

SHORT COMMUNICATION

Molecular evidence of *Babesia* infections in Spinose ear tick, *Otobius megnini* infesting stabled horses in Nuwara Eliya racecourse: A case study

G.C.P. Diyes^{1,2}, R.P.V.J. Rajapakse³ and R.S. Rajakaruna^{1,2,*}

¹Department of Zoology, Faculty of Science, University of Peradeniya, Peradeniya 20400, Sri Lanka

²The Postgraduate Institute of Science, University of Peradeniya, Peradeniya 20400, Sri Lanka

³Department of Veterinary Pathobiology, Faculty of Veterinary Medicine & Animal Science, University of Peradeniya, Peradeniya 20400, Sri Lanka

Received:26/04/2018; Accepted:02/08/2018

Abstract: Spinose ear tick, *Otobius megnini* (Family Argasidae) is a one-host soft tick that parasitizes domesticated animals and occasionally humans. It causes otoacariasis or parasitic otitis in humans and animals and also known to carry infectious agents. Intra aural infestations of *O. megnini* is a serious health problem in the well-groomed race horses in Nuwara Eliya. *Otobius megnini* collected from the ear canal of stabled horses in Nuwara Eliya racecourse were tested for three possible infections, *Rickettsia*, *Theileria* and *Babesia*. Genomic DNA was extracted from 22 ticks collected from 11 horses and then pathogen-specific DNA was amplified using PCR. Ticks from two horses (one thoroughbred and one stallion) tested PCR positive for *Babesia* but not for other infections. None of the horses however, showed any clinical symptoms of babesiosis. This is the first record of *Babesia* infections in *O. megnini*. However, the presence of *Babesia* DNA in *O. megnini* doesn't qualify it as the vector of equine babesiosis, horse blood has to be tested for the presence of parasite DNA or antibodies.

Keywords: Spinose ear tick, Babesiosis, Horses, Otoacariasis, *Rickettsia*, *Theileria*.

INTRODUCTION

The spinose ear tick, *Otobius megnini* (Dugès 1883) (Acari: Ixodida: Argasidae) is an economically important soft tick, parasitizes livestock mostly cattle, goats, sheep, and horses and infests humans as well (Naudé *et al.*, 2001; Keirans and Pound, 2003; Ariyarante *et al.*, 2016). It is a one-host tick from the New World with a wide geographical distribution and its original centre of distribution is considered to be the southwestern North America, from where it spread to Central and South America and then to the rest of the world (Estrada-Pena *et al.*, 1999; Keirans and Pound, 2003). Since the larva and the nymph of this tick feed inside the ear canal of the host for a long period, it allows the tick to be distributed over a vast geographic region transcontinentally through the distribution of the host animals. *Otobius megnini* is thought to have reached India in mid-1930s together with cattle or horses brought from Southern Africa. This tick species is recorded in race horses brought from farms in northern India for an auction at the Madras

Race Club (Joseph, 1982). There is a speculation that *O. megnini* was introduced to Sri Lanka from India via horse trading. The first report of *O. megnini* in Sri Lanka is in 2010 from stable workers and jockeys as an intra-aural infestation (Ariyaratne *et al.*, 2010). In Sri Lanka, *O. megnini* appears to have a limited distribution with no records of it infesting any other domesticated animals other than horses in the racecourses (Diyes and Rajakaruna, 2016).

Otobius megnini is the causative agent of horse otoacariasis or parasitic otitis. This condition can cause serious injuries and occasionally death in horses (Wall and Shearer, 2008). The common clinical signs include abnormal head carriage, head shaking and head rubbing (Perris, 1995). Early studies report nervous disease (Ramanujachari and Alwar, 1955) and auricular nerve paralysis (FAO, 1958) due to the presence of *O. megnini* in the ear canal of horses. However, there is no conclusive evidence to support the classification of *O. megnini* as a paralysis tick. The fact that this tick feeds within the ears of its hosts where inflammatory reactions could affect the balance of the host and lead to symptoms that could be interpreted as being neurological in origin should be considered. Intermittent painful muscle cramps were described in horses that were severely infested with *O. megnini* (Madigan *et al.*, 1995). Between muscle cramps, horses appear to be normal and once the ticks are removed clinical signs are reduced and recovered within 12 to 36 hrs (Madigan *et al.*, 1995). Recently, from Northern Mexico a two-year-old quarter breed was reported having myotonia and colic associated with the infestation of *O. megnini* (Zarate-Ramos *et al.*, 2014).

Otobius megnini is known to carry some infectious agents. *Coxiella burnetii*, the causative agent of Q fever was recovered from *O. megnini* collected from dairy cattle in Southern California (Jellison *et al.*, 1948). Nymphs of *O. megnini* were reported infecting two patients with Spotted fever group rickettsioses from Mexico (Bustamante *et al.*, 1946). Studies have shown that *O. megnini* can be naturally infected with *Ehrlichia canis* but does not transmit the

*Corresponding Author's Email: rupikar@pdn.ac.lk

 <http://orcid.org/0000-0001-7939-947X>



agent (Ewing *et al.*, 1990). A study carried out in South Africa reported *Borrelia burgdorferi* seropositive cases of three horse riders and owner of a stable, 71 horses and five dogs but the *O. megnini* specimens collected from these hosts were negative for the infection (Fivaz *et al.*, 1991).

Equine piroplasmiasis and rickettsial infections are common among horses but there are no records of these infections in horses in Sri Lanka. However, human pathogenic *Orientia tsutsugamushi*, *Rickettsia typhi* and *Rickettsia conorii* (Kularatne *et al.*, 2003; Kularatne *et al.*, 2013), *Theileria orientalis*, *Theileria annulata* (among water buffaloes) (Sivakumar *et al.*, 2012), *Babesia bovis*, *Babesia bigemina* (among cattle), *Babesia gibsoni* and *Babesia canis* (among dogs) have been reported (Sivakumar *et al.*, 2012) in Sri Lanka. However, little is known about their prevalence, distribution and the vector-tick species. Hence, the present study examined the presence of three common tick-borne pathogens: *Rickettsia*, *Theileria* and *Babesia* in *O. megnini* infesting stabled horses in Nuwara Eliya.

MATERIALS AND METHODS

Ticks were collected from the ear canals of 11 thoroughbred horses in Nuwara Eliya racecourses during 2014 using small pieces of white open wove cotton bandages (new, cleaned bandage for every removal). The ticks together with earwax were placed in 100 ml plastic vials and brought to the laboratory. From each horse, two fully engorged nymphs were cleaned and preserved in absolute ethanol and were kept at -20 °C. A total of 22 samples were used to analyze from 11 horses for the presence of *Rickettsia*, *Theileria* and *Babesia* infections in the tick.

Molecular analysis

Each nymph was washed in a series of ethanol (70%, 50%, 30%, 10%) keeping 1 hour in each solution and were finally transferred into distilled water for 1 hour. Genomic DNA was extracted using QIAGEN DNeasy Blood and Tissue kit (Cat no: 69504, German) according to manufacturer's instructions. Eluted DNA was kept at -20 °C until the PCR amplification. The PCR reactions for the detection of pathogenic DNA from ticks were performed with 5 µl of extracted DNA, 1.5 µl of each primer (10 mM), 5 µl of 5x PCR buffer, 3 µl of MgCl₂ (25 mM), 2 µl of dNTPs (2 mM), 0.25 µl Taq DNA polymerase and 6.75 µl DEPC water in a final reaction volume of 25 µl. After amplification, the PCR products were visualized by gel electrophoresis (1.5% W/V) with a DNA molecular weight marker (GelPilot 100 bp Plus DNA Ladder, product no. 239045, QIAGEN).

Detection of *Rickettsia*

DNA extracts were screened for 190 kDa protein antigen (*ompA*) gene using a nested PCR assay. Outer primers used for the PCR assays were Rr 190-70 with gene sequence of, 5'-ATGGCGAATATTTCTCCAAAA-3' and Rr 190-701 with gene sequence of 5'-GTTCCGTTAATGGCAGCATCT-3' to amplify a 631bp segment. For the nested stage, Rr 190.70 (5'-ATGGCGAATATTTCTCCAAAA-3') and Rr 190.602 (5'-AGTGCAGCATTCGCTCCCCCT-3') inner

primers were used to amplify a 530-bp fragment of the Rickettsial *ompA* gene (Fournier *et al.*, 1998; Santibáñez *et al.*, 2013). A nested PCR was performed in 25 µl volumes as stated above with the primers and 2 µl of the first PCR product as the DNA template. The reaction mixtures were subjected to an initial denaturation step at 95 °C for 5 min, followed by 42 repeated cycles at 95 °C for 40 seconds (denaturation), 57 °C for 40 seconds (annealing), 68 °C for 40 seconds (extension). Amplification was completed by a further 10 min step at 72°C (final extension). Same thermocycler parameters were used for the nested PCR with 30 cycles.

Detection of *Babesia*

Detection of *Babesia* isolates in DNA extracts were performed by amplifying a 284bp fragment of 18S rRNA gene by using Bab 18S forward primer 5'-TAG(AG)GATTGGAGGTCGTCA-3' and Bab 18S reverse primer 5'-AACGGAATTAACCAGACAAA-3' (Hildebrandt *et al.*, 2007). Thermal profile used for the amplification was 5 min initial denaturation at 94 °C, followed by repeated 30 cycles of 30 seconds denaturation at 94 °C, 30 seconds annealing at 50 °C, 30 seconds extension at 72 °C. The final extension was 2 min at 72 °C.

Detection of *Theileria*

Theileria genus-specific, forward primer 989F (5'-AGTTTCTGACCTATCAG-3') and reverse primer 990R (5'-TTGCCTTAACTTCCTTG-3') were used to amplify a 1098-bp fragment of SSU rRNA gene (Durrani and Kamal, 2008). Amplification was done under the following thermocycler conditions: 95 °C for 5 min (initial denaturation), followed by 30 cycles of denaturation, annealing and extension at 94 °C for 30 seconds; 55 °C for 30 seconds, 72 °C for 45 seconds respectively. The final extension was at 72 °C for 7 min.

RESULTS AND DISCUSSION

Analysis of 22 samples of *O. megnini* from 11 race horses for the presence of *Rickettsia*, *Babesia* and *Theileria* infections revealed that only two ticks (18.2%) collected from one thoroughbred and one stallion were PCR positive for *Babesia* infections. This study provides the first evidence of presence of *Babesia* in *O. megnini*. Although the DNA isolated from the tick was sent for sequencing to confirm the *Babesia* species, the results were not satisfactory. It is important to examine the horse blood for either parasite DNA or antibodies to confirm whether *O. megnini* acts as a vector for equine babesiosis. None of the horses however showed any symptoms of babesiosis. Recently, microscopic examination of blood samples from some of the horses in this stable has shown no positive results for *Babesia* or any other infection of blood parasites (Dissanayake *et al.*, 2017). It could be possible that microscopy may have not picked the low parasitemia levels.

It is not clear the way in which *O. megnini* became infected with the pathogen. Many of the race horses in Nuwara Eliya stables including the two horses with *Babesia* infected ticks were imported from India. Therefore, it can be suspected that infected ticks may have been introduced to Sri Lanka

via horse trading. It is important that the horses undergo a proper and thorough quarantine process. Once the larvae attach to the horse ear canal, they feed, moult and come out as a fully engorged nymph. Adults are non-feeding, free living stage. Since, *O. megnini* is a one host tick and the adult females do not feed, spreading of infectious diseases from one host to another is limited unless the infectious agent shows both transovarial and transstadial transmission like *Rickettsia bellii* maintained in *Ixodes loricatus* (Horta et al., 2006).

Equine babesiosis (Equine piroplasmiasis) is established in tropical, subtropical regions of the world and it is an important intra-erythrocytic protozoan that causes great economic loss to equine industry (De Waal, 1992). In Asia, equine babesiosis is frequently reported from India, China, Korea and Iran (Gautam and Dwivedi, 1976; Wise et al., 2013). So far, *Babesia caballi* and *B. equi* (some reported as *Theileria equi* but remains controversial) are frequently reported among horses and other equid species including donkeys, mules and zebras (Friedhoff and Soule, 1996). The information on soft ticks as vectors of these two *Babesia* species is scant. Recently, under laboratory conditions, *Ornithodoros moubata* has been identified as a suitable candidate to transmit *B. equi* (Battsetseg et al., 2007).

There was no indication of the presence of *Rickettsia* and *Theilaria* infections in *O. megnini* infesting the racehorses in Nuwara Eliya. The information on the distribution of *Rickettsia* spp. associating horses is limited. Besides, the relationship between soft ticks and *Rickettsia* spp. is poorly documented except for *Carios capensis*, *Carios sawaii* and *Ornithodoros erraticus* (Kawabata et al., 2006; Raoult and Parola, 2007). Although spotted fever group rickettsial infections have been recorded in hard ticks collected from some wild and domesticated animals in Sri Lanka, the list doesn't include horses (Liyanarachchi et al., 2015). *Coxiella burnetii* has been reported in some areas of Sri Lanka and has the capability of causing abortion, stillbirth and neonatal deaths in horses (Angelakis et al., 2012).

In Sri Lanka, *O. megnini* is reported only from horses in Nuwara Eliya racecourse. Dynamic nature of the life cycle and high population stability has enabled *O. megnini* to become an invasive tick in Nuwara Eliya racecourses (Diyes and Rajakaruna, 2016; 2017). Different strategies have been implemented to control the infestations of *O. megnini* in the stabled horses but none of them was successful. Recently, the use of flumethrin based acaricides (Diyes et al., 2016), parasitoid scuttle fly, *Megaselia scalaris* (Diyes et al., 2015) and predator ant species *Tapinoma melanocephalum* (Diyes et al., 2017) have shown to be effective in controlling the free-living stages of *O. megnini*. In Sri Lanka *O. megnini* is reported only from Nuwara Eliya racecourse. Though *Babesia* was found in *O. megnini*, it does not confirm its vector capacity. Presence of pathogenic nucleic acid in a tick only indicates the carrier status of the tick but not the interspecies transmission (Estrada-Peña and de la Fuente, 2014). Therefore, further studies are required to elucidate the vector capacity of *O. megnini* for equine babesiosis and to confirm the species status. This tick has a high affinity

to infest cattle posing a potential risk of spreading the infestation and associated tick-borne diseases into the nearby dairy farms.

ACKNOWLEDGEMENT

Authors thank the management of the racecourse in Nuwara Eliya, Kaushalya Karunathilake in the Department of Veterinary Pathobiology, Faculty of Veterinary Medicine & Animal Science, University of Peradeniya, Rasika Alawatte, T.D.B. Lenadora, Punsiri Tennakoon and the National Research Council (Grant 11-44) for financial support.

REFERENCES

- Angelakis, E., Munasinghe, A., Yaddhige, I., Liyanapathirana, V., Thevanesam, V., Bregliano, A., Socolovschi, C., Edouard, S., Fournier, P.E., Raoult, D. and Parola, P. (2012). Detection of rickettsioses and Q fever in Sri Lanka. *The American Journal of Tropical Medicine and Hygiene* **86**(4):711-712.
- Ariyaratne, S., Apanaskevich, D.A., Amarasinghe, P.H. and Rajakaruna, R.S. (2016). Diversity and distribution of tick species (Acari: Ixodidae) associated with human otoacariasis and socio-ecological risk factors of tick infestations in Sri Lanka. *Experimental and Applied Acarology* **70**(1):99-123.
- Ariyaratne, S., Rajakaruna, R.S., Ekanayake, D.H., Dilrukshi, P.R.M.P., Balasooriya, P.S. and Amarasinghe, P.H. (2010). Occurrence and distribution of tick species associated with human otoacariasis in selected districts of Sri Lanka. *Proceedings of the 15th Peradeniya University Research Sessions (PURSE) Kandy, Sri Lanka*.
- Battsetseg, B., Matsuo, T., Xuan, X., Boldbaatar, D., Chee, S.H., Umemiya, R., Sakaguchi, T., Hata, T., Zhou, J., Verdida, A.R., Taylor, D. and Fujisaki, K. (2007). *Babesia* parasites develop and are transmitted by the non-vector soft tick *Ornithodoros moubata* (Acari: Argasidae). *Parasitology* **134**(1):1-8.
- Bustamante, M.E., Varela, G. and Ortiz Mariotte, C. (1946). Estudios de fiebre manchada en Mexico. *Fiebre manchada en la laguna. Revista del Instituto de Salubridad y Enfermedades Tropicales* **7**:39-49.
- De Waal, D.T. (1992). Equine piroplasmiasis: a review. *British Veterinary Journal* **148**(1):6-14.
- Desjardins, I., Joulié, A., Pradier, S., Lecollinet, S., Beck, C., Vial, L., Dufour, P., Gasqui, P., Legrand, L., Edouard, S. and Sidi-Boumedine, K. (2018). Seroprevalence of horses to *Coxiella burnetii* in an Q fever endemic area. *Veterinary microbiology* **215**:49-56.
- Dissanayake, S., Rajapakse, R.P.V.J. and Rajakaruna, R.S. (2017). Gastrointestinal parasites of domesticated and feral horses (*Equus caballus*) in Sri Lanka. *Ceylon Journal of Science* **46**:17-26.
- Diyes, G.C.P. and Rajakaruna, R.S. (2016). Seasonal dynamics of spinose ear tick *Otobius megnini* associated with horse otoacariasis in Sri Lanka. *Acta Tropica* **159**: 170-175.

- Diyes, G.C.P. and Rajakaruna, R.S. (2017). Life cycle of Spinose ear tick, *Otobius megnini* (Acari: Argasidae) infesting the race horses in Nuwara Eliya, Sri Lanka. *Acta Tropica* **166**:164-176.
- Diyes, G.C.P., Karunarathna, N.B., Silva, T.H.S.E., Karunaratne W.A.I.P. and Rajakaruna R.S. (2017). Ants as predators of the Spinose Ear Tick, *Otobius megnini* (Dugès) in Sri Lanka *Acarologia* **57**(4):747-753.
- Diyes, G.C.P., Bandara, K.M.U.J., Rajakaruna, R.S. and Karunaratne, S.H.P.P. (2016). Assessment of susceptibility of the Spinose ear tick *Otobius megnini* (Acari: Argasidae) to selected acaricides. *Proceedings of Peradeniya University Research Sessions (iPURSE), Sri Lanka* **20**: Pp.300.
- Diyes, G.C.P., Karunaratne, W.A.I.P., Tomberlin, J.K. and Rajakaruna, R.S. (2015). Case Study First record of *Megaselia scalaris* (Loew) (Diptera: Phoridae) infesting a spinose ear tick, *Otobius megnini*, colony in Sri Lanka. *Tropical Biomedicine* **32**(4):791-795.
- Durrani, A.Z. and Kamal, N. (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. *Tropical animal health and production* **40**(6): 441-447.
- Estrada-Peña, A. and de la Fuente, J. (2014). The ecology of ticks and epidemiology of tick-borne viral diseases. *Antiviral research* **108**:104-128.
- Estrada-Peña, A. and Jongejan, F. (1999). Ticks feeding on humans: a review of records on human-biting Ixodidae with special reference to pathogen transmission. *Experimental and applied acarology* **23**(9):685-715.
- Ewing, S.A., Harkness, J.R., Kocan, K.M., Barker, R. W., Fox, J.C., Tyler, R.D., Cowell, R.L. and Morton, R.B. (1990). Failure to transmit *Ehrlichia canis* (Rickettsiales: Ehrlichieae) with *Otobius megnini* (Acari: Argasidae). *Journal of Medical Entomology* **27**: 803-806.
- Fivaz, B.H., Botha, P. and Cairns, L.M. (1991). A putative outbreak of equine Lyme borreliosis in Natal. *Journal of the South African Veterinary Association* **61**:128-129.
- Food and Agriculture Organization (1958). First meeting of the joint FAO/OIE expert panel on tick borne diseases of livestock, London, England: 24-29.
- Fournier, P.E., Dubourg, G., Raoult, D. (2014). Clinical detection and characterization of bacterial pathogens in the genomics era. *Genome Medicine* **6**: 114.
- Fournier, P.E., Roux, V. and Raoult, D. (1998). Phylogenetic analysis of spotted fever group rickettsiae by study of the outer surface protein rOmpA. *International Journal of Systematic Bacteriology* **48**:839-849.
- Friedhoff, K.T. and Soule, C. (1996). An account on equine babesiosis. *Revue scientifique technique (International Office of Epizootics)* **15**(3):1191.
- Gautam, O.P. and Dwivedi, S.K. (1976). *Equine babesiosis: A severe outbreak in a stud farm at Hisar. Indian Veterinary Journal* **53**:546-551.
- Hildebrandt, A., Hunfeld, K.P., Baier, M., Krumbholz, A., Sachse, S., Lorenzen, T., Kiehltopf, M., Fricke, H.J. and Straube, E. (2007). First confirmed autochthonous case of human *Babesia microti* infection in Europe. *European Journal of Clinical Microbiology and Infectious Diseases* **26**(8):595-601.
- Jellison, W.L., Bell, E.J., Huebner, R.J., Parker, R.R. and Welsh, H.H. (1948). Q fever studies in Southern California. IV. Occurrence of *Coxiella burnetii* in the spinose ear tick, *Otobius megnini*. *Public Health Reports* **63**:1483-1489.
- Joseph, S.A. (1982). Studies on the ecology, zoophilic and anthropophilic habits of the argasid ticks of Tamil Nadu. *Cheiron* **11**:266-269.
- Kawabata, H., Ando, S., Kishimoto, T., Kurane, I., Takano, A., Nogami, S., Fujita, H., Tsurumi, M., Nakamura, N., Sato, F., Takahashi, M., Ushijima, Y., Fukunaga, M. and Watanabe, H. (2006). First Detection of *Rickettsia* in Soft-bodied Ticks Associated with Seabirds, Japan. *Microbiology and Immunology* **50**(5):403-406.
- Keirans, J.E. and Pound, J.W. (2003). An annotated bibliography of the spinose ear tick, *Otobius megnini* (Dugès, 1883) (Acari: Ixodida: Argasidae) 1883-2000. *Systematic and Applied Acarology Special Publication* **13**:1-68.
- Kularatne, S.A.M., Edirisingha, J.S., Gawarammana, I.B., Urakami, H., Chenchittikul, M. and Kaiho, I. (2003). Emerging rickettsial infections in Sri Lanka: the pattern in the hilly Central Province. *Tropical Medicine and International Health* **8**(9):803-811.
- Kularatne, S.A.M., Rajapakse, R.P.V.J., Wickramasinghe, W.M.R.S., Nanayakkara, D.M., Budagoda, S.S., Weerakoon, K.G.A.D., Edirisinghe, J.S. and Premaratna, R. (2013). Rickettsioses in the central hills of Sri Lanka: serological evidence of increasing burden of spotted fever group. *International Journal of Infectious Diseases* **17**(11):988-992.
- Liyanaarachchi, D.R., Rajakaruna, R.S. and Rajapakse, R.P.V.J. (2015). Spotted fever group rickettsia in ticks infesting humans, wild and domesticated animals of Sri Lanka: one health approach. *Ceylon Journal of Science (Biological Sciences)* **44**(2):67-74
- Madigan, J.E., Valberg, S.J., Ragle, C. and Moody J.L. (1995). Muscle spasms associated with ear tick (*Otobius megnini*) infestations in five horses. *Journal of the American Veterinary Medical Association* **207**: 74-76.
- Naudé, T.W., Heyne, H., van der Merwe, I.R. and Benic, M.J. (2001). Spinose ear tick, *Otobius megnini* (Dugès, 1884) as the cause of an incident of painful otitis externa in humans. *Journal of the South African Veterinary Association* **72**:118-119.
- Perris, E.E. (1995). Parasitic dermatoses that cause pruritus in horses. *Veterinary Clinics of North America Equine Practice* **11**:11-28.
- Ramanujachari, G. and Alwar, V.S. (1955). Notes of parasitological interest. *Ceylon Veterinary Journal* **3**: 38-40.
- Raoult, D. and Parola, P. (2007). *Rickettsial diseases*. New York: Informa Healthcare USA.
- Santibáñez, S., Portillo, A., Santibáñez, P., Palomar, A.M. and Oteo, J.A. (2013). Usefulness of rickettsial PCR assays for the molecular diagnosis of human rickettsioses. *Enfermedades Infecciosas y Microbiología Clínica* **31**(5):283-288.

- Sivakumar, T., Kothalawala, H., Abeyratne, S.A., Vimalakumar, S.C., Meewewa, A.S., Hadirampela, D.T., Puvirajan, T., Sukumar, S., Kuleswarakumar, K., Chandrasiri, A.D., Igarashi, I. and Yokoyama, N. (2012). A PCR-based survey of selected *Babesia* and *Theileria* parasites in cattle in Sri Lanka. *Veterinary parasitology* **190**(1):263-267.
- Tissot-Dupont H, Amadei, M.A., Nezri, M. and Raoult, D. (2004). Wind in November, Q fever in December. *Emerging Infectious Diseases* **10**(7):1264-1269.
- Wall, R.L. and Shearer, D. (2008). *Veterinary ectoparasites: biology, pathology and control*. John Wiley and Sons.
- Wise, L.N., Kappmeyer, L.S., Mealey, R.H. and Knowles, D.P. (2013). Review of equine piroplasmiasis. *Journal of Veterinary Internal Medicine* **27**(6):1334-1346.
- Zarate-Ramos, J.J., Garza, A.M.N., Avila, D.E.Z. and Tovar, L.E.R. (2014). Myotonia and Colic Associated with the Spinose Ear Tick, *Otobius megnini*, in a Horse in Northern Mexico *Journal of Parasitology Research* **9**:16-20.
-