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ORIGINAL PAPER

# Frequency distribution of hard ticks (Acari: Ixodidae) infesting bubaline population of district Toba Tek Singh, Punjab, Pakistan

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**Abstract** The current research was conducted to define the epidemiological parameters related to the prevalence and associated risk factors of tick infestation in buffaloes in the Toba Tek Singh District of central Punjab, Pakistan. The prevalence of ticks on buffaloes was 31.21 % (352/1,128). Among the species of ticks, the prevalence of *Hyalomma marginatum* (75.56 %; 266/352) was higher ($P<0.05$; odd's ratios (OR)=3.09) than *Rhipicephalus microplus* (24.44 %; 86/352). Female buffaloes (69.60 %; 245/352) and younger animals (59.09 %; 208/352) were more heavily infested than males (30.40 %; 107/352) and adult animals (40.91 %; 144/352), respectively, whereas breed was not a determinant ($P>0.05$). With regard to management and husbandry practices, the prevalence of ticks was higher in animals kept on uncemented flooring (54.55 %; 192/352; OR=1.90) followed in order by partially cemented (28.69 %; 101/352; OR=1.71) and fully cemented flooring (16.76 %; 59/352). With regard to feeding systems, grazing animals (64.20 %; 226/352) were more burdened compared to stall-fed animals (35.80 %; 126/352). The highest tick prevalence was recorded in closed housing systems (52.27 %; 184/352), followed by semi-closed (34.09 %; 120/352; OR=1.53), and open housing systems (13.64 %; 48/352). Rope-tied animals (70.73 %; 249/352) were more parasitized ($P>0.05$) than open (29.27 %; 103/352). Prevalence in the study district was highest in tehsil Kamalia followed in order by T.T. Singh and Gojra. The primary body area of infestation by ticks (head, neck, ear, dewlap, back, abdomen, foreleg, shoulder, hind leg, congenital areas, and tail) ranged from highest at inside thigh (17 %) to lowest at rump. In the present survey, the highest prevalence was recorded in July and lowest in December. Comparison of hematological changes showed remarkable differences between infested and non-infested animals, in the form of low values of infested animals, whereas an increment in biochemical parameter values was observed in tick-infested animals. The present study provides significant data to enhance planning for tick control program in the study area.

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## Introduction

Buffalo (*Bos bubalus bubalis*) is a multipurpose animal as it has extensively been used for agricultural land preparation, racking, carting and transportation of goods, and threshing and crushing of sugarcane and oil seeds in the rural areas of the continent (Islam et al. 2006). Pakistan produces about 1.6 billion tons of milk and four million hides per year from buffaloes (Anonymous 2010). Among many constraints, parasitism is thought to be major hindrance in the development of livestock population including buffaloes (Jabber and Green 1983). Hard ticks (Acari: Ixodidae) are obligatory ectoparasites and are a potential source of direct and indirect losses to the buffalo production (Rahbari et al. 2007). They are known to cause lowered productivity (Sajid et al. 2007), mortality (Niyonzema and Kiltz 1986), and transmission of diseases such as babesiosis, theileriosis, and anaplasmosis (Norval et al. 1984). In Pakistan, a few reports are available from different regions concerning prevalence of tick infestation, taxonomy, and associated determinants in different livestock species (Chaudhry et al. 1969; Siddiqi and Jan 1986; Khan et al. 1998; Sajid et al. 2008a, 2009, 2011). A recent study conducted in two districts of lower Punjab indicates that the prevalence of bovine tick infestation (BTI) exceeds 50 % (Sajid et al. 2008a, b). The diverse agro-

climatic conditions, animal husbandry practices, and pasture management largely determine the variability and severity of prevalence of BTI (Sajid et al. 2009) which necessitates the need of an epidemiological survey of different agro-climatic zones of the country which may provide better understanding and strategic control to the small holder dairy farming community of Pakistan. The present cross-sectional study describes the epidemiology of tick infestations of buffaloes in district T.T. Singh, Punjab, Pakistan. The results of the present study would provide a basis for evolving strategic and tactical control of ticks and tick-borne diseases in the district T.T. Singh, Punjab, Pakistan.

## Materials and methods

### Study area

District T.T. Singh, located in the central Punjab between 30°33′–31°2′° N and 72°08′–72°48′ E, contains three tehsils, viz, Gojra, Kamalia, and T.T. Singh or 82 union councils. Most of its area is low land flooded from the Ravi River which runs along the southern and southeastern borders of the district. According to Pakistan Livestock Survey (2010), the average population of domestic animals in district T.T. Singh is 1.25 million. The climate of the study area is extremely hot in summer and cold in winter. There are four seasons in the study area: summer (May to July), autumn (August to October), winter (November to January), and spring (February to April). The annual rainfall varies from 254 to 381 mm; June and July being the hotter months of the year with maximum average temperature of 39 °C. The excessive heat is followed by the monsoon rains in August. December and January are the colder months of the year with an average minimum temperature of 6 °C. Cattle, buffaloes, goats, sheep, and camels are major agricultural commodities of T.T. Singh in descending order of importance. About 439,000 buffalo heads are present in the district T.T. Singh (Anonymous 2010) which is under threat of parasitism. A majority of dairy farmers have traditional stall feeding systems with poor husbandry practices including unhygienic conditions, rope-tied practices, and uncemented floor.

### Selection of study population

Multistage cluster random sampling was used to select a total of 1,128 buffaloes. The sample size of primary units (union councils), secondary units (farms), and elementary units (animals) were randomly selected using formulae given by Thrusfield (2007). Minimum number of animals per farm required to enter into the sampling frame was ten. According to this criterion, 150 farms from 35 union councils were surveyed. Categories of animals based on their age ranges were as follows: (1) buffalo male/female (5–10 years) and (2) buffalo young stock (3–4 years). The breeds under study included (1) Nili Ravi and (2) Kundi. A total of 376 buffaloes from each tehsil of study district were examined to determine if agro-climatic conditions had any impact on tick infestation.

### Prevalence and associated determinants

The technically trained personnel of the Laboratory of Epidemiology, Department of Parasitology, University of Agriculture, Faisalabad, Pakistan visited the selected animals twice a month from April 2010 to March 2011. A questionnaire based on dichotomous and multiple choice types of closed questions was prepared and refined through formal and informal testing (Thrusfield 2007). Quantification of variation in the prevalence of tick infestation with respect to host (age, species, sex, and breed), agent (species), and environment (geographical area, climate, and husbandry) were determined from the information collected through the above-mentioned questionnaire. The metrological data of district T.T. Singh for the study year were procured from the Meteorological Department of Pakistan.

### Collection and identification of ticks

Ticks were collected from animals enrolled in the study using forceps without damaging their mouthparts (Soulsby 1982). These specimens were brought to the Epidemiology Laboratory of the Department of Parasitology for taxonomic identification according to the keys given by Walker et al. (2007). In addition to examining various body parts, an area of 7×7 cm on the mid-dewlap of the selected animals was also counted to compare the intensity of tick infestation in this area with that of one side of the body as previously done by Teglas et al. (2005) and Sajid et al. (2008a). The handling of animals was done as per recommendations of the "Ethics committee in Animal Research" of the Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan.

### Statistical analyses

The data obtained for prevalence of tick infestation and its influencing determinants including age, sex, breed, species, climate, and husbandry were analyzed using multiple logistic regression. Association between prevalence with its possible influencing determinants was measured by odd's ratios (ORs). All the data were analyzed using SAS (2010) software package.

## Results

The prevalence of ticks in buffaloes was recorded as 31.21 % (352/1,128). Among various species of ticks, the

prevalence of *Hyalomma marginatum* (75.56 %; 266/352) was higher ($P<0.05$; OR=3.09) as compared with *Rhipicephalus microplus* (24.44 %; 86/352). Regarding host determinants, female buffaloes (69.60 %; 245/352) and younger animals (59.09 %; 208/352) were found more heavily infested than males (30.40 %; 107/352) and adult animals (40.91 %; 144/352), respectively. Whereas, breed of the host was found equally susceptible ($P>0.05$) to ticks. In different observed management and husbandry practices, the prevalence of ticks was found to be higher in animals kept on uncemented floor (54.55 %; 192/352; OR=1.90) followed in order by partially cemented (28.69 %; 101/352; OR=1.71) and cemented floor (16.76 %; 59/352), and in feeding system, grazing animals (64.20 %; 226/352) were more burdened as compared to stall-fed animals (35.80 %; 126/352). The highest tick prevalence was recorded in closed-type housing system (52.27 %; 184/352) followed in order by semi-closed (34.09 %; 120/352; OR=1.53) and open type (13.64 %; 48/352), and rope-tied animals (70.73 %; 249/352) were found more closely associated with the prevalence of ticks ($P>0.05$) as compared to the open (29.27 %; 103/352). Tehsil-wise prevalence in the study district was highest in tehsil Kamalia followed in order by T.T. Singh and Gojra (Table 1). The highest tick burden was recorded inside thigh (17 %) followed in order by scrotum (13 %), perianal area (13 %), udder (11 %), rump, dewlap and prepuce (8 % each), ear (5 %), shoulder (6 %), eyes, mane, hind legs, tail and fore head (3 % each), foreleg, and flank (2 % each). Highest and lowest prevalence of buffalo ticks were recorded in July and December, respectively (Fig. 1). Hematological profile revealed significantly ($P<0.05$) lower values in the infested than those of non-infested buffaloes; however, biochemical parameters were higher in the former (Table 2).

## Discussion

The prevalence of tick infestation in ruminants is much higher in developing countries in Asia (Siddiqi and Jan 1986; Khan et

**Table 1** Prevalence and associated determinants of ticks infesting buffaloes (*Bos bubalus bubalis*) of district Toba Tek Singh

| Buffaloes screened for ticks ($N$=1,128) | | | | | | |
|---|---|---|---|---|---|---|
| Infested animals ($n$) | | Prevalence (%)=$n/N\times100$ | | | | |
| 352 | | 31.21 | | | | |
| Associated determinants | Levels | Prevalence (%) | $P$ value | 95 % C.I. | | Odds ratio |
| | | | | Lower limit | Upper limit | |
| Sex | Female | 69.60 % (245/352) | 0.000 | 64.64 | 74.37 | 2.29 |
| | Male | 30.40 % (107/352) | – | 25.76 | 35.36 | – |
| Age | Young | 59.09 % (208/352) | 0.005 | 53.89 | 64.14 | 1.44 |
| | Adult | 40.91 % (144/352) | – | 35.86 | 46.11 | – |
| Breed | Nili Ravi | 54.26 % (191/352) | 0.204 | 49.03 | 59.42 | 1.19 |
| | Kundi | 45.74 % (161/352) | – | 40.58 | 50.97 | – |
| Tick species | *Hyalomma marginatum* | 75.56 % (266/352) | 0.000 | 70.87 | 79.84 | 3.09 |
| | *Rhipicephalus microplus* | 24.44 % (86/352) | – | 20.16 | 29.13 | – |
| Feeding system | Grazing | 64.20 % (226/352) | 0.000 | 59.09 | 69.09 | 1.79 |
| | Stall feeding | 35.80 % (126/352) | – | 30.91 | 40.91 | – |
| Housing system | Close | 52.27 % (184/352) | 0.002 | 47.05 | 57.46 | 1.53 |
| | Semi-close | 34.09 % (120/352) | 0.000 | 29.28 | 39.16 | 2.50 |
| | Open | 13.64 % (48/352) | – | 10.34 | 17.53 | – |
| Floor pattern | Uncemented | 54.55 % (192/352) | 0.000 | 49.32 | 59.70 | 1.90 |
| | Partially cemented | 28.69 % (101/352) | 0.002 | 24.15 | 33.59 | 1.71 |
| | Cemented | 16.76 % (59/352) | – | 13.13 | 20.94 | – |
| Animal keeping | Rope tied | 70.73 % (249/352) | 0.000 | 65.82 | 75.32 | 2.42 |
| | Open | 29.27 % (103/352) | – | 24.68 | 34.18 | – |
| Area | Kamalia | 43.75 % (154/352) | 0.008 | 38.63 | 48.97 | 1.48 |
| | Toba Tek Singh | 29.54 % (104/352) | 0.547 | 24.95 | 34.47 | 1.11 |
| | Gojra | 26.70 % (94/352) | – | 22.28 | 31.51 | – |

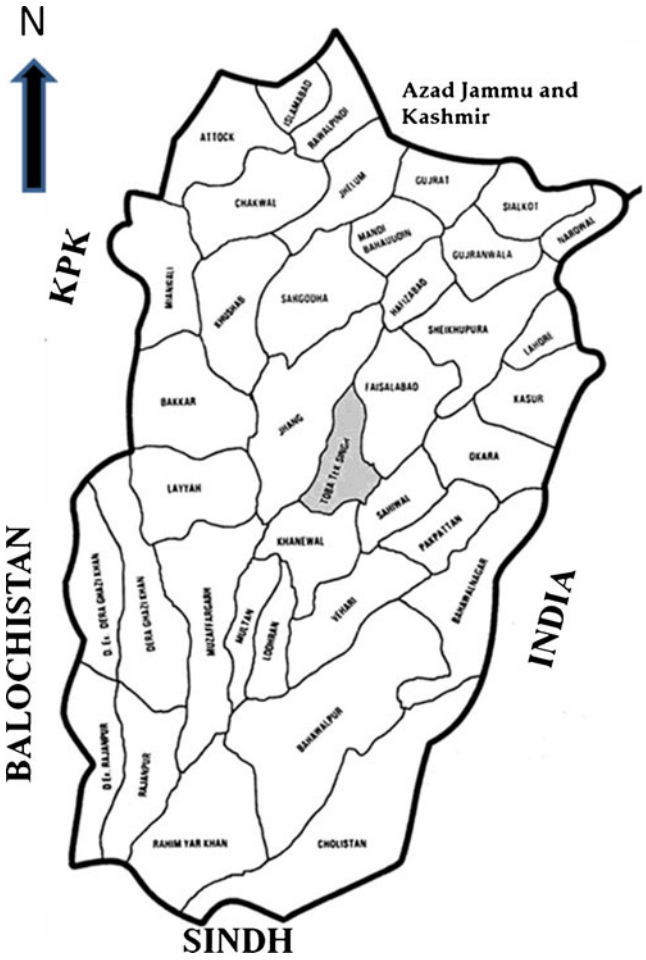


**Fig. 1** Physical map of Punjab Province showing districts and boundaries. The *gray filled* district is the study area (Toba Tek Singh)

al. 1993; Sajid 2007; Sajid et al. 2011) and Africa (Bouattour et al. 1996; Walker and Koney 1999), where control measures are more rarely applied, than in Australia (Springell 1974), Europe (Papadopoulos et al. 1996), and the Americas (Guimaraes et al. 2001). Ticks identified from domesticated livestock populations in Pakistan include *Rhipicephalus* (*Boophilus*) *microplus*, *Rhipicephalus annulatus*, *Rhipicephalus sanguineus*, *Hyalomma anatolicum anatolicum*, *Hyalomma aegyptium*, and *Dermacentor marginatus* from Faisalabad (Khan et al. 1993); *H. a. anatolicum* and *R. sanguineus* from the districts of Layyah and Muzaffargarh (Sajid et al. 2009); *R. microplus*, *Rhipicephalus sharifi*, *Dermacentor raskimensis*, *Haemaphysalis cormupunctata*, *Haemaphysalis montgonervi*, *H. a. anatolicum*, *Hyalomma excavatum*, *Hyalomma detritum*, *Hyalomma dromedarii*, *Hyalomma marginatum toranicum*, *Rhipicephalus haemaphysaloides*, and *R. sanguineus* from Khyber Pakhtunkhwa (formerly known as NWFP) (Siddiqi and Jan 1986); and *H. a. anatolicum* and *Rhipicephalus turanicus* as most predominant among 16 species reported from five different agro-ecological zones of Sind Province (Hussain and Kumar 1986).

**Table 2** Comparative hematological and serum biochemical profile of tick-infested- and non-infested buffaloes (*Bos bubalus bubalis*) of district Toba Tek Singh, Punjab, Pakistan

| Components | Healthy animal (mean±S.E.) | Infested animal (mean±S.E.) |
|---|---|---|
| Hb (g/100 ml) | 13.9±0.13 | 10.48±0.733 |
| PCV (%) | 26.4±0.22 | 23.5±0.95 |
| TEC ($10^6$/mm$^3$) | 8.2±0.7 | 6.64±0.44 |
| TLC ($10^3$/mm$^3$) | 9.1±0.16 | 8.27±0.32 |
| Lymphocytes | 64±0.87 | 74.76±3.2 |
| Neutrophils | 27±0.87 | 15.17±1.72 |
| Eosinophils | 7±0.7 | 9.27±1.17 |
| Monocytes | 4±0.42 | 2.17±0.29 |
| Total protein (g/dl) | 9.24±2.1 | 10.26±2.4 |
| Albumin (g/dl) | 6.61±1.6 | 7.34±1.6 |
| Globulin (g/dl) | 2.91±1.1 | 3.22±1.9 |

High average temperatures in Asia and Africa may be one of the factors which favor tick growth and development. Other determinants may include standards of animal housing, husbandry, and drug administration protocols (Sajid et al. 2011). Many factors can affect infestation rates, including season, habitat (Teel et al. 1996), altitude (Perret et al. 2004; Jouda et al. 2004), breed, sex, lactation stage, and nutritional status (Springell 1974). Geographic variation in the prevalence of tick infestation on buffaloes has been previously documented in Pakistan (Chowdhury 1992; Khan et al. 1993; Islam et al. 2006; Sajid et al. 2007; Ramzan et al. 2008; Sajid et al. 2009). The data on tick infestation from the current study are not different from the previous reports from districts Layyah and Muzaffargarh despite different geo-climates (Sajid et al. 2007). The summer temperature of Pakistan in general and Punjab Province specifically (38–48 °C) is well-suited for the activity, growth, development, and reproduction of ticks (Fig. 2) (Nicholson et al. 2009). Monthly variation in tick populations also influences the questing population of ticks (Perret et al. 2004).

The current study found a higher prevalence of ticks on younger and female buffaloes. Reasons for higher infestation in younger animals include (a) less developed immune system that has yet to be challenged by exposure to tick infestation and (b) softer skin and tissue, facilitating easy penetration of mouth parts of ticks into the body of the host (Swai et al. 2005; Sajid et al. 2009; Kabir et al. 2011). In Pakistan, buffaloes are used for milk, drought, breeding, and sacrificial purposes; males are preferred for the latter three. Hence, extra attention paid to the management of male animals may make them less prone to tick infestation and may explain the significantly lower prevalence in males vs. females. In addition, stress in females due to milking, pregnancy, and parturition could lead to hormonal disturbances

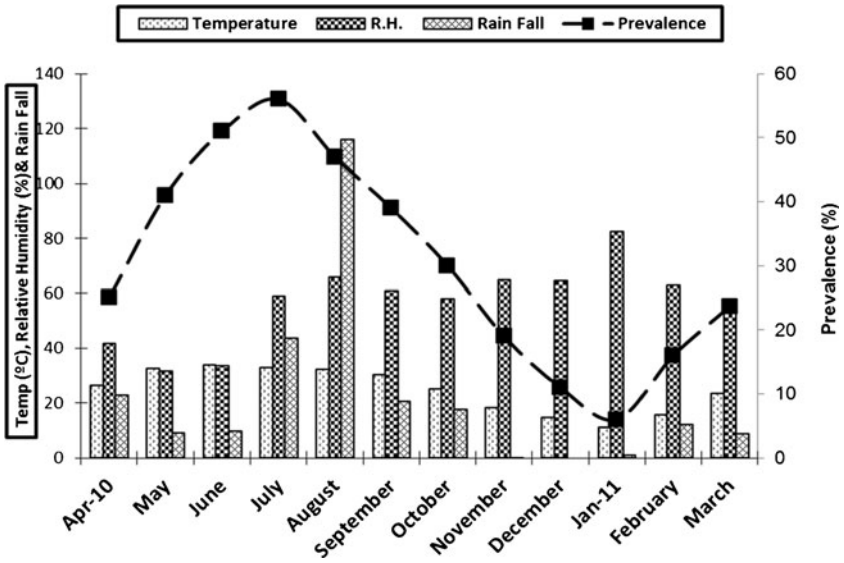


**Fig. 2** Prevalence (in percent) of tick infestation in buffaloes (*Bos bubalus babalis*) with respect to temperature (degree Celsius), relative humidity (in millimeter), and rainfall (in millimeter) of district Toba Tek Singh, Punjab, Pakistan

that weaken immune status, which could contribute to higher tick infestation rates in females (Sajid et al. 2009; Kabir et al. 2011). Breed was not found as an associated risk factor ($P \geq 0.05$) influencing prevalence of ticks which may be due to the equal nutritional and management practices in the study area. Both breeds of buffaloes in this study were local and indigenous to the study area, so it is not surprising that no significant difference in the prevalence of tick infestation was found between them.

The present study found a higher prevalence of tick infestation on field grazing animals than on stall feeding animals. Although the cause of higher prevalence of tick infestation in animals cannot be explained, it can be hypothesized that regular washing of barns and animals and more regular treatment with an acaricide accounts for the difference (Hussain and Kumar 1986; Kabir et al. 2011). Closed-type housing made the animals more prone to tick infestation. Similar findings have been reported by Geden et al. (1990), with higher infestation in closed-type housing than open-type housing. It is hypothesized that less exposure to sunlight favors the retention of humidity in heaps of dung cakes and stacks of bricks in the closed houses, providing more and better breeding places for ticks. In addition, female ticks generally lay eggs in cracks and crevices in the walls of the animal sheds, which provide a favorable environment for the tick growth and development (Muhammad et al. 2008). Moreover, closed farms create a favorable sheltering place for egg laying and hatching of ticks throughout the year (Jouda et al. 2004) and animals kept in this type of housing systems are more prone to tick infestation compared to those housed in an open system.

Higher levels of infestation in rope-tied animals may be due to confinement of animals in specific areas, which reduces movement; it may increase the chances of tick burden over the animals by preventing escape from sources of new parasites.

Variation in the frequency of tick infestation on different body parts as noted here have been previously reported; Kabir et al. (2011) reported that the ears, base of the horn, neck, tail, ventral abdomen, mammary gland, udder, groin, and perianal region were preferred sites of infestation of ticks in buffaloes. Similarly, Yakhchali and Hasanzadehzarza (2004) found that hard tick infestation on the groin and mammary glands was most prevalent in buffaloes. L'Hostis et al. (1994) and Rahbari et al. (2007) reported that attachment sites were the axilla, udder/groin, neck, dewlap, and flank in buffaloes. In our study, animals kept on uncemented flooring were more frequently infested (OR=2.17) than animals housed on partially and fully cemented flooring. Non-cemented floors generate mud around the animals, which fosters tick burden in animals, in accordance with the previous reports (Sajid et al. 2007).

Since ticks are obligatory hematophagous ectoparasites, infestation may result in a serious decrease in hematological parameters such as hemoglobin, packed cell volume, total erythrocyte count (Biswal et al. 1988; Stuti et al. 2008; Raut et al. 2008), neutrophils, and monocytes (Mamak et al. 2006), which might be correlated with blood loss anemia (Soulsby 1982). A post-tick infestation eosinophilia and lymphocytosis was reported by Sanjay et al. (2007), which is understandable due to increased parameters in the serum biochemical profile of tick-infested buffaloes (Soulsby 1982) that might be correlated with increased level of total proteins (Arvind et al. 2010).

## Recommendations

In the light of results from the present study, the following recommendations are advisable for the small holder buffalo

farmers: (a) preventive therapy may be useful to minimize tick infestations before the start of summer, focusing on the most frequently infested parts of body as previously suggested (Sajid et al. 2009); (b) young and female animals should be given special attention as they are more prone to tick infestation; and (c) husbandry practices like stall feeding, confinement via rope-tie and housing on non-cement flooring should be discouraged as they increase susceptibility of tick infestation.

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