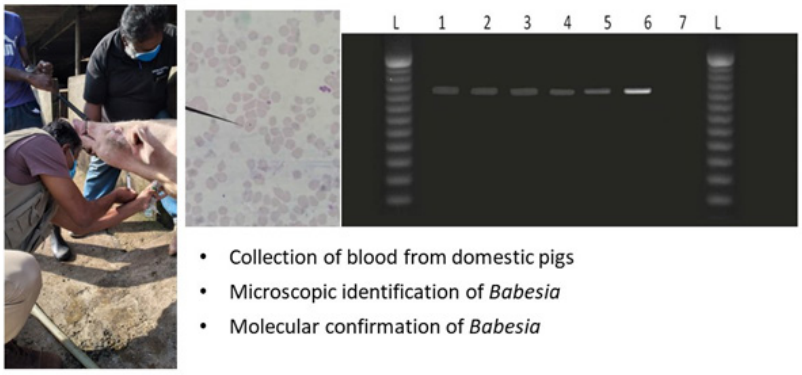
SHORT COMMUNICATION

First record of porcine babesiosis in Sri Lanka

R.A.S. Ranatunga, A. Dangolla, A.P Bodahewa, K.H.A.T.D. Peiris, R.M.B.K., Rajakaruna, R.M.A.N. Senevirathne and R.S. Rajakaruna*



Highlights

- This study provides the first record of porcine babesiosis in Sri Lanka
- 23% of the pigs in the farm were infected with *Babesia* sp.
- Microscopy results revealed the likely presence of *B. perroncitoi*
- DNA analysis confirmed the infection with *Babesia* sp.
- All infected pigs were asymptomatic

Ceylon Journal of Science 52 (1) 2023: 107-110
DOI: http://doi.org/10.4038/cjs.v52i1.8110

SHORT COMMUNICATION

First record of porcine babesiosis in Sri Lanka

R.A.S. Ranatunga[a], A. Dangolla[b], A.P Bodahewa[c], K.H.A.T.D. Peiris[b], R.M.B.K., Rajakaruna[b], R.M.A.N. Senevirathne[b] and R.S. Rajakaruna[d*]

[a]Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya, Peradeniya, 20400, Sri Lanka
[b]Department of Veterinary Clinical Science, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya, 20400, Sri Lanka
[c]Government Veterinary Surgeon, Teldeniya, 20900, Sri Lanka
[d]Department of Zoology, Faculty of Science, University of Peradeniya, Peradeniya, 20400, Sri Lanka

Received: 15.08.2022; Accepted: 21.01.2023

Abstract: Babesiosis is a tick-borne disease caused by hemoprotozoan parasites of the genus *Babesia*. Porcine babesiosis has not been reported in Sri Lanka. Two species have been identified infecting pigs: *Babesia trautmanni* and *Babesia perroncitoi*. Blood samples were collected from 100 healthy pigs of different age categories (piglets, weaners, fatteners, sows and boars) in a piggery at Talatuoya (Kandy District) and were subjected to Giemsa staining and microscopic examination. Selected smear-positive samples were subjected to molecular analysis using PCR with genus-specific primers. Twenty three percent of the pigs in the farm were infected. The microscopic measurement results revealed the likely presence of *B. perroncitoi* (the mean diameter of the parasite was 0.94±0.20 µm), and PCR results confirmed that it is *Babesia* sp. Still, sequence data and phylogenetic analysis are required for confirmation. All infected pigs were asymptomatic. This study provides the first record of porcine babesiosis in Sri Lanka. Investigations are underway to determine whether babesiosis is connected to the immunocompromised status of the pigs due to an outbreak of Porcine Reproductive and Respiratory Syndrome (PRRS) in the piggery.

***Keywords*:** Porcine babesiosis, *Babesia perroncitoi, 18S rRNA*, Piggery farm

INTRODUCTION

Babesiosis is a tick-borne disease caused by an intraerythrocytic hemoprotozoan belonging to the genus *Babesia* with a worldwide distribution (Schnittger *et al*., 2012). The condition is more prevalent in the tropics and subtropics, where tick distribution and prevalence are high (Laha *et al*., 2015). It has significant economic and medical importance, raising veterinary and public health concerns as an emerging zoonotic disease (Kjemtrup and Conrad, 2000; Colwell *et al*., 2012; Schnittger *et al*., 2012; Lobo *et al*., 2013). Although the infection is common among domestic animals like dogs, cattle and small ruminants, it is less frequently reported in cats, horses and pigs. Especially, reports on porcine babesiosis are less common, although it is known to cause severe production losses in many regions throughout the globe, including Southern Europe, China, Africa and Russia (Zobba *et al*., 2011; 2014; De Waal, 2019).

Porcine babesiosis is primarily caused by *Babesia trautmanni* or, less frequently, by *B. perroncitoi*; the two species vary in size and shape: *B. trautmanni,* the large piroplasms (size 1–3.3 µm), and *B. perroncitoi,* the small ring-shaped piroplasms (size 0.7–2 µm; Uilenberg, 2006; Zobba *et al*., 2011; De Waal *et al*., 2019). Porcine babesiosis produces antemortem clinical signs similar to bovine babesiosis (Purnell, 1981). Studies have shown that *B. trautmanni* in Italy and *B. perroncitoi* in China cause significant mortality during outbreaks (Ligios and Scala 1993; Guo *et al*., 1997). However, porcine babesiosis outbreaks are rarely described and remain an overlooked and neglected disease, with a limited number of cases reported worldwide. In Sri Lanka, babesiosis has been reported in domestic and wild animals including dogs, cattle, and water buffaloes (Seneviratna, 1965; Sivakumar *et al*., 2012; 2014; Ranatunga *et al*., 2022), but there were no cases in domesticated pigs. This study provides the first evidence of porcine babesiosis in a piggery in Sri Lanka.

MATERIALS AND METHODS

Blood samples from domestic pigs (*Sus scrofa domesticus*) reared in a piggery at Talatuoya (Kandy District) were collected and analysed.

Study site

The piggery is a 15-year-old, privately owned farm located on top of a hill in a peri-urban area 10 km away from the city. It has about 30 sows, 400 weaners and 2,000 fatteners at a time. Animals are primarily fed by unboiled swill collected from various hotels and food outlets in and around the city close by, and about 20% by commercial creep feed. For fatteners, 90% on swill and 10% on creep feed are given. The farm buys a few piglets from piggeries in Chillaw, but 90% are from their sows. The farm has employed eight

**Corresponding Author's Email: *rupika.rajakaruna@sci.pdn.ac.lk*
https://orcid.org/0000-0001-7939-947X

causal labourers to feed the animals and clean the piggery manually. Slaughtering is done in at least eight months. The veterinary and sanitary conditions of the farm cannot be considered up to the standard. No designated veterinary surgeon is employed, but hired service is sought when necessary. Therefore, no regular disease investigation or routine veterinary procedures are adopted. Physical injuries, wounds, poor body condition, and poor body weight gain were notable observations in many animals. There was no treatment for ectoparasites, but ivermectin is injected for mange about twice a year. About 15 cows and a few goats are also individually managed by caretakers in their homes within a 1 km radius. There is a thick nearby jungle with wild animals, including wild boar, rabbits, deer, mongoose, and stray dogs and cats are common in and around the farm premises.

Sample collection

Hundred clinically normal pigs were selected from piglets, sows, weaners, fatteners and boars (20 from each category). Thin smears were prepared from the blood samples obtained from the auricular vein, brought to the laboratory and stained with Giemsa. The smears were examined under a light microscope for hemoparasites. Another EDTA-preserved fresh blood sample (500 µl) from the smear-positive animals was collected, brought to the laboratory, and used for molecular identification.

Molecular analysis

DNA from the blood samples was extracted using ReliaPrep™ Blood gDNA Miniprep System (Promega, Madison, USA) and was subjected to PCR using *Babesia* genus-specific primers: *BJ1* (GTCTTGTAATTGGAATGATGG) and *BN2* (TAGTTTATGGTTAGGACTACG) to amplify a 411-450 bp region of 18S rRNA gene of *Babesia* spp. (Casati *et al*., 2006). Each PCR reaction mixture (30 µl) was composed of 15 µl of Go Taq master mix, 0.5 µl of each forward and reverse primer, 1.5 µl of template DNA, 1 µl of BSA and adequate nuclease-free water. The amplification process was as follows: initial denaturation at 94 °C for 10 mins, and this was followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 2 min, and the final extension was at 72 °C for 5 mins (Casati *et al*., 2006). PCR was carried out in a thermal cycler (Eppendorf Mastercycler® gradient), and then, the amplified products were visualized in ethidium bromide-stained agarose gel (1%) electrophoresis under UV illumination.

RESULTS AND DISCUSSION

Of the 100 blood smears examined, 23 (23%) animals, including two sows, 12 weaners, and nine fatteners were infected with *Babesia.* None of the piglets or boars was infected. Microscopical measurement results indicated the infection with small ring-stage piroplasms with an average diameter of 0.94±0.20 µm, suggesting a likely infection by *B. perroncitoi*. The PCR results confirmed the *Babesia* infection in pigs as it generated the predicted band size in 1% agarose gel electrophoresis (Figure 1).

Babesia infection can range from asymptomatic to life threatening (CDC, 2022). All positive pigs were asymptomatic; none had clinical signs of babesiosis except for reduced weight in some animals. The absence of clinical signs indicates that the pigs may be chronically or subclinically infected with these haemoparasites or have clinical-pathological abnormalities and organ dysfunctions (Dantas-Torres and Otranto, 2014). Chronic infections may not threaten the animals, but these pigs remain possible reservoirs of infection. In addition, stressful conditions, concurrent illnesses, and parturition may induce clinical signs in chronically infected animals (Harrus *et al*., 1999). At the same time, it could also be possible that the host displays infection tolerance traits, which reduces the infection's damage. The mechanisms and research on the tolerance phenomenon in porcine babesiosis have relevance for disease control measures targeting natural reservoirs (Roy and Kirchner, 2000).

Of the 100 blood samples microscopically tested, only a subsample of the positive ones was tested for parasite DNA. However, if all the blood samples were subjected to DNA testing, the percentage infected could be higher as microscopy doesn't always pick the parasites, especially during low parasitemia levels. For example, a recent study on canine babesiosis showed that 45.2% of the dogs are microscopically positive, while 78.6% of them are DNA positive, indicating a large percentage of infected dogs (66.7%) are undetected through microscopy (Ranatunga *et al*., 2022).

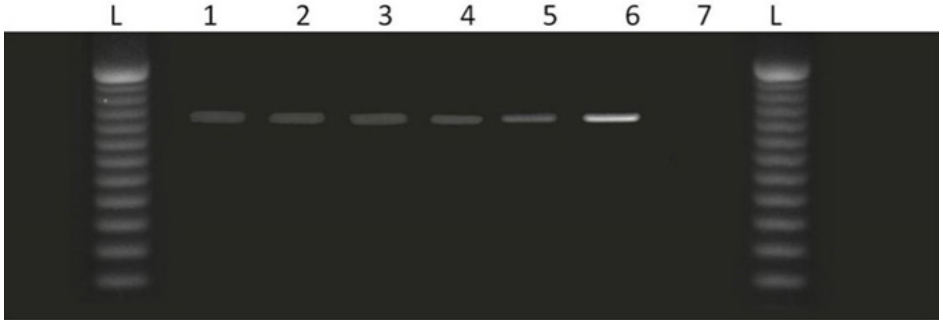


Figure 1: PCR amplification products for *Babesia* by the genus specific primers *BJ1* and *BN2* which amplify *18S rRNA* gene. The band size and the presence or absence of the parasite were visualized in 1% agarose gel using 50 bp molecular markers (L). Lanes 1-5, showing PCR products for pig blood DNA infected with *Babesia* parasite. Lane 6 has the positive control for *Babesia* and lane 7 has the negative control

There was a speculation that the island-wide outbreak of Porcine Respiratory and Reproductive Syndrome (PRRS) in Sri Lanka in 2009 has a connection to babesiosis (Personal communication with veterinarians). Recently, a piggery located in the Central Province of Sri Lanka started losing production and reported an unusual death rate, especially among weaning piglets found to be infected with PRRS although vaccinated (Personal communications with the veterinarian). During the investigations into these deaths, examining the presence of hemoparasites was also carried out. In most cases, infected animals exhibit no signs of disease, but there is a high mortality rate. It is possible that the immune-compromised status of pigs due to PRRS (Montaner-Tarbes *et al*., 2019) could leave the pigs at a higher risk for other infections or vice versa as one of the major risk factors of babesiosis is impaired immune function.

Though flies and mosquitos were present in and around the farm, none of the pigs had ectoparasites like ticks, lice or fleas at the sampling time. Studies have shown that some *Babesia* species can be transmitted by ixodid tick vectors (Boozer and Macintyre, 2003), via blood transfusion (Stegeman *et al*., 2003) and the placenta (Fukumoto *et al*., 2005). In the absence of ticks in the farm, congenital transmission is a likely explanation. However, the presence of other livestock animals and wildlife in the vicinity of the farm highlights the importance of eco-epidemiological studies to examine the reservoirs and epizootic and enzootic cycles. Moreover, studies on infection prevalence, geographical distribution, tick vectors, phylogenetic affinities of the parasite, and clinical aspects of porcine babesiosis in Sri Lanka are essential because the disease may lead to veterinary health issue in the future, causing significant economic losses.

ACKNOWLEDGEMENT

Financial support by the National Science Foundation [Grant no. RG/2019/BT/01], Sri Lanka is acknowledged.

Statement of conflict of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

REFERENCES

Ahedor, B., Kothalawala, H., Kanagaratnam, R., Vimalakumar, S.C., Otgonsuren, D., Tuvshintulga, B., Batmagnai, E., Silva, S.S.P., Sivakumar, T. and Yokoyama, N. (2022). First detection of *Theileria equi* in free-roaming donkeys (*Equus africanus asinus*) in Sri Lanka. *Infection, Genetics and Evolution,* **99**, 105244. DOI https://doi.org/10.1016/j.meegid.2022.105244.

Boozer, A.L. and Macintire, D.K. (2003). Canine babesiosis. *Veterinary Clinics of North America- Small Animal Practice,* **33**(4), 885-904. DOI https://doi.org/10.1016/S0195-5616(03)00039-1.

Casati, S., Sager, H., Gern, L. and Piffaretti, J.C. (2006). Presence of potentially pathogenic *Babesia* sp. for human in *Ixodes ricinus* in Switzerland. *Annals Agricultural and Environmental Medicine,* **13**(1), 65–70.

Centers for Disease Control and Prevention. Babesiosis surveillance—18 states, 2011. MMWR 2012;61: 505–9. https://www.cdc.gov/ticks/tickbornediseases/babesiosis.html#:~:text=Babesia%20infection%20can%20range%20from,)%2C%20and%20impaired%20immune%20function. Accessed on Jan 2022.

Colwell, D.D., Dantas-Torres, F. and Otranto, D. (2011). Vector-borne parasitic zoonoses: Emerging scenarios and new perspectives. *Veterinary Parasitology,* **182**(1), 14–21. DOI https://doi.org/10.1016/j.vetpar.2011.07.012.

Dantas-Torres, F. and Otranto, D. (2014). Dogs, cats, parasites, and humans in Brazil: Opening the black box. *Parasites & Vectors,* **7**(22), 1–25. https://doi.org/10.1186/1756-3305-7-22.

De Waal, D.T. (2019). Porcine babesiosis. In: Coetzer JAW, Thomson GE, Maclachlan NJ, Penrith ML, editors. *Infectious Diseases of Livestock.* Anipedia. Available from: https://www.anipedia.org/resources/infectious-diseases-of-livestock/196 Accessed March 30 2021.

Fukumoto, S., Suzuki, H., Igarashi, I. and Xuan, X. (2005). Fatal experimental transplacental *Babesia gibsoni* infections in dogs. *International Journal for Parasitology,* **35**(9), 1031-1035. DOI https://doi.org/10.1016/j.ijpara.2005.03.018.

Guo, Y., Yang, X., Huhebater, Bai, Y. and Liu, Z. (1997). The cure of acute *Babesiosis perroncitoi* in swine. *Tropical Animal Health and Production,* **29**(S4), 64S–65S. DOI 10.1007/BF02632934.

Harrus, S., Waner, T., Bark, H., Jongejan, F. and Cornelissen, A.W.C.A. (1999). Minireview Recent advances in determining the pathogenesis of canine monocytic ehrlichiosis. *Journal of Clinical Microbiology*, **37**(9), 2745–2749. DOI https://doi.org/10.1128/jcm.37.9.2745-2749.1999.

Kjemtrup, A.M. and Conrad, P.A. (2000). Human babesiosis: An emerging tick-borne disease. *International Journal of Parasitology*, **30**, 1323–1337.

Laha, R., Das, M. and Sen, A. (2015). Morphology, epidemiology, and phylogeny of *Babesia*: an overview. *Tropical Parasitology*, **5**, 94–100. DOI: 10.4103/2229-5070.162490.

Ligios, C., Idini, G. and Scala, A. (1993). An outbreak of pig babesiosis in Sardinia. *Atti della Societa Italiana delle Scienze Veterinarie,* **47**, 1379–1383. (Italy.)

Lobo, C.A., Cursino-Santos, J.R., Alhassan, A. and Rodrigues, M. (2013). *Babesia*: An emerging infectious threat in transfusion medicine. *PLoS Pathogens,* **9**(7), e1003387.

Montaner-Tarbes, S., Dell Portillo, H.A., Montoya, M. and Fraile, L. (2019). Key gaps in the knowledge of the porcine respiratory reproductive syndrome virus (PRRSV). *Frontiers in Veterinary Science,* **6**, 1-15. DOI https://doi.org/10.3389/fvets.2019.00038.

Purnell, R. E. (1981). Babesiosis in various hosts, p. 25–63. *In* M. Ristic and J. P. Kreier (ed.), *Babesiosis*. Academic Press, New York, NY.

Ranatunga, R.A.S., Dangolla, A., Sooriyapathirana, S.D.S.S.

and Rajakaruna, R.S. (2022). High Asymptomatic Cases of Babesiosis in Dogs and Comparison of Diagnostic Performance of Conventional PCR vs Blood Smears. *Acta Parasitologica*, **67**(3), 1217–1223. DOI https://doi.org/10.1007/s11686-022-00549-x.

Roy, B.A. and Kirchner, J.W. (2000) Evolutionary dynamics of pathogen resistance and tolerance. *Evolution* **54**: 51-63.

Schnittger, L., Rodriguez, A.E., Florin-Christensen, M. and Morrison, D.A. (2012). *Babesia*: A world emerging. *Infection, Genetics and Evolution*, **12**(8),1788–1809.

Seneviratna, P. (1965). Studies of *Babesia gibsoni* infections of dogs in Ceylon. *Ceylon Veterinary Journal,* **13**, 107-110.

Sivakumar, T., Kothalawala, H., Abeyratne, S.A.E., Vimalakumar, S.C., Meewewa, A.S., Hadirampela, D.T., Puvirajan, T., Sukumar, S., Kuleswarakumar, K., Chandrasiri, A.D.N., Igarashi, I. and Yokoyama, N. (2012). A PCR-based survey of selected *Babesia* and *Theileria* parasites in cattle in Sri Lanka. *Veterinary Parasitology,* **190**(1-2), 263-267. DOI https://doi.org/10.1016/j.vetpar.2012.05.014.

Sivakumar, T., Tattiyapong, M., Fukushi, S., Hayashida, K., Kothalawala, H., Silva, S.S.P., Vimalakumar, C., Kanagaratnam, R., Meewewa, A.S., Suthaharan, K., Puvirajan, T., De Silva, W.K., Igarashi, I. and Yokoyama, N. (2014). Genetic characterization of *Babesia* and *Theileria* parasites in water buffaloes in Sri Lanka. *Veterinary Parasitology,* **200**, 24-30. DOI https://doi.org/10.1016/j.vetpar.2013.11.029.

Stegeman, J.R., Birkenheuer, A.J., Kruger, J.M. and Breitschwerdt, E.B. (2003). Transfusion-associated *Babesia gibsoni* infection in a dog. *Journal-American Veterinary Medical Association*, **222**(7), 959-963.

Uilenberg, G. (2006). *Babesia*-A historical overview. *Veterinary Parasitology,* **138**(1-2), 3–10. DOI https://doi.org/10.1016/j.vetpar.2006.01.035.

Zobba, R., Nuvoli, A.M., Sotgiu, F., Lecis, R., Spezzigu, A., Dore, G.M., Masia, M.A., Cacciotto, C., Parpaglia, M.L.P., Dessì, D., Pittau, M. and Alberti, A. (2014). Molecular epizootiology and diagnosis of porcine Babesiosis in sardinia, Italy. *Vector-Borne Zoonotic Diseases,* **14**(10), 716–723. DOI https://doi.org/10.1089/vbz.2014.1648.

Zobba, R., Parpaglia, M.L.P., Spezzigu, A., Pittau, M. and Alberti, A. (2011). First molecular identification and phylogeny of a *Babesia* sp. from a symptomatic sow (*Sus scrofa Linnaeus* 1758). *Journal of Clinical Microbiology,* **49**(6), 2321–2324. DOI https://doi.org/10.1128/JCM.00312-11.