 0.2L and 11.4 ± 0.45L whereas milk yield decreased 4.7±0.43L, 4.9±0.41L and 6±0.37L respectively in diseased groups 72 hours after the start of infection. In recovered animals milk production increased to 7.6 ± 0.2L, 9.0 ± 0.35L and 9.3 ± 0.23L up to 120 days in each species of *Bos taurus* (Table-8).

3- Effect on Fertility (Estrous Cycle)

a- In Babesiosis affected Animals

In healthy animals of *B. bubalis* and *Bos* (indigenous, exotic and crossbred) species the estrous cycle duration is normally 21 days. The Babesia infected animals which were treated with drug of choice, 72 hours after the start of the infection the duration of estrous cycle was recorded as 76.0±4.4 days, 47.0 ± 4.2 days, 65.0 ± 3.29 days and 58 ± 5.2 days respectively (Table-9).

b- In Theileriosis Affected Animals

The duration of estrous cycle observed in animals of *Bos* species (indigenous, crossbred and exotic) was 31.0 ± 5.7 days, 68.0 ± 10.0 days and 63.0 ± 8.57 days respectively (Table-10).

Table 1: Babesiosis effect on body weight (Kg) of adult animals of *B. taurus* and *B. bubalis* species after appearance of symptoms up to 72 hours.

Species			Body Weight		
			24 hours Mean ± S.E	48 hours Mean ± S.E	72 hours Mean ± S.E
<i>B. bubalis</i>		Control	430.75 ± 2.25	430.75 ± 2.25	430.75 ± 2.25
		Diseased	427.0 ± 2.02 ^{NS}	424.7 ± 1.7 ^{NS}	395.2 ± 3.05 ^{***}
<i>B. taurus</i>	Cross bred	Control	286.5 ± 3.1	286.5 ± 3.1	286.5 ± 3.1
		Diseased	282.0 ± 2.9 ^{NS}	280.0 ± 3.3 ^{NS}	251.0 ± 4.2 ^{**}
	Indigenous	Control	334.0 ± 2.3	334.0 ± 2.3	334.0 ± 2.3
		Diseased	330.0 ± 2.6 ^{NS}	328.0 ± 2.5 ^{NS}	308.0 ± 1.1 ^{**}
	Exotic	Control	328.5 ± 1.4	328.5 ± 1.4	328.5 ± 1.4
		Diseased	325.0 ± 1.6 ^{NS}	323.0 ± 1.7 ^{NS}	302.0 ± 2.0 ^{**}
NS: Non-significant; ^{**} : P <0.01; ^{***} :P<0.001					

Table 2: Post treatment increase in weight (Kg) of adult animals of *B. bubalis* and *Bos* species affected with Babesiosis.

Species		Treatment	Day 0	Day 30	Day 60	Day 90	Day 120
<i>B. bubalis</i>		Control	430.75 ± 2.25	430.75 ± 2.25	431.0 ±2.25	430.0 ±2.25	432.0 ±2.25
		Treated	395.2 ±3.05 ^{***}	400.7 ± 3.25 ^{***}	403.7 ±1.25 ^{***}	412.0 ±1.47 ^{**}	416.2 ±1.25 [*]
<i>B. taurus</i>	Indigenous	Control	334.2 ± 2.3	334.2 ± 2.3	335.0 ± 2.3	337.0 ±2.4	336.2 ±2.3
		Treated	308.2 ± 1.18 ^{**}	310.7 ± 1.49 ^{**}	315.0 ± 0.6 ^{**}	318.0 ±0.81 ^{**}	323.0 ±1.2 ^{**}
	Cross bred	Control	286.5 ± 3.12	284.0 ± 3.1	385.4 ± 3.	285.0 ±3.2	287.0 ±3.3
		Treated	251.2 ± 4.2 ^{**}	256.2 ± 5.54 ^{**}	260.7 ± 5.2 ^{**}	265.5 ±5.04 ^{**}	269.5 ±4.7 ^{**}
	Exotic	Control	328.5 ± 1.4	329.5 ± 1.4	329.0 ± 1.4	330.0 ±1.3	330.4 ±1.3
		Treated	302.5 ± 2.0 ^{**}	305.0 ± 2.87 ^{**}	312.5 ± 1.4 ^{**}	317.5 ±0.95 [*]	319.5 ±1.25 [*]
* :P <0.05;		** :P <0.01					

Table 3: Effect of Theileriosis on body weight (Kg) of adult animals of *B. taurus* after appearance of symptoms up to 72 hours.

Species			Body weight (Kg)		
			24 hours Mean ± S.E	48 hours Mean ± S.E	72 hours Mean ± S.E
<i>B. taurus</i>	Exotic	Control	328.5 ± 1.4	328.5 ± 1.4	328.5 ± 1.4
		Diseased	325.0 ± 2.0 ^{NS}	322.0 ± 1.7 ^{NS}	303.0 ± 2.8 ^{**}
	Cross bred	Control	286.5 ± 3.12	287.5 ± 2.12	286.5 ± 3.12

Indigenous	Diseased	284.0 ± 2.2 ^{NS}	280.0 ± 4.1 ^{NS}	253.0 ± 6.2 [*]
	Control	334.2 ± 2.3	335.0 ± 2.0	333.0 ± 1.4
	Diseased	333.0 ± 2.3	329.0 ± 2.4 ^{NS}	307.0 ± 1.37 ^{**}

NS: Non-significant; ^{*}:P<0.05; ^{**}:P<0.01

Table 4: Effect of Theileriosis on the body weight (Kg) of calves of *Bos* species after onset of disease up to 72 hours.

			Body weight (Kg)		
Species			24 hours Mean ± S.E	48 hours Mean ± S.E	72 hours Mean ± S.E
<i>B. taurus</i>	Exotic	Control	79.0 ± 1.4	79.0 ± 1.4	79.0 ± 1.4
		Diseased	76.0 ± 2.1 ^{NS}	76.0 ± 1.3 ^{NS}	64.0 ± 2.6 ^{**}
	Cross bred	Control	69.0 ± 4.3	69.0 ± 4.3	69.0 ± 4.3
		Diseased	66.0 ± 2.1 ^{NS}	65.0 ± 1.7 ^{NS}	55.0 ± 1.7 [*]
	Indigenous	Control	72.0 ± 1.3	72.0 ± 1.3	72.0 ± 1.3
		Diseased	70.0 ± 1.0 ^{NS}	68.0 ± 1.5 ^{NS}	55.0 ± 2.36 ^{**}

NS: Non-Significant; ^{*}:P <0.05; ^{**}:P <0.01

Table 5: Post treatment increase in weight (Kg) of adult animals of *B. taurus* affected with Theileriosis.

Species	Treatment	Day 0	Day 30	Day 60	Day 90	Day 120
<i>B. Taurus</i> (Exotic)	Control	329.0 ± 1.4	329.5 ± 1.4	329.5 ± 1.4	330.5 ± 1.4	330.4 ± 1.4
	Treated	303.0 ± 3.22 ^{**}	305.2 ± 2.93 ^{**}	309.0 ± 2.58 [*]	319.0 ± 1.5 [*]	322.2 ± 1.64 [*]
<i>B. Taurus</i> (Cross bred)	Control	286.5 ± 3.12	284.1 ± 3.1	285.4 ± 3.1	285.0 ± 3.0	287.0 ± 3.1
	Treated	253.0 ± 6.2 [*]	259.5 ± 5.93 [*]	263.5 ± 4.99 [*]	267.2 ± 4.6 [*]	271.2 ± 4.3
<i>B. Taurus</i> (Indigenous)	Control	334.2 ± 2.3	335.2 ± 2.3	336.0 ± 2.3	337.0 ± 2.3	338.0 ± 2.3
	Treated	307.0 ± 1.37 ^{**}	308.7 ± 2.01 ^{**}	315.2 ± 1.12 ^{**}	318.0 ± 0.5 ^{**}	321.7 ± 1.28

^{*}:P<0.05; ^{**}:P<0.01

Table 6: Effect of Theileriosis on body weight (Kg) of calves of *B. taurus*.

Species	Treatment	Day 0	Day 30	Day 60	Day 90	Day 120
<i>B. Taurus</i> (Exotic)	Control	79.0 ± 1.4	82.8 ± 1.76	86.0 ± 1.11	92.5 ± 2.02	101.0 ± 1.38
	Treated	64.0 ± 2.6 ^{***}	65.0 ± 2.87 ^{***}	67.0 ± 2.14 ^{***}	71.0 ± 2.7 ^{***}	76.0 ± 2.35 ^{***}
<i>B. Taurus</i> (Cross bred)	Control	69.0 ± 4.3	72.0 ± 1.6	76.0 ± 2.8	80.0 ± 4.39	87.0 ± 4.6
	Treated	55.0 ± 1.7 [*]	58.0 ± 1.7 [*]	61.0 ± 2.10 [*]	64.0 ± 1.19 [*]	71.0 ± 3.0
<i>B. Taurus</i> (Indigenous)	Control	72.0 ± 1.3	73.0 ± 1.47	78.0 ± 4.66	81.0 ± 3.51	85.0 ± 4.11
	Treated	55.5 ± 2.36 [*]	58.0 ± 2.4 [*]	62.0 ± 2.06	65.0 ± 2.12 [*]	71.0 ± 1.49

^{*}:P<0.05; ^{***}:P<0.001

Table 7: Effect of Babesiosis on milk production of animals of *B. bubalis* and *B. taurus*.

Species	Treatment	Day 0	Day 30	Day 60	Day 90	Day 120
<i>B. bubalis</i>	Control	12.1 ± 0.31	12.3 ± 0.3	12.1 ± 0.3	12.3 ± 0.3	12.3 ± 0.3
	Diseased	4.7 ± 0.02 ^{***}	5.3 ± 0.37 ^{***}	6.5 ± 0.45 ^{**}	8.1 ± 0.42 ^{**}	8.5 ± 0.24 ^{**}
<i>B. taurus</i> (Exotic)	Control	14.6 ± 0.23	14.6 ± 0.23	14.7 ± 0.23	14.6 ± 0.23	14.7 ± 0.23L
	Diseased	6.1 ± 0.5 ^{**}	6.3 ± 0.24 ^{***}	7.2 ± 0.14 ^{**}	8.8 ± 0.24 ^{**}	9.8 ± 0.24L [*]
<i>B. taurus</i> (Cross bred)	Control	13.3 ± 0.2	13.4 ± 0.2	13.4 ± 0.2	13.3 ± 0.2	13.5 ± 0.2L
	Diseased	4.3 ± 0.32 ^{***}	6.1 ± 0.5 ^{**}	7.9 ± 0.42 ^{**}	9.8 ± 0.32 ^{**}	10.1 ± 0.23L [*]
<i>B. taurus</i> (Indigenous)	Control	11.5 ± 0.4	11.6 ± 0.4	11.6 ± 0.4	11.5 ± 0.4	11.3 ± 0.45L
	Diseased	4.3 ± 0.24 ^{***}	5.9 ± 0.21 ^{**}	7.5 ± 0.20 ^{**}	8.2 ± 0.14 [*]	8.7 ± 0.25L [*]

^{*}:P <0.05; ^{**}:P <0.01; ^{***}:P <0.001

Table 8: Effect of Theileriosis on milk yield of animals of *B. taurus*.

Species	Treatment	Day 0	Day 30	Day 60	Day 90	Day 120
<i>B. taurus</i> (Exotic)	Control	14.6 ± 0.23	14.5 ± 0.23	14.8 ± 0.23	14.6 ± 0.23	14.6 ± 0.23
	Diseased	4.9 ± 0.41***	5.8 ± 0.25**	6.67 ± 0.28	8.3 ± 0.35**	4.7±0.43L **
<i>B. taurus</i> (Cross bred)	Control	13.3 ± 0.2	13.4 ± 0.2	13.4 ± 0.2	13.5 ± 0.2	13.4 ± 0.2
	Diseased	5.6 ± 0.37***	7.12 ± 0.23***	7.7 ± 0.32***	8.9 ± 0.25	4.9±0.41L
<i>B. taurus</i> (Indigenous)	Control	11.5 ± 0.45	11.3 ± 0.45	11.5 ± 0.45	11.4 ± 0.45	11.4 ± 0.45L
	Diseased	4.7 ± 0.43***	5.7 ± 0.46**	6.9 ± 0.6*	7.1 ± 0.0**	6±0.37L **

*:P <0.05;

** :P <0.01;

***:P <0.001

Table-9: Effect of Babesiosis on the estrous cycle duration of animals of *B. bubalis* and *B. taurus*.

Days after which estrous was observed							
<i>B. bubalis</i>		<i>B. taurus</i>					
		Indigenous		Exotic		Cross bred	
Healthy (n=4)	Treated (n=4)	Healthy (n=4)	Treated (n=4)	Healthy (n=4)	Treated (n=4)	Healthy (n=4)	Treated (n=4)
21 days	76 ± 4.4**	21 days	47 ± 4.2*	21 days	65 ± 3.29*	21 days	58 ± 5.24**

*:P<0.05; ** :P<0.01;

***:P<0.001

Table-10: Effect of Theileriosis on estrous cycle duration of animals *B. taurus*.

Days after which estrous was observed					
<i>B. taurus</i>					
Indigenous		Cross bred		Exotic	
Healthy (n=4)	Treated (n=4)	Healthy (n=4)	Treated (n=4)	Healthy (n=4)	Treated (n=4)
21 days	31 ± 5.7	21 days	68 ± 10.0* [/]	21 days	63 ± 8.57*

*:P<0.05.

DISCUSSION

Ticks are considered economically significant ecto-parasites of the livestock. The veterinary importance of ticks lies in their habit of feeding on the blood of the host thus directly or indirectly leading to disease. In cattle and buffaloes tick infestation leads to anemia, stress, decrease milk production, weight loss, toxicity, low value of hides and hypersensitivity reactions resulting in secondary bacterial infections. Some tick species serve as vectors of various bacterial, viral and protozoan pathogens of veterinary importance thus causing various tick-borne diseases and affecting production and health of the livestock.

A substantial decrease in the weight gain of the affected animals was observed from the findings of the current project. Calves affected with Theileriosis gained weight at slower rate after they were recovered from the disease as compared to the animals in control groups. These findings are in line with the results of earlier studies as done by Mottoli (1995) who reported that *B. bigemina* infection accompanied with *Amblyomma variegatum* and *Hyalomma* infestation significantly affected weight gain in *Bos*. Significant loss in weight due to theileria infections was also illustrated by Patil *et*

al. (1995) in *Bos* calves. Significant weight loss (P<0.01) was also reported by Jonsson and Mayer (1999) and by Jonsson *et al.* (2008) due to *Haematobia irritans exigua* in *Bos species* and a significant decrease in the milk yield in infected animals was observed. The decrease in milk yield is a general phenomenon observed in animals affected with Babesiosis and Theileriosis. These findings are in agreement with that of Bock *et al.* (2004).

Another aspect observed to be affected in this study, was fertility of the animals. The healthy *Bos taurus* and *B. bubalis* have regular estrous cycle 21 days while irregular and prolonged estrous cycles were observed in the diseased or recovered animals. This irregularity of the estrous cycle may be due to haemoglobinuria, anemia and emaciation. Such type of disturbance in ovarian activity was also noted by El-Moghazy (2011). In acute cases of Theileriosis abortion was also observed in cross bred animals which might be due to their lesser resistance against this disease or it could be the case of individual resistance. The high fever during babesiosis may cause abortion in pregnant animals and reduced rate of fertility as previously observed by Ferreri *et al.* (2008).

The current project aims at taking steps for the development of vaccines against theileria and babesia species with their proper control so as to avert the losses

in terms of reduced weight gain, lower milk production and reproductive disorders.

REFERENCES

- Ahmed, S., M. Numan, A. W. Manzoor and F. A. Ali (2012). Investigations into Ixodidae ticks in cattle in Lahore, Pakistan. *Vet. Ital.* 48(2): 185-191.
- Bock, R., L. Jackson, D. Vos and W. Jorgensen (2004). Babesiosis of cattle. *Parasitol.* (129): 247-249.
- Durrani, A. Z., A. R. Shakoori and N. Kamal (2008). Bionomics of hyalomma ticks in three districts of Punjab Pakistan. *Journal of Animal and Plant Sciences*, 18(1). .
- Estrada-Pena, A., A. Bouattour, J. L. Camicas and A.R. Walker (2004). Ticks of domestic animals in the Mediterranean Region: *A guide to identification of Species. University of Zaragoza, Pza, San Francisco s/n, 50001-Zaragoza, Spain*
- El-Moghazy, F. M. (2011). Impact of Parasitic Infestation on Ovarian Activity in Buffaloes-Heifers with Emphasis on Ascariasis. *World Journal of Zoology*, 6 (2), 196-203.
- Ferreri, L., D. Benitez, M. Dominguez, A. Rodriguez, G. Asenzo and M. Mesplet (2008). Water buffalos as carriers of Babesia bovis in Argentina. *Ann. N. Y. Acad. Sci.* (1149):149-151.
- Ghosh, S., G.C. Bansal, S.C. Gupta, D. Ray, M.Q. Khan, H. Irshad, M. Shahiduzzaman, U. Seitzer and J.S. Ahmed (2007). Status of tick distribution in Bangladesh, India and Pakistan. *Parasitol. Res.* 101(2), 207-216.
- Guglielmone, A. A., R. G. Robbing, D. A. Apanaskevich, T. N. Petney, A. Estrada-Peña, I. G. Horak, R. Shao, and S. C. Barker (2010). The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida) of the world, a list of valid species names. *Zootaxa.*, (2528) 1-28.
- Heyman, P., C. Cochez, A. Hofhuis, J. Van der Giessen, H. Sprong, S. R. Porter, B. Losson, C. Saegerman, O. Donoso-Mantke, M. Niedrig, and A. Papa (2010). A clear and present danger: tick-borne diseases in Europe. *Expert. Rev. Anti. Infect. Ther.* 8(1), 33-50.
- Jonsson, N. N. and D. G. Mayer (1999). Estimation of the effects of buffalo fly (*Haematobia tritans exigua*) on the milk production of dairy cattle based on a meta-analysis of literature data. *Med. Vet. Entamol.* (13): 372-376.
- Jonsson, N.N., R.E Bock and W. K. Jorgensen, (2008). Productivity and health effects of anaplasmosis and babesiosis on *Bos indicus* cattle and their crosses and the effects of differing intensity of tick control in Australia. *Veterinary Parasitology*, (155): 1-9.
- Kaiser, M. N. and H. Hoogstraal, (1964). The Hyalomma ticks of Pakistan, India and Ceylon with key to sub-genera and species. *Acarologia*, (6): 257-286.
- L'hostis, M., H. Seegers (2002). Tick-borne parasitic diseases in cattle: Current knowledge and prospective risk analysis related to the ongoing evolution in French cattle farming systems. *Veterinary Research* (33): 599-611.
- McCarthy, V. C. (1967). Ixodid ticks (Acarina, Ixodidae) of West Pakistan. *Ph.D. diss.*, Faculty of Graduate School of the University of Maryland, USA.
- Mottioli, R. C., M. S. Kora, M. Cassama and D. J. Clifford (1995). Susceptibility to different tick generain Gambian N/Dama and Gobra Zebu cattle exposed to naturally occurring tick infestation. *Trop. Anim. Hlth. Prod.* 27(2): 95-105.
- Muhammad, G., A. Naureen, S. Firyal and M. Saqib (2008). Tick control strategies in dairy production medicine. *Pakistan Veterinary Journal*, 28(1): 43-50.
- Mohammed, E. S., and I. Elshahawy (2017). The Current Prevalence of Bovine Babesiosis and Theileriosis Infection in Egypt. *Clin Med Images Int J*, 1(1): 00004.
- Patil, N. A., K. R. Lakshmiah, and M. D. Harapanahalli, (1995). Efficacy of Butalex (Buparva- quone) in the treatment of clinical cases borne tropical theileriosis. *Ind. J. Anim. Res.*, 29(1): 59-61.
- Rajput, Z. I., S. H. Hu, W. J. Chen, A. G. Arijo and C. W. Xiao, (2006). Importance of ticks and their chemical and immunological control in livestock. *J. Zhejiang Univ Sci B.*, 7(11): 912-921.
- Raza, A. M., Z. Iqbal, A. Jabbar, and M. Yaseen, (2007). Point prevalence of gastrointestinal helminthiasis in ruminants in Southern Punjab, Pakistan. *J. Helminthol.*, (81): 323-328.
- Sharma, A. K., R. C. Katoch, K. B. Nagal, R. S. Kishtwaria, and S. K. Sharma, (2000). Bovine Babesiosis in Palam valley of Himachal Pradesh. *Indian Vet. J.*, (77): 731-732.