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Seasonal dynamics of spinose ear tick *Otobius megnini* associated with horse otoacariasis in Sri Lanka

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ABSTRACT

Otobius megnini is a one host, nidicolous soft tick (Family Argasidae) whose larvae and nymphs parasitize the external ear canal of many wild and domestic animals and occasionally humans. The present study was conducted to determine the seasonal dynamics of *O. megnini* infesting stabled horses in Nuwara Eliya racecourses. Ticks were sampled biweekly for two years from May 2013 to May 2015 from the ear canal of seven thoroughbred male horses. Weather data: temperature, rainfall and humidity for the study period were obtained from the Meteorological Department, Colombo. A total of 23, 287 ticks of *O. megnini* were collected from the ear canal of horses and all of which were immature stages comprising 41.4% larvae and 59.6% nymphs. Larval counts were high during warmer months of the year with minimum numbers recording September–November. High larval abundance may be due to the high hatching rate of eggs at high temperatures. Larval abundance was significantly affected by maximum temperature, minimum relative humidity and average relative humidity (Multiple regression; $r^2 = 0.5$, F = 3.7, $p = 0.01$). Population peaks for nymphs occurred in May, October and January. Nymph counts were low in March and April. Weather conditions did not have any correlation with the abundance of nymphs. Rainfall had no effect on both larvae and nymph counts. Even though control measures have been taken, *O. megnini* infestation is a serious problem for the stable owners especially because these horses are used in racing and other recreational purposes. Further, there is a high risk of spreading this tick within the country due to the presence of suitable hosts and the adaptability of the tick to survive in changing climatic conditions. Information on the seasonal dynamics of the tick population is important for employing control measures.

1. Introduction

Seasonal dynamics of nidicolous ticks (live in or near nests, burrows or shelters of their respective host) vary with the migratory pattern of its host. Nidicolous ticks that parasitize migratory hosts such as birds and bats have a distinct seasonal pattern compared to the ticks that parasitize non-migratory animals (Gray et al., 2013). For example, *Ornithodoros erraticus* and *Ornithodoros tholozani* which parasitize rodents, *Ornithodoros procinus* and *Ornithodoros moubata* which parasitize humans and pigs do not have a predictable seasonal activity (Peirce, 1974; Jori and Bastos, 2009). However, *Argas arboreus* and *Ornithodoros maritimus* which parasitize migratory buff-backed heron and seabirds appeared to have synchronized their life cycle with bird migratory and nesting pattern with pronounced seasonal activity (Sonenshine, 1993; Anderson, 2002; Oorebeek and Kleindorfer, 2008; Gray et al., 2013). Yet, host migratory pattern have no clear effect on the population dynamics of ticks with wider host rage and therefore absence of a particular host will not reduce the tick abundance (Sonenshine, 1993).

Many abiotic factors like relative humidity, temperature, rainfall and photoperiod; biotic factors like host migration, host stimuli act as driving forces of population dynamics, off-host survival, rate of development, questing behaviour and diapause of many hard and soft tick species (Sonenshine, 1993). Due to the constant exposure to changing climatic conditions, non-nidicolous ticks must be adapted to cope with the seasonal fluctuations of environmental extremes in tropical and subtropical regions. However, niche conditions of nidicolous ticks are relatively uniform hence, are less exposed to extreme environmental conditions. Nevertheless, soft ticks are highly resistant to water and temperature stresses (Vial, 2009).

In nidicolous ticks that has distinct seasonal activity, reproductive diapause with respect to temperature and photoperiod has been suggested as the main mechanism which controls the seasonal activity. However, ambient climatic conditions needed for better survival of ticks greatly vary from region to region (Sonenshine,

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1993; Cumming, 2002; Monello and Gompper, 2007). Therefore, there is a high geographic variation in seasonal population dynamics of ticks. In temperate region where distinct seasonal pattern is pronounced the activity of different development stages of ticks are directly linked with the changing environmental condition. However, in the tropics the altering of rainy and dry periods has been identified as main contributing factors of tick seasonal activity (Sonenshine, 1993).

Otobius megnini is a medically and veterinary important soft tick that infest wide range of wild and domesticated animals (e.g. goat, cattle, zebra, deer, swine, pony, mule, horse, donkey, dog, cat) and occasionally humans (Jensen et al., 1982; Burchard et al., 1984; Naudé et al., 2001; Keirans and Pound, 2003). They also can cause paralysis, irritations, toxic conditions, allergies, eardrum perforation, muscle spasms, myotonia, severe otitis and also act as vectors of spotted fever and Q fever (Bustamante and Varela, 1946; Jellison et al., 1948; Estrada-Pena and Jongejan, 1999; Ramos et al., 2014). Studies have shown that sometimes deaths of domestic cattle and horses may occur as a result of heavy infestation of *O. megnini* (Herms, 1917; Rich, 1957; Hoogstraal and Aeschlimann, 1982). *O. megnini* is adapted to survive in diverse ecological niches in tropics, subtropics. From its center of origin Southwestern North America, it has distributed over a vast geographic region including Central and South America, Africa and South Asia (Keirans and Pound, 2003; Estrada-Pena and Jongejan, 1999). Need for horses during the Boer Wars (1980–1981; 1899–1902, between the United Kingdom and the Boers of the South African Republic) have introduced this tick to South Africa. From there, it was introduced to neighboring countries and also to India on cattle or horses (Keirans and Pound, 2003; Estrada-Pena and Jongejan, 1999). It is speculated that *O. megnini* was introduced to Sri Lanka from India *via* horse-trading and was first reported in Sri Lanka by Ramanujachari and Alwar (1955) as a tick with great potential to cause neurological disorders in horses. Much later, Ariyarathne et al. (2010) reported this tick from patients visiting the ENT (Ear, Nose, Throat) clinic at Nuwara Eliya General Hospital as an intra-aural infestation. All these patients were jockeys or worker in Nuwara Eliya racecourses. In Sri Lanka, this tick appears to have a limited distribution and there were no records of it infesting any other domesticated animals other than horses in the racecourses in Nuwara Eliya.

Seasonal dynamics of *O. megnini* is important in controlling populations because studies have shown that sanitary measures and applications of biocides considering the seasonal dynamics have been able to minimize the abundance (Nava and Guglielmone, 2009a,b). Previous studies conducted in Argentina (Guglielmone and Mangold, 1986; Nava and Guglielmone, 2009a,b), South Africa (Theiler and Salisbury, 1958; Dreyer et al., 1998), Texas and USA (Drummond, 1967) have reported discordant results for seasonal activity for *O. megnini* indicating absence of a clear seasonal pattern. Further, climatic factors such as annual rainfall, temperature and altitude appear to have no profound effect on distribution and seasonal dynamics of *O. megnini* populations in Argentina and Union of South Africa (Theiler and Salisbury, 1958; Guglielmone and Mangold, 1986; Guglielmone et al., 1992). This study was conducted to determine the seasonal variation of *O. megnini* on horses over two-year period and to investigate the weather conditions that affect the tick abundance in the stabled horses in Nuwara Eliya racecourse.

2. Materials and methods

2.1. Study site

One of the stables in Nuwara Eliya racecourse (GPS: 6.9628 N, 80.7692 E) was selected as the study site. The racecourse is located in central hills of Sri Lanka approximately 1800 m above sea level. The temperature is ranging from 9.9 °C to 22.2 °C with an average of 15.9 °C. The warmest and coldest months according to mean monthly temperature are April and January, respectively. The annual rainfall ranges from 1900 to 2300 mm (monthly between 70 and 225 mm) with no distinct dry period.

2.2. Study animals

A total of 23 horses (13 males; 6 females; 4 foals) were in the stable of which many are thoroughbred horses (16) and some are Marwari (2) or Stallion (1) and their cross breeds (4). Majority of the horses was imported from India (e.g. Bangalore, Chennai) and few were locally bred ones. All these horses are raised mainly for racing and after the racing season from April to June, the horses are either used for breeding, riding or any other recreational purposes. They are usually fed with cut grass and horse feed, which is given twice a day. The diet during racing season includes boiled chickpea and cowpea in addition to the normal diet. All the body hairs except in the tail and mane are trimmed during the racing period. Regular, individual care is given every morning for grooming and hoof care. Most of the jockeys who maintain the horses do not have any professional experience or training.

Walls of the stable are made of wood and the floor is covered with sawdust. Most of the time, horses are kept inside the stables but during the daytime they may be released to paddock near the stables and were allowed to free graze for few hours depending on the weather conditions.

2.3. Tick collection

Seven thoroughbred male horses were selected for the study. During the initial survey, the horses were ranked depending on the total tick count (larvae and nymphs of *O. megnini*). The individuals with lower tick counts were not considered for the sampling to eliminate the effect of host resistance to ticks. Ticks that infest the ear canals were collected regularly at every 14 days for two years from May, 2013 to May, 2015. Ticks were manually removed from both left and right ear canals using a small piece of white open wove cotton bandage. Collection was done in the morning between 0730 h to 1100 h. Ticks were transferred into small 100 ml plastic vials for subsequent counting and were brought to the Parasitology laboratory in the Department of Zoology, University of Peradeniya and were separated according to their life stage (larvae/nymph).

2.4. Environmental data

Weather data: daily measurements of minimum, maximum and average relative humidity (RH; 3 hourly measurements), minimum, maximum and daily average temperatures and rainfall (RF) were obtained from Meteorological Department, Colombo for the entire study period.

2.5. Data analysis

Mean abundance of ticks per month at each weather variable (average RH, average T and monthly RF) were plotted to determine the relationship between the two variables. Monthly weather variables were calculated by considering the same intervals of collection of ticks. To examine the effect of weather on tick abundance, linear regression was used with tick (larvae/nymph/total) abundance as the dependent variable and the weather variables as the independent variables. Pearson correlation was performed for each weather variable (minimum, maximum and average RH; minimum, maximum and average T and RF) and total tick counts (larvae/nymph/total tick population) to determine a relationship.

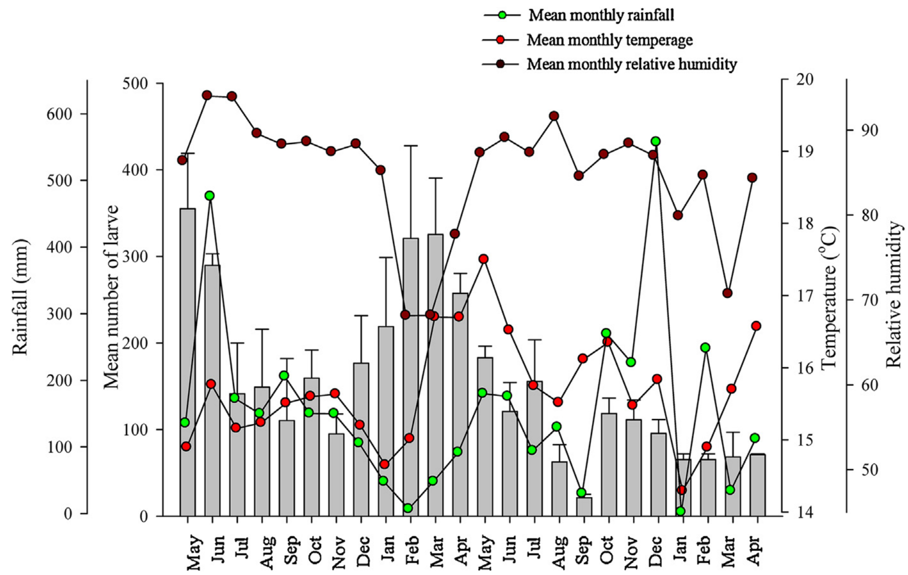

Fig. 1. Abundance of *Otobius megnini* larvae on horses in Nuwara Eliya Racecourse for the two year study period together with the weather conditions.

Table 1
Statistical relationship between the weather conditions and tick counts on horses over a 24-month period.

Weather variable	Pearson correlation coefficient r (p)		
	Larvae	Nymphs	Total
Minimum temperature	−0.1 (0.4)	0.0 (0.9)	0.0 (0.7)
Maximum temperature	0.5 (0.01)[a]	0.1 (0.7)	0.2 (0.1)
Mean temperature	0.0 (0.9)	−0.2 (0.4)	−0.1 (0.7)
Minimum RH	−0.3 (0.01)[a]	0.1 (0.5)	−0.1 (0.6)
Maximum RH	−0.3 (0.03)[a]	0.1 (0.5)	−0.1 (0.5)
Mean RH	−0.3 (0.1)	0.2 (0.5)	−0.1 (0.7)
Monthly rainfall	−0.1 (0.5)	−0.1 (0.7)	−0.1 (0.5)

[a] Statistically significant.

Later, the contribution of several independent variables as predictors of seasonality were investigated by stepwise regression until the best model is reached to explain the factors that influenced the maximum abundance of ticks within the ear canal. The multiple regression analysis was carried out considering all the weather factors together. The data were analyzed using the Minitab® version 16 statistical software.

3. Results

During the study period a total of 23, 287 ticks of *O. megnini* were collected and all of which were immature stages comprising 9, 646 (41.4%) larvae and 13, 641 nymphs (59.6%). Larvae and nymphs were observed only inside the ear canals and nowhere else on the body. All the horses in the racecourse were infested with *O. megnini* except for one lactating mare which was a three quarter breed (one of the grandparents was a pony) and her foal were found with no tick infestation. Of the total ticks, 68.3% were collected during the first year and the rest (31.7%) were collected during the subsequent year.

Seasonal dynamics of larvae with respect to the meteorological data (e.g., average RH, average temperature and monthly RF are shown in Fig. 1). The mean number of larvae per horse varied from 37.1 ± 23.7 (first year) to 17.3 ± 11.6 (second year). Highest abundance of larvae occurred in May–June (24.8% of the total larvae counts for year 1) and February–March (24.7% of the total larvae counts for year 1) during the first sampling year. During the second year, highest abundance of larvae was observed only in May (16.1% of the total larvae counts for year 2). Minimum number of larvae was recorded in November 2013 (3.7% of the total larvae counts for year 1) and September 2014 (1.9% of the total larvae counts for year 2) during the first and the second years of the study, respectively. However, in both sampling years, mid-September had lower larval abundance (~0.3% of the total larvae; according to biweekly observations). Generally, larval abundance declined from May–September and then it increased gradually till March in the following year (Fig. 1). Larval abundance during the first and the second sampling year was approximately similar though it did not fully coincide.

Nymph abundance varied throughout the study (Fig. 2). However, population peaks occurred in May 2013 (13.7% of the total nymph counts for year 1), October 2013 (8.4% of the total nymph counts for year 1) and January 2014 (13.6% of the total nymph counts for year 1) during the first sampling year. During the second sampling year, a seasonal peak was observed only in May 2014 (22.7% of the total nymph counts for year 2). Lower nymph counts were observed in March 2014 for the first sampling year (2.2% of the total nymph counts for year 1). In the second year, minimum infestation was observed in April (1.9% of the total nymph counts for year 2). Nymph counts were remarkably increased during the late-December 2013 where the nymph population increased by 7.1 fold compared to mid-December 2013 population. Similarly, tick population suddenly dropped by 5.4 folds after late-February 2014. Nymph counts periodically oscillated. However, predictable seasonal pattern was not observed.

Fig. 3 indicates the seasonal dynamics of both larvae and nymphs of *O. megnini*. Highest tick abundance occurred in May 2013 (12.4% of the total tick counts for year 1), January 2014 (11.9% of the total tick counts for year 1) and February 2014 (12.3% of the total tick counts for year 1). Minimum tick abundance occurred in November (3.0% of the total tick counts for year 1) during the first sampling year. During the second year, tick population was decreased from May (19.7% of the total tick counts for year 2) to September (3.8% of the total tick counts for year 2). Subsequently, population was increased till November 2014 (8.5% of the total tick counts for year 2) and it was gradually declined to a minimum of 3.0% of the total tick counts for year 2, in April 2015.

Abundance of larvae and nymphs were different with weather data where larval counts were high when the temperature was high and the relative humidity was low (Fig. 1) and the nymph counts

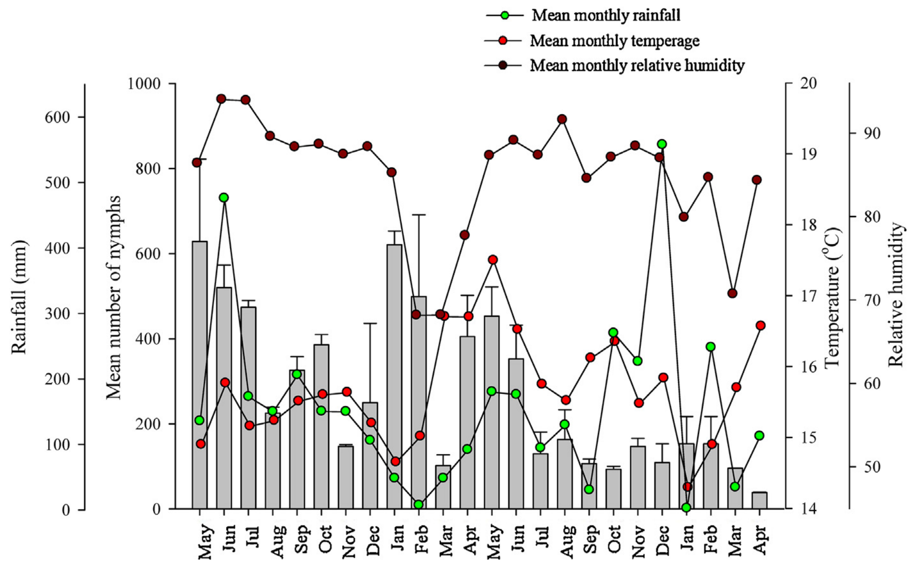

Fig. 2. Abundance of *Otobius megnini* nymphs on horses in Nuwara Eliya Racecourse for the two year study period together with the weather conditions.

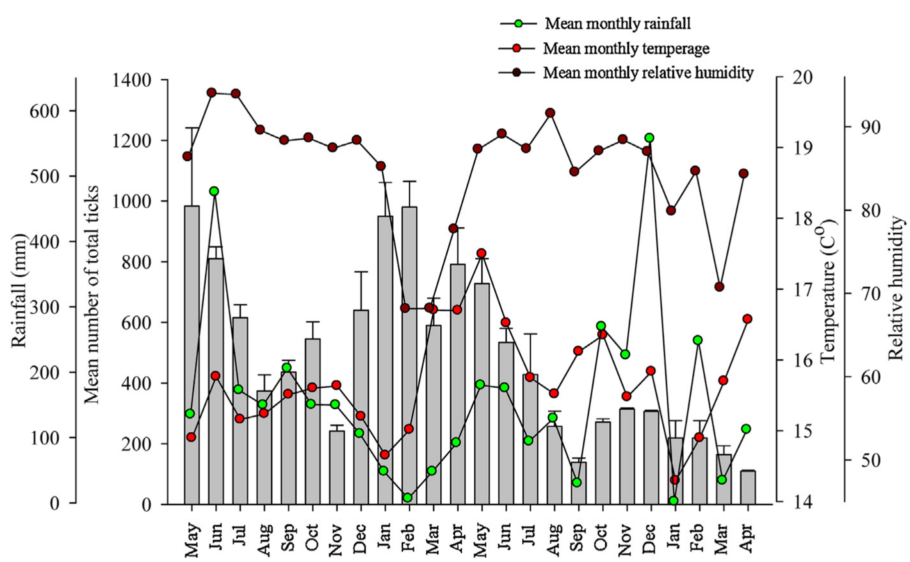

Fig. 3. Abundance of total population of *Otobius megnini* on horses in Nuwara Eliya Racecourse for the two year study period together with the weather conditions.

were high when the temperature and relative humidity were high (Fig. 2). However, a statistically significant positive correlation was shown only with larval abundance and maximum temperature. Similarly, larval abundance increased significantly with the decrease of minimum and maximum RH (Table 1). Nevertheless, there was no significant association between the tick counts and the mean monthly temperatures and RH. Rainfall had no effect on both larvae and nymph counts but the tick counts went down with the starting of rainy season when there is high relative humidity (RH) and low temperature (Fig. 1 and 3 ; Table 1). Further, high tick abundance was observed in dryer and warmer months. Larval abundance was significantly affected only by monthly maximum temperature (Multiple regression; t = 3.3, $p = 0.002$) and monthly minimum relative humidity (Multiple regression; t = 3.2, $p = 0.005$) and monthly average relative humidity (Multiple regression; t = 3.3, $p = 0.004$) (Regression fit; $r^2 = 0.5$, F = 4.3, $p = 0.01$; Monthly counts of larvae = – 863 + 46.5 maximum T + 22.4 minimum RH – 20.1 Average RH). Nevertheless, nymphs were not affected by any weather variables significantly. Total tick population significantly depended on maximum temperature (Multiple regression; t = 2.6, $p = 0.02$), minimum relative humidity (Multiple regression; t = 2.7, $p = 0.01$) and maximum relative humidity (Multiple regression; t = 2.21, $p = 0.02$) (Regression fit; $r^2 = 0.4$, F = 2.8, $p = 0.04$; Monthly counts of total ticks = – 2226 + 141 maximum T – 50.2 maximum RH + 55.1 minimum RH).

Maximum abundance of tick within the ear canal of horses was reached when the maximum temperature was above 19 °C and the relative humidity below 85. The larval counts were high at 20 °C–22 °C when there was 66–80 relative humidity. However, the total number of nymphs was independent of both temperature and saturation deficit.

4. Discussion

Current study was conducted in one of the racecourse stables in Nuwara Eliya, located in high-altitude with high relative humidity, low temperature and high rainfall. Under these weather conditions *O. megnini* exhibited high population stability with the potential of infesting horses throughout the year. The abundance of ticks differed in two sampling years. Yet, the larvae showed a clear pattern with weather variables where larval counts were high during high temperature and low relative humidity but nymph had no predictable seasonal pattern over the study period. Abundance of larvae declined from May to September and nymphs from May to December during the study period. This could be because at high temperature, hatching rate of the eggs is higher which was observed under laboratory conditions (Diyes and Rajakaruna, 2014) resulting more larvae. Further, it was observed that wet and warm conditions seemingly regulate the seasonal activity of Sri Lankan *O. megnini* population. Similarly, Guglielmone and Mangold (1986) and Theiler and Salisbury (1958) reported low population of *O. megnini* in areas with high annual rainfall and high population of *O. megnini* in warmer months (Dreyer et al., 1998).

Seasonality of *O. megnini* varies in different populations. A recent study conducted in Cordoba, Argentina (Southern hemisphere; humid subtropical hot summer climate with dry winters and moderate seasonality) has reported high larval activity in April-May (autumn; first sampling year) and August-October (spring; second sampling year) and high nymphal activity during January-April (mid-Summer–mid-autumn; first sampling year) and July–August (winter; second sampling year) (Nava and Guglielmone, 2009a,b). Nava and Guglielmone (2009a,b), Guglielmone and Mangold (1986) have shown seasonal activity of *O. megnini* in Argentina, where larvae and nymphs present in high abundance during spring (September–November) and autumn (March–May), respectively. Further, Medrano and Suárez, (1983) reported highest population of *O. megnini* in La Pampa, Argentina during the autumn. Glass et al. (2013) determined the general seasonal activity of *O. megnini* at Fossil Rim Wildlife Centre, Texas (Northern hemisphere; hot, humid summers and generally mild–cool winters) where highest seasonal abundance of larvae was found in May 2012 (spring) and November 2012 (autumn) in animal shelters than open areas (e.g., woodland, hay, grassland) but no seasonal trends have been observed (Glass et al., 2013). A different study conducted in South-Western Texas reported high seasonal activity of ticks during late summer and early fall with no predictable seasonal activity. Theiler and Salisbury (1958) and Dreyer et al. (1998) have found no seasonal trends for *O. megnini* in Union of South Africa (Southern hemisphere). Most of the above-mentioned studies have reported lack of pronounced seasonality nether have they reported adequate information on weather conditions and therefore the factors affecting the seasonal activity of *O. megnini* are largely unknown.

Nymphs live inside the ear canal of horses where the niche conditions are more uniform. The weather variables may not have a pronounced impact on parasitic nymphs and hence exhibit a weak association with macroclimatic conditions. Even though engorged larvae are live inside the ear canals, unfed larvae are continuously being exposed to variable environmental conditions owing to their host seeking behaviour and prolong survival (Diyes and Rajakaruna, 2014). Previous studies on seasonal dynamics of ticks have shown that ticks are highly associated with environmental conditions during their off host development which requires high relative humidity (>85%) and ambient temperatures (Needham and Teel 1991; Sonenshine, 1993). If the abundance of free-living larvae were measured together with parasitic stages, the data would elaborate a better model to predict the effect of weather conditions on *O. megnini*.

In some months, there was a considerable reduction of nymphs and larvae. This suggest the occurrence of development or behavioural (absence of questing behaviour) diapause (Sonenshine, 1993) as it is observed for nymphs of *O. megnini* (Diyes and Rajakaruna; unpublished data). Lower abundance of ticks during the second sampling year might be as a result of diapause, due to the development of resistance in horses or otherwise it is simply due to the constant removal of ticks during sampling. However, there are other horses in the same stable and other stables nearby with heavy infestation which were not sampled. Hence, constant removal may not have a significant effect on the overall population density of *O. megnini*.

Tick infestation appeared to be influenced by body hair of the horse and the breed. The infestation is severe in pure bred, well groomed horses but not in the mixed bred horses (Eg. three quarter breed horses, ponies) as well as in those with hairs in and around the ears. These horses were not considered for sampling. Hair prevents the entering of the immature stages to some extent. Therefore, if the horses were not shaved during racing off-season (July–March) tick infestation could be controlled substantially. As it might limit the entry of the ticks into the ears, cleaning of ears and manually removing the ticks on daily basis can further reduce the infestation. Further, the information on seasonal dynamics of *O. megnini* in the present study can be used for developing a strategic and cost effective tick control method.

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