Pertanika J. Trop. Agric. Sci. 49 (4): 1957 - 1975 (2026)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Brucella spp. Surveillance in Wild Long-Tailed Macaques (*Macaca fascicularis*) in Selangor, Malaysia

Atiqah Zulhisam[1], Siti-Khairani Bejo[2], Abdul Rahman Omar[2], Annas Salleh[3], Yaro Sallau Saidu[1], Safawati Zawani Zamri[1], Mazlina Mazlan[2], and Azlan Che-Amat[1]*

[1]Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM), 43400 UPM Serdang, Selangor, Malaysia
[2]Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM), 43400 UPM Serdang, Selangor, Malaysia
[3]Department of Veterinary Laboratory Diagnostics, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM), 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Brucellosis is a zoonotic disease that is caused by the intracellular Gram-negative coccobacillus *Brucella* spp., which threatens public health and local socio-economics, with wild and captive non-human primates known to be susceptible to several *Brucella* biovars. In Malaysia, wild long-tailed macaques (*Macaca fascicularis*) frequently inhabit areas near human settlements, increasing human-wildlife conflict and potential disease spillover risk. This study aimed to determine the seroprevalence of anti-*Brucella* antibodies and the presence of *Brucella* antigens in wild long-tailed macaques from selected areas in Selangor, Malaysia. 100 fresh carcasses of the wild long-tailed macaques were retrieved from the Department of Wildlife and National Parks of Peninsular Malaysia (PERHILITAN), and pathological abnormalities were observed in several organs, including the reproductive, hepatosplenic, and lymphatic organs, during necropsy. Serum samples (n=100) were screened using the Rose Bengal Plate Test (RBPT), and reactive samples were confirmed by the Complement Fixation Test (CFT). Reproductive tissues (ovaries, uteri, placentas, testes) were collected for DNA extraction and analysed by qPCR targeting *Brucella* spp. genes. RBPT screening showed 1% positivity (1/100, 95% CI: 0.37%-1.63%), but all sera tested negative by the confirmatory CFT. qPCR analysis of all 100 reproductive tissue samples yielded no amplification of the targeted *Brucella* gene, indicating a 0% antigen presence (0/100, 95% CI: 0-0.627%). Consequently, the overall prevalence of *Brucella* antibodies or antigens in

ARTICLE INFO

Article history:
Received: 08 July 2025
Accepted: 07 July 2026
Published: 14 August 2026

DOI: https://doi.org/10.47836/pjtas.49.4.18

E-mail addresses:
atiqahzulhisam@gmail.com (Atiqah Zulhisam)
skhairani@upm.edu.my (Siti-Khairani Bejo)
aro@upm.edu.my (Abdul Rahman Omar)
annas@upm.edu.my (Annas Salleh)
sallausaidu3@gmail.com (Yaro Sallau Saidu)
watie212@yahoo.com (Safawati Zawani Zamri)
m_mazlina@upm.edu.my (Mazlina Mazlan)
c_azlan@upm.edu.my (Azlan Che-Amat)
* Corresponding author

wild long-tailed macaques in the study areas ranged from 0% to 1.67% (95% CI). These findings suggest that exposure to *Brucella* spp. and active infection range from very low to non-existent in these wild macaque populations. Nevertheless, continued surveillance is warranted given the zoonotic potential and the proximity of the wildlife population to human settlements.

Keywords: Brucella spp., CFT, human-wildlife conflict, *Macaca fascicularis*, qPCR, RBPT

INTRODUCTION

This bacterial disease was first discovered in the 1850s, plaguing the citizens of Malta Island, located in the Mediterranean Sea. The newly emerging disease was initially named Malta fever before being discovered by a British army surgeon, David Bruce, and then named brucellosis (Vassallo, 1992). Humans, livestock, wildlife, and marine animals are known to be susceptible to brucellosis (Wyatt, 2013). Typically, this zoonosis is transmitted between farmed animals, where it is also transmissible to humans and wildlife through direct contact, consumption of raw milk, dairy products, and meat; exposure to infected animals and their bodily secretions; and exposure to contaminated aerosol and bacteria cultures can cause infection via mucous membranes and conjunctiva (Küplülü et al., 2004; Piao et al., 2020; Pereira et al., 2020; Sack et al., 2018).

The clinical manifestations of brucellosis in animals primarily involve reproductive complications such as abortion, orchitis, epididymitis, sterility, and infertility. Alternatively, this disease also induces pathological alterations in various body systems, including the musculoskeletal, cardiovascular, lymphatic, hepatobiliary, renal, and nervous systems (Pradeepkiran et al., 2021). Moreover, Mazlan (2016) detailed the pathological manifestations in animals infected with *Brucella*, including swollen tissue, inflammation, necrosis, petechial haemorrhages, mastitis, endometritis, and cystitis. Additionally, Christopher et al. (2010) have documented that the presence of pathological lesions in brucellosis cases, especially in the reproductive organs and reticuloendothelial systems, has resulted in abortion, as well as enlargement of the liver (hepatomegaly) and spleen (splenomegaly).

On top of that, individuals in direct and frequent contact with infected animals, such as farmers, abattoir workers, and veterinary experts, face an elevated likelihood of contracting *Brucella (Dadar et al., 2022)*. Due to a lack of research and demand, there are currently no commercially available vaccinations for humans against *Brucella*. Hence, the laboratory personnel working on diagnosing the samples from infected individuals and creating the vaccines against these bacterial species are equally at risk of contracting laboratory-acquired brucellosis (Sun et al., 2021).

Research concerning brucellosis zoonotic transmission from wild fauna to humans, particularly non-human primates, is still in its infancy. Interestingly, non-human primates

possess analogous anatomical and physiological traits to their human counterparts, rendering them the most suitable subjects for investigating the epidemiology of human brucellosis and developing vaccines against *Brucella* in humans (Henning et al., 2012). The depletion of animal habitats and natural resources compels wild species to seek refuge and sustenance in human settlements, which offer ample food and shelter for their continued survival (Magouras et al., 2020). This would ultimately escalate the human-wildlife conflict and heighten the likelihood of zoonotic illnesses being transmitted from wildlife to humans when they coexist in close proximity.

The wild long-tailed macaque (*Macaca fascicularis*) is distributed widely across Southeast Asian countries, like Malaysia, Singapore, Thailand, Indonesia, the Philippines, Laos, Vietnam, Cambodia and Bangladesh (Liedigk et al., 2015). This species is one of the arboreal frugivorous non-human primates more commonly responsible for human-wildlife conflicts in Malaysia. Public reports to the Wildlife Department in Peninsular Malaysia indicate that long-tailed macaques are Malaysia's most prevalent non-human primates that instigate conflicts between humans and wildlife (Perhilitan, 2018). Nevertheless, several potential risk of wildlife zoonosis transmission that originating from close interaction with wild macaques can leads to wildlife-human conflict and exposes humans to multitude arrays of diseases such as malaria, tuberculosis, leptospirosis, and meliodosis (Lekko et al., 2021; Saechan et al., 2022).

In order to enhance the efficacy of *Brucella* detection and provide a more precise diagnosis for early illness management, a molecular diagnosis of *Brucella* antigen is strongly advised, in addition to serological findings. Although a bacterial culture is regarded as the gold standard to identify bacteria and provides stronger assurance for true positive results, given its prevalence as a laboratory-acquired infection, brucellosis poses potential risks to laboratory personnel due to its lengthy incubation period, low positivity rate, and possible violation of biosafety protocols (Traxler et al., 2013; Song et al., 2021). Moreover, Yagupsky et al. (2019) reported that bacterial isolation and *Brucella* strain identification also require extended time and effort, which may compromise the diagnosis process and the epidemiological surveillance for mitigating outbreaks. Hence, molecular assays were employed to highlight the amplification of nucleic acids to detect the *Brucella* antigen, facilitating prompt identification and verification of the bacterial species.

Brucella antigen detection through molecular qPCR has a high specificity of 100% and a high sensitivity of 93.14% (Che et al., 2019). When extracting samples for molecular diagnosis, different kinds of samples can be extracted, such as bacterial cultures, physiological fluids (e.g., blood, milk, and semen), and tissues (e.g., placenta, aborted foetuses, reproductive organs, lungs, and lymph nodes) (Amin et al., 2001; Gallien et al., 1998; Ilhan et al., 2008; Manivannan et al., 2021; Nyarku et al., 2020; Yu & Nielsen, 2010). Then, multiplex real-time PCR is an effective method for quantifying antigen DNA

copies, identifying *Brucella* strains or biovars, and differentiating false-negative results from serological assays, as was found by Dal et al. (2019). Similarly, Che et al. (2019) reported that qPCR detected high *Brucella* DNA loads in some of the negative samples tested by Plate Agglutination Test (PAT) and Serum Agglutination Test (SAT), a detection that demonstrates how molecular tests like PCR are capable of aiding in the diagnosis of early exposure and acute brucellosis. These reports are useful for elucidating the process of transmission of zoonotic diseases, particularly between humans and wildlife (Chisi et al., 2017; Katsiolis et al., 2022).

To date, only one preliminary serological study (Yong, 2017) has assessed *Brucella* exposure in long-tailed macaques in Peninsular Malaysia, using stored sera and RBPT alone without confirmatory testing or molecular detection. No study has combined serological confirmation like CFT with direct antigen detection like qPCR in reproductive tissues, the primary sites of *Brucella* persistence and shedding. This gap is critical because long-tailed macaques in Selangor frequently inhabit densely populated human settlements, where they contact humans, domestic animals, and contaminated environments. Without baseline data on both antibody prevalence and active infection, the zoonotic risk posed by these peri-urban macaques cannot be assessed, and public health authorities lack evidence to guide surveillance or mitigation strategies. Therefore, this study aimed to fill that gap by addressing the following objectives: (1) to screen wild long-tailed macaques from selected areas in Selangor for anti-Brucella antibodies using RBPT and confirm with CFT; (2) to detect *Brucella* spp. DNA in reproductive tissues via qPCR; (3) to estimate the combined prevalence of exposure and infection; and (4) to provide baseline data for zoonotic risk assessment in human-macaque conflict zones.

MATERIALS AND METHODS

Study Design

The study was conducted in Selangor, Peninsular Malaysia, which lies between latitudes 2°35‘ N and 3°60’ N and longitudes 101°14‘ E and 101°46’ E. The region has a tropical rainforest climate with average annual rainfall of 2,500 mm, mean daily temperatures ranging from 27 °C to 32 °C, and relative humidity of 80-85%. The target population was the finite population of wild long-tailed macaques (*Macaca fascicularis*) in Peninsular Malaysia, estimated at 133,403 individuals (Karuppannan et al., 2014). The sample size was calculated using Open-Epi Version 3 with the standard formula for frequency in a finite population as in Equation 1:

$$n = [DEFF \times N \times p(1-p)] / [(d^2/Z^2_{1-\alpha/2} \times (N-1)) + p(1-p)] \quad [1]$$

In this formula, N represents the population size, around 133,403; p is the expected prevalence, which was set at 50% to maximise sample size; d is the desired confidence limit

at 5%, and DEFF is the design effect for cluster surveys, which was set at 1. The minimum required sample size was 95, which was rounded up to 100. A cross-sectional study design was employed. Using convenience sampling, 100 wild long-tailed macaques were obtained from the Malaysia Wildlife Department (Perhilitan) between July 2022 and March 2023.

Post-mortem Examination and Sample Collection

Fresh carcasses were collected from the Perhilitan Selangor district office immediately after chemical euthanasia. All carcasses were transported to the post-mortem laboratory at Universiti Putra Malaysia (UPM) within six hours. A complete necropsy was performed on each carcass. The signalment of each animal was recorded, and any pathological abnormalities or lesions were documented. To minimise the risk of zoonotic transmission, particularly aerosol, wound, and mucous membrane exposure to *Brucella* spp., strict biosafety precautions were followed throughout the necropsy.

Blood was collected from each fresh carcass as soon as possible after arrival. Blood was drawn using three methods: vena cava venipuncture, suborbital vein puncture, and intracardiac venipuncture. All blood was transferred into plain tubes and allowed to clot, after which the serum was separated by centrifugation. Reproductive organs, including the uterus, ovaries, and testes, were harvested. Associated tissues such as amniotic fluid, placenta, and foetus were also collected when available. All tissue samples were stored at -20 °C until further analysis.

Serological and Molecular Detection

Rose Bengal Plate Test (RBPT)

The Rose Bengal Plate Test (RBPT) is a rapid, qualitative serum agglutination assay that detects anti-*Brucella* antibodies (Monlab, 2013). For each serum sample, a drop of Rose Bengal reagent was placed next to a drop of serum on a glass plate. Positive and negative control sera were included in each run. A separate stirrer was used for each sample to mix the reagent with the serum. The plate was then rocked gently for four minutes. The presence of any agglutination or visible clumping was interpreted as a seropositive result (Department of Veterinary Services, 2020).

Complement Fixation Test (CFT)

All sera, regardless of RBPT results, were sent to the Veterinary Research Institute (VRI) in Ipoh, Perak, Malaysia, an accredited diagnostic laboratory, for confirmatory testing. The Complement Fixation Test (CFT) was performed strictly according to the World Organisation for Animal Health (WOAH) (2022). Sera producing a titre of 20 ICFTU/mL (International Complement Fixation Test Units per millilitre) or higher were classified as

positive for anti-*Brucella* antibodies. Samples showing any degree of haemolysis could not be accurately analysed and were therefore recorded as negative. As reported by Legesse et al. (2023), the CFT has 100% specificity but lower sensitivity compared to RBPT or I-ELISA.

Real-Time Polymerase Chain Reaction (RT-PCR)

DNA Extraction. Genital tissue samples such as testes, uteri, ovaries, and placenta from all 100 macaques were subjected to DNA extraction. The specific numbers of each tissue type were: 56 uteri and ovaries, 6 placentas, and 46 testes. Extraction was performed using the DNeasy Blood & Tissue Kit (69,506, Qiagen, Germany) following the manufacturer's instructions. DNA concentration was measured using a TECAN Nanoquant spectrophotometer (Hinić et al., 2008; Saytekin & Ak, 2018).

Primer and Probe Design. Two genetic targets were selected for *Brucella* detection, as shown in Table 1, which were the 16s-23s rDNA intergenic region and the insertion element IS711 (Nyarku et al., 2020; Probert et al., 2004; Zakaria et al., 2019). Primers and probes were designed using NCBI Primer-BLAST, Primer3, Integrated DNA Technologies tools, and OligoAnalyzer. These tools were used to check for primer-dimer and homodimer formation and to ensure target specificity.

Table 1
The probe, forward and reverse primers for the gene targets 16s-23s rDNA and IS711

Gene Target	Taqman		Gene Sequence
16s-23s rDNA	Primer	Forward	GTCGGCCTTGCGAAGCT
		Reverse	GCCCAGATACCGACGCAAA
	Probe		FAM-ATCTGTGGATCGCGTAGTA-MG
IS711	Primer	Forward	GCTTGAAGCTTGCGGACAGT
		Reverse	GGCCTACCGCTGCGAAT
	Probe		FAM-AAGCCAACACCCGGCCATTATGGTBHQ-1

Source: Nyarku et al., 2020; Probert et al., 2004; Zakaria et al., 2019

qPCR Optimisation. Before running the study samples, real-time PCR conditions were optimised. The optimal annealing temperature for both gene targets was determined to be 63 °C. The optimal primer concentration was 0.4 µL per reaction, and the optimal probe concentration was 2 µL per reaction. These values were selected after testing a range of concentrations (primers at 0.8, 0.6, and 0.4 µL; probes at 0.4, 0.3, and 0.2 µL) and temperatures (61 °C to 63 °C).

Standard Curve. A standard curve was generated for each gene target using ten-fold serial dilutions of a positive control (Masjedian Jazi et al., 2017). Each dilution was run in triplicate. Acceptable qPCR efficiency ranges from 90% to 100%, which corresponds to a slope of -3.3 or lower due to a difference of 3.3 cycles between each of the ten-fold dilutions. In this study, the standard curve for 16s-23s rDNA had an R^2 value of 96%, and the curve for IS711 had an R^2 value of 95%. These values confirmed that the qPCR method achieved near-optimal efficiency.

qPCR protocol. All 100 DNA extracts were run in triplicate on a real-time PCR thermal cycler. Each run included negative controls and positive controls, which were a strand of known *Brucella* DNA. A sample was considered positive for *Brucella* antigen if amplification of the target genes, either 16s-23s rDNA or IS711, was detected within the defined threshold cycle (Ct) value established from the standard curve. Samples showing no amplification or Ct values above the threshold were recorded as negative.

RESULTS

After the wild cynomolgus macaque was caught using a metal wall trap and chemically euthanised using barbiturates, all carcasses were collected from the Perhilitan office, where the necropsy was performed in a Biosafety Level (BSL) 2 post-mortem laboratory located at the Faculty of Veterinary Medicine, Universiti Putra Malaysia. The post-mortem examination findings of all wild long-tailed macaque carcasses (N = 100) revealed that 54% of the wild long-tailed macaques (*Macaca fascicularis*) were females, and 6 macaques, or 11% of the 54 females, were discovered to be pregnant. In comparison, 46% were males. Furthermore, other necropsy findings revealed that 2% of the carcasses were emaciated, and ulcerated wounds were found on 2% of the macaques. Besides that, multiple whitish granulomas were found in the gastrointestinal systems, spreading across the stomach until the small intestines in 5% of the samples, which may indicate encysted gastrointestinal endoparasites.

Moreover, the hepatobiliary system demonstrated in Figure 1 had the highest numbers of macroscopic pathological changes (16%), like hepatomegaly at 1%, focal hepatic necrosis at 2%, hepatic granuloma at 5%, hepatic lipidosis at 6%, and hepatic ecchymotic haemorrhage at 2%. Besides that, 8% of the wild long-tailed macaques showed splenomegaly, 1% had a spleen infarction, and 4% had granulomatous lesions on the spleen. Next, the reproductive system (12%) revealed 1% with a vaginal prolapse, 3% with a uterus ecchymotic haemorrhage, 1% with uterus congestion, and 5% with undescended testes, which are indicative of a young age, around less than one year old or juvenile. In comparison, 2% of male macaques showed unilateral testicular hypoplasia. For the renal system, only 1% were discovered to have a unilateral right renomegaly; for the

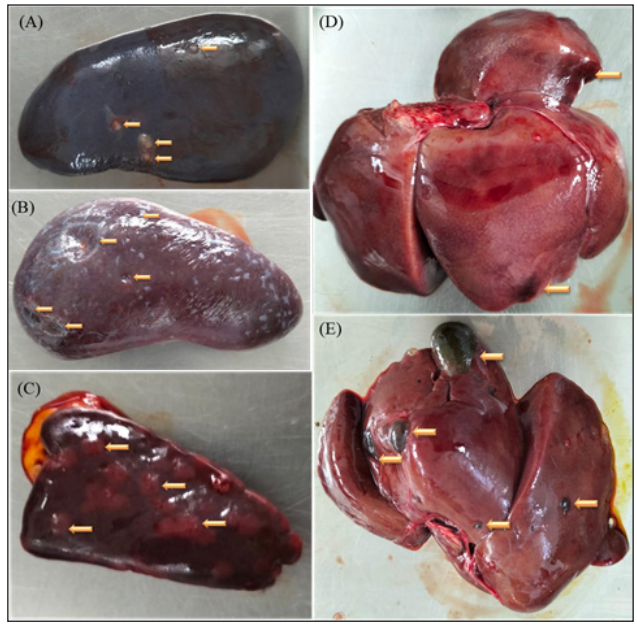

Figure 1. (A) Multiple whitish, raised foci suggestive of granulomatous splenitis (arrows). (B) Multiple depressions and pits of contraction (arrows) were observed on the surface of a spleen with a change in consistency suggestive of splenic infarction. (C) Multiple whitish, raised foci in the parenchyma of a spleen are suggestive of granulomatous splenitis. (D) Presence of a focus of dark red discolouration at the edge of the liver's median lobe and a generalised red discolouration of the liver, suggestive of hepatic haemorrhage and congestion. (E) Multiple cysts of various sizes are embedded in the liver parenchyma as well as on the liver surface (arrows), consistent with multiple hepatic cysts

lymphatic systems, 1% had enlarged submandibular lymph nodes, and 3% had ecchymotic haemorrhage at bilateral inguinal lymph nodes.

Serology

Only one macaque exhibited a positive reaction (1%) against the RBPT through an agglutination reaction observed in Figure 2, indicating the potential presence of antibodies against the *Brucella* spp. antigen in this individual, as shown in Table 2.

Table 2
The Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT) results of free-ranging long-tailed macaques in Selangor

Serological Tests	Reactions	Results	Percentage of Reaction
RBPT	Positive	1	1%
	Negative	99	99%
CFT	Positive	0	0%
	Negative	100	100%

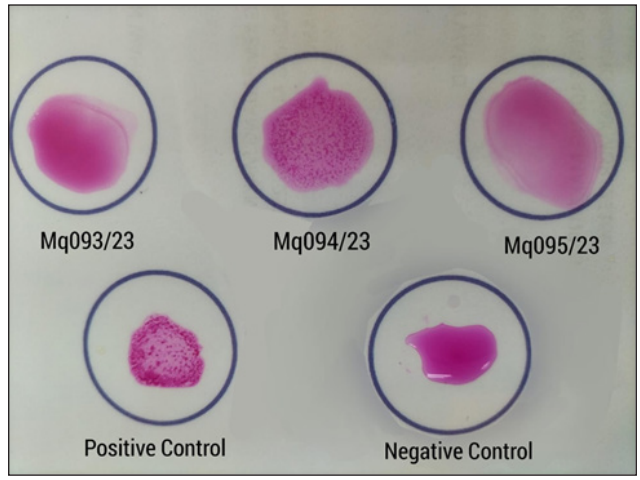

Figure 2. Rose Bengal Plate Test (RBPT) was performed concurrently with positive and negative controls on one hundred sera collected from wild long-tailed macaques

Conversely, the remaining 99% of the wild long-tailed macaques exhibited an adverse response or showed no apparent agglutination of blood cells when exposed to the Rose Bengal stain containing *Brucella* spp. antigen. According to the CFT results, all sera were determined to be negative, including the serum with a positive reaction on the RBPT.

Molecular

The 16s-23s rDNA and IS711 gene targets had threshold cycle (CT) values for the positive control of 24.98 and 24.69, as mentioned in Table 3. Whereas the negative control had CT values of 34.51 for 16s-23s rDNA and 36.77 for IS711 gene targets. Figure 3 revealed that for both the 16s-23s rDNA and IS711 gene targets, 0% of the extracted DNA samples from the related reproductive tissues in the wild long-tailed macaques (*Macaca fascicularis*) had CT values near the cut-off CT values that were established for both IS711 and 16s-23s rDNA, indicating negative results for the Brucella antigen. The qPCR results for all the reproductive tissues would be interpreted as positive if the CT value had been lower than the cut-off value, and if the CT values had exceeded the cut-off points, then it would have been considered a negative result. By simple logistic regression (Logit), the Limits of

Table 3
The CT values for 16s-23s and IS711 to identify the Brucella *spp. gene target from the associated reproductive organs of the cynomolgus macaques*

CT Values Range	16s-23s	IS711
0-5	0%	0%
5-10	0%	0%
10-15	0%	0%
15-20	0%	0%
20-25	20%	5%
25-30	20%	20%
30-35	45%	50%
>35	15%	25%

Detection (LODs) (95% detection rate) for the real-time PCR assays developed in this study and confirmed using a 10-fold serial dilution in triplicate for two different gene targets ranged from 10^6 to 10^{10} copies/µL. As anticipated, all tested genes were successfully identified without any cross-detection, and the amplification signals remained specific to the target gene up to at least cycle 25. The LODs were approximately 4,717 copies per reaction for the target gene 16s-23s rDNA, while there were 3,305 copies per reaction for the IS711 target gene.

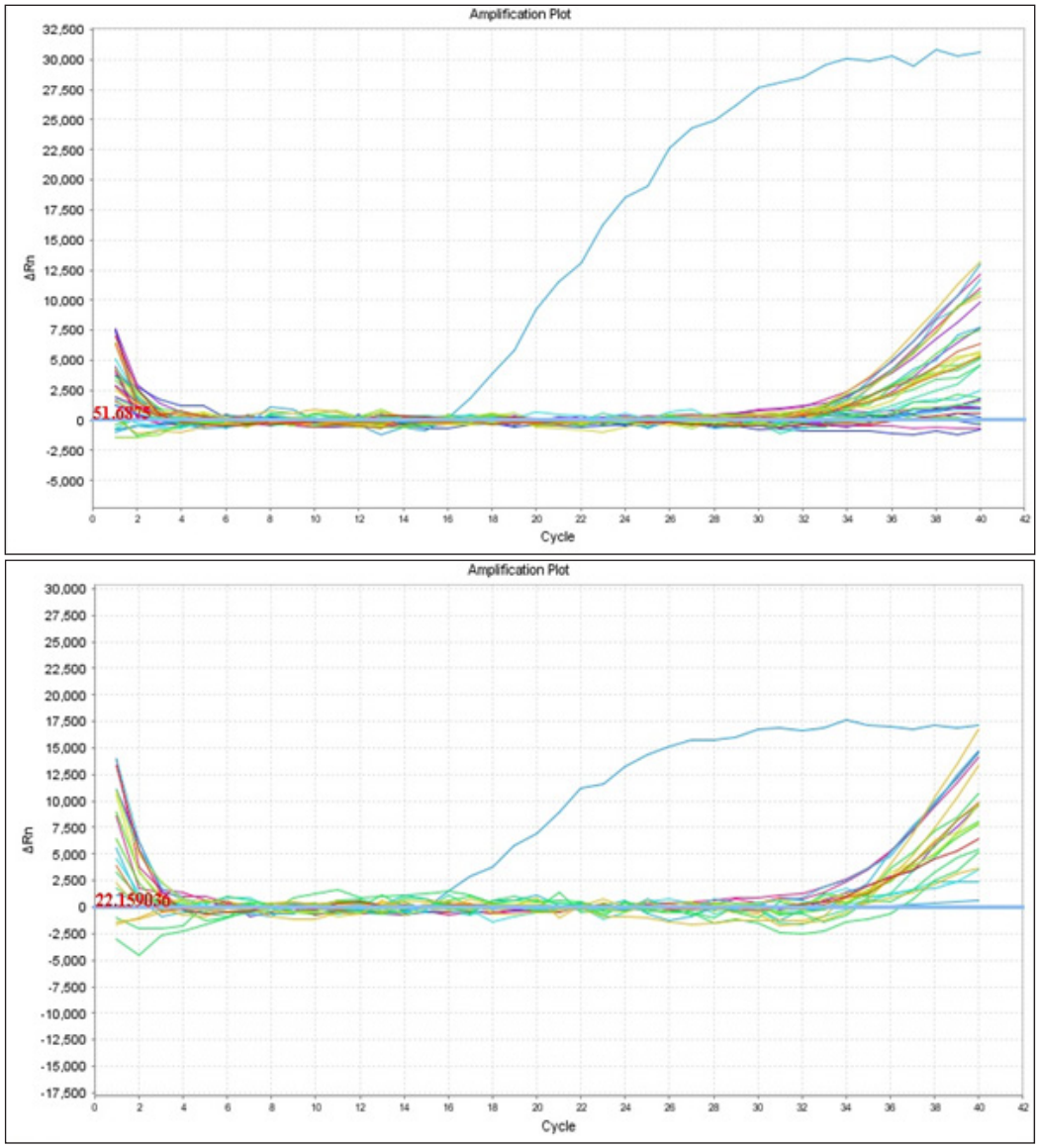

Figure 3. The real-time PCR amplification plot results of the gene sequence to detect *Brucella* spp. antigen. 16s-23s rDNA (upper); IS711 (lower)

DISCUSSION

Brucellosis is a zoonotic infection that affects various species, including non-human primates. Nevertheless, other non-human primate species that were caught from the wild and kept in captivity either through natural infection or through experiment were found to be susceptible to various *Brucella* spp. biovars that are also susceptible to humans (De Mello et al., 1964). Like in certain cases where a new strain of *Brucella* spp. called *B. papionis* was found in Baboons which opens up the possibilities of a new strain of Brucella to occur in other NHP (Huddleson et al., 1929; Schlabritz-Loutsevitch et al., 2009; Whatmore et al., 2014). Interestingly, there are evidence of *B. papionis* that was susceptible to humans trophoblast *in-vitro* where the normal physiological function of the cells were modified (García-Méndez et al., 2019).

This zoonosis is primarily characterised by macroscopic pathological lesions that predominantly target the reproductive system. These lesions can manifest in different clinical forms, including infertility, metritis, retained placenta, abortion, and orchiepididymitis. Furthermore, as highlighted by Mazlan (2016), *Brucella* spp. infection in animals can also lead to additional symptoms, including tissue swelling, inflammation, necrosis, petechial haemorrhages, mastitis, endometritis, and cystitis. In this study conducted on wild long-tailed macaques (*Macaca fascicularis*), 12% of the animals examined were found to have pathological lesions in their reproductive organs, including unilateral testis hypoplasia or undescended testes (1%), uterine ecchymotic haemorrhage (3%), and uterine congestion (1%). These lesions, while consistent with brucellosis, are not pathognomonic and require differentiation from other infectious causes.

Magden et al. (2015) furthered our understanding of differential diagnoses associated with different types of pathological lesions in the reproductive organs, liver, and spleen. Some other infectious diseases have overlapping symptoms with brucellosis, such as leptospirosis, campylobacteriosis, yersiniosis, and tuberculosis, all of which are zoonotic. These diseases can also affect other non-human primates, leading to similar pathological findings in both the acute and chronic stages of testing (Magden et al., 2015; Thayaparan, 2014). Therefore, the presence of reproductive lesions alone cannot confirm *Brucella* infection, and the negative serological and molecular results in this study further support the absence of brucellosis as the cause of those pathologies.

In addition to reproductive system abnormalities, further pathological findings in the hepatobiliary system (16%) included hepatic granulomas in 5% of the cases, hepatomegaly in 1%, hepatic necrosis in 2%, and hepatic haemorrhage in another 2%. Moreover, 13% of splenic abnormalities were observed, with splenomegaly accounting for 8%, splenic infarction making up only 1%, and splenic granulomas accounting for 4%. Then, lymphatic tissues were also affected, affecting the inguinal lymph nodes of 3% of macaques and the submandibular lymph nodes of 1% of macaques. The proximity of the inguinal lymph

nodes to the reproductive organs suggests that there could have been a septic cross-infection between the bloodstream and lymphatic systems. However, as with the reproductive lesions, these hepatosplenic and lymphatic findings are non-specific and were not correlated with any evidence of *Brucella* infection in this study.

The serological testing of 100 wild long-tailed macaques revealed that 99% of blood samples were negative for serum agglutination when exposed to the RBPT with *Brucella* spp. antigen. Consequently, these sera were classified as negative since they showed no detectable antibodies against *Brucella* spp. However, 1% of the macaques' sera tested positive for antibodies, indicating a positive reaction and suggesting that wild long-tailed macaques, similar to rhesus macaques (*Macaca mulatta*), may be susceptible to *Brucella* spp. infection, as has been demonstrated in previous studies involving exposure to contaminated aerosols (Yingst et al., 2010). Nevertheless, all sera were confirmed negative (0%, 95% confidence interval) upon further testing with the CFT. This single RBPT-positive result is therefore most likely a false positive, a known limitation of RBPT due to its lower specificity compared to CFT (Lighter, 1953).

These findings are consistent with a previous serological survey of wildlife in Selangor and Pahang by Zulhisam et al. (2026), which tested 105 archived sera from wild long-tailed macaques (N = 54), wild boars (N = 29), and Asian elephants (N = 22) using RBPT. That study similarly found only one positive result, which came from a wild boar, while all macaque and elephant samples were negative. The authors concluded that the lack of human-livestock-wildlife interface in the study areas likely limits brucellosis transmission cycles, leading to a low prevalence of brucellosis in Malaysian wildlife (Zulhisam et al., 2026). Importantly, that study also emphasised that serological screening results should always be supported with a confirmatory test such as CFT or ELISA, a recommendation that aligns directly with the methodology employed in the present study.

In terms of test performance, the RBPT is known to have higher sensitivity (95.6%) but lower specificity (94.8%) when compared to the CFT, which has higher specificity (96.8%) but lower sensitivity (74.4%) (Teng et al., 2017). As an easy, quick in situ serological screening test, RBPT is always recommended by the WOAH brucellosis protocol but has to be compared with a confirmatory serological test like CFT or ELISA. In contrast, Erdenliğ Gürbilek et al. (2017) reported that CFT, while a reliable test, can yield fewer positive results compared to other serological assays, often due to limitations such as requiring larger serum volumes, being costly, and sometimes providing false-negative outcomes. Despite the limitations, CFT and RBPT were found to have comparable sensitivities (95%) in some studies, as described by Legesse et al. (2023), making them both viable tools for serological tests for antibody detection. The complete concordance between negative CFT results and negative qPCR results in this study reinforces the conclusion that no active or past infection was confirmed.

For this study, molecular detection using real-time PCR (qPCR) was also conducted on the macaques for the purpose of analysing the presence of the *Brucella* spp. antigen. However, no amplification of DNA was observed in any of the 100 macaque samples, indicating the absence of the *Brucella* spp. antigen in reproductive tissue samples, as confirmed by the cycle threshold (CT) values. The failure to detect *Brucella* spp. DNA precludes the ability to accurately quantify *Brucella* spp. in the macaques‘ tissues using qPCR. This finding is consistent with the negative CFT results and strongly suggests that none of the 100 macaques carried an active *Brucella* spp. infection in the reproductive tissues examined.

Seeing as there was a lack of amplification in qPCR results in conjunction with the single positive RBPT result, which was not confirmed by CFT, the RBPT-positive macaque may have previously been exposed to *Brucella* spp., leading to transient antibody development without persistent bacterial colonisation. However, given that the CFT, a more specific test, was negative, the more parsimonious explanation is a false-positive RBPT. The likelihood of detecting the antigen during molecular analysis can be impacted by the selection of the proper tissue samples, namely tissues from reproductive, hepatosplenic, or lymphatic organs. As González-Espinoza et al. (2021) and Preena et al. (2024) have noted, organs such as the lymph nodes, spleen, liver, genital organs, and reticuloendothelial organs are considered "golden organs" for detecting *Brucella* spp. through bacterial isolation (Carvalho et al., 2023). Future approaches will improve the sensitivity of molecular detection by including a wider range of these organs, which may increase the chances of identifying latent or persistent *Brucella* infections in wild long-tailed macaques.

Despite the low prevalence of brucellosis in Malaysian livestock such as goats (1%) and the absence of evidence suggesting livestock-to-wildlife transmission, the detection of a single RBPT-positive result, even if a false positive, highlights the importance of continued surveillance. Zulhisam et al. (2026) similarly argued that while the lack of a human-livestock-wildlife interface may break transmission cycles, routine screening of wildlife brucellosis remains crucial, as it may affect the reproductive performance of wild fauna and have implications for conservation. This risk is further reduced by the Malaysian Department of Veterinary Services‘ proactive vaccination and eradication campaigns against brucellosis in farm animals (Department of Veterinary Services, 2020). Nevertheless, the potential of wild macaques acting as reservoirs for *Brucella* spp. cannot be entirely dismissed, as the bacterium can still cause reproductive issues in both wildlife and livestock, therefore posing a potential risk to both local ecological balance and public health (Godfroid, 2002; Schlabritz-Loutsevitch et al., 2009).

Furthermore, previous studies such as those by Huddleson and Hallman (1929), Mense et al. (2004), and Percy et al. (1972) demonstrated that non-human primates, including macaques, are susceptible to various *Brucella* species such as *B. melitensis*, *B. suis*, *B. abortus*, and *B. canis*. Understanding the exact *Brucella* biovars present in wildlife is

essential, particularly given the potential impact on human health. The findings of this study contribute to the comprehensive understanding of *Brucella* spp. infections in wild long-tailed macaques and underscore the importance of using a variety of diagnostic methods, including serological and molecular tests, to accurately detect and monitor the disease, a point also emphasised by Zulhisam et al. (2026) in their recommendation that serological screening results should always be supported with confirmatory tests. In order to prevent future outbreaks and mitigate the risks posed by *Brucella* spp. in non-human primates, surveillance of human-wildlife interactions should be continued, and wildlife-specific diagnostic methods should be further developed.

CONCLUSION

This study found no confirmed anti-*Brucella* antibodies after CFT verification and no detectable *Brucella* DNA in reproductive tissues, indicating no active infection in the sampled macaques. The combined prevalence of exposure or infection was effectively zero, with the 95% confidence interval upper bound below two per cent. Wild long-tailed macaques in the studied areas of Selangor thus pose a negligible risk for brucellosis transmission to humans. The observed pathological lesions in reproductive and hepatobiliary organs were unrelated to brucellosis. Limitations include convenient sampling and the restriction of molecular analysis to reproductive tissues, leaving lymph nodes, spleen, and liver untested. Future studies should broaden tissue panels, incorporate bacterial culture where safe, and expand zoonotic surveillance to strengthen One Health reinforcement.

ACKNOWLEDGEMENT

Special thanks to the Malaysia Wildlife Department (Perhilitan), Department of Veterinary Services (DVS), Veterinary Research Institute (VRI) and Veterinary Laboratory Services Unit, UPM. Moreover, deepest gratitude to SEAOHUN and USAID for the One Health Scholarship for providing financial support for the research.

REFERENCES

Amin, A. S., Hamdy, M. E., & Ibrahim, A. K. (2001). Detection of *Brucella melitensis* in semen using the polymerase chain reaction assay. *Veterinary Microbiology*, *83*(1), 37-44. https://doi.org/10.1016/S0378-1135(01)00401-1

Carvalho, T. P. d., Silva, L. A. d., Castanheira, T. L. L., Souza, T. D. d, Paixão, T. A. d., Lazaro-Anton, L., Tsolis, R. M., & Santos, R. L. (2023). Cell and tissue tropism of *Brucella* spp. *Infection and Immunity*, *91*(5), Article e00062-23. https://doi.org/10.1128/iai.00062-23

Che, L. -h., Qi, C., Bao, W. -g, Ji, X. -feng, Liu, J., Du, N., Gao, L., Zhang, K. -y, & Li, Y. -x. (2019). Monitoring the course of *Brucella* infection with qPCR-based detection. *International Journal of Infectious Diseases*, *89*, 66-71. https://doi.org/10.1016/j.ijid.2019.09.013

Chisi, S. L., Schmidt, T., Akol, G. W., & van Heerden, H. (2017). Use of *Brucella abortus* species specific polymerase chain reaction assay for the diagnosis of bovine brucellosis. *Journal of the South African Veterinary Association*, *88*, Article e1433. https://doi.org/10.4102/jsava.v88i0.1433

Christopher, S., Umapathy, B. L., & Ravikumar, K. L. (2010). Brucellosis: Review on the recent trends in pathogenicity and laboratory diagnosis. *Journal of Laboratory Physicians*, *2*(2), 55-60. https://doi.org/10.4103/0974-2727.72149

Dadar, M., Tabibi, R., Alamian, S., Caraballo-Arias, Y., Mrema, E. J., Mlimbila, J., Chandrasekar, S., Dzhusupov, K., Sulaimanova, C., Alekesheva, L. Z., Manar, S. A., Toguzbayeva, K. K., Wickramatillake, A., & Mirzaei, B. (2022). Safety concerns and potential hazards of occupational brucellosis in developing countries: A review. *Journal of Public Health*, *31*, 1681-1690. https://doi.org/10.1007/s10389-022-01732-0

Dal, T., Kara, S. S., Cikman, A., Balkan, C. E., Acikgoz, Z. C., Zeybek, H., Uslu, H., & Durmaz, R. (2019). Comparison of multiplex real-time polymerase chain reaction with serological tests and culture for diagnosing human brucellosis. *Journal of Infection and Public Health*, *12*(3), 337-342. https://doi.org/10.1016/j.jiph.2018.11.008

Department of Veterinary Services. (2020). *Protokol veterinar Malaysia: Brucellosis (PVM 1(4):2/2020)* [Malaysian veterinary protocol: Brucellosis (PVM 1(4):2/2020)]. Ministry of Agriculture and Food Industries Malaysia. https://www.dvs.gov.my/dvs/resources/user_1/2021/BKPBV/KAWALAN%20PENYAKIT%20&%20ZOONOTIK/PVM_Brucellosis-ver2.pdf

Gallien, P., Dorn, C., Alban, G., Staak, C., & Protz, D. (1998). Detection of *Brucella* species in organs of naturally infected cattle by polymerase chain reaction. *Veterinary Record*, *142*(19), 512-514. https://doi.org/10.1136/vr.142.19.512

García-Méndez, K. B., Hielpos, S. M., Soler-Llorens, P. F., Arce-Gorvel, V., Hale, C., Gorvel, J. -P., O'Callaghan, D., & Keriel, A. (2019). Infection by *Brucella melitensis* or *Brucella papionis* modifies essential physiological functions of human trophoblasts. *Cellular Microbiology*, *21*(7), Article e13019. https://doi.org/10.1111/cmi.13019

Godfroid, J. (2002). Brucellosis in wildlife. *Revue Scientifique et Technique (International Office of Epizootics)*, *21*(2), 277-286. https://doi.org/10.20506/rst.21.2.1333

González-Espinoza, G., Arce-Gorvel, V., Mémet, S., & Gorvel, J. -P. (2021). *Brucella*: Reservoirs and niches in animals and humans. *Pathogens*, *10*(2), Article 186. https://doi.org/10.3390/pathogens10020186

Erdenliğ Gürbilek, S., Tel, O. Y., & Keskin, O. (2017). Comparative evaluation of three serological tests for the detection of *Brucella* antibodies from infected cattle herds. *Journal of Applied Animal Research*, *45*(1), 557-559. https://doi.org/10.1080/09712119.2016.1222942

Henning, L. N., Miller, S. M., Pak, D. H., Lindsay, A., Fisher, D. A., Barnewall, R. E., Briscoe, C. M., Anderson, M. S., & Warren, R. L. (2012). Pathophysiology of the rhesus macaque model for inhalational brucellosis. *Infection and Immunity*, *80*(1). https://doi.org/10.1128/IAI.05878-11

Hinić, V., Brodard, I., Thomann, A., Cvetnić, Ž., Makaya, P. V., Frey, J., & Abril, C. (2008). Novel identification and differentiation of *Brucella melitensis*, *B. abortus*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotomae* suitable for both conventional and real-time PCR systems. *Journal of Microbiological Methods*, *75*(2), 375-378. https://doi.org/10.1016/j.mimet.2008.07.002

Huddleson, I. F., & Hallman, E. T. (1929). The pathogenicity of the species of the genus *Brucella* for monkeys. *The Journal of Infectious Diseases*, *45*(4), 293-303. https://doi.org/10.1093/infdis/45.4.293

Ilhan, Z., Aksakal, A., Ekin, I. H., Gülhan, T., Solmaz, H., & Erdenlig, S. (2008). Comparison of culture and PCR for the detection of *Brucella melitensis* in blood and lymphoid tissues of serologically positive and negative slaughtered sheep. *Letters in Applied Microbiology*, *46*(3), 301-306. https://doi.org/10.1111/j.1472-765X.2007.02309.x

Karuppannan, K., Saaban, S., Mustapa, A., Zainal Abidin, F. A., Azimat, A., & Keliang, C. (2014). Population status of long-tailed macaque (*Macaca fascicularis*) in Peninsular Malaysia. *Journal of Primatology*, *3*(1), 1-4. https://doi.org/10.4172/2167-6801.1000118

Katsiolis, A., Papanikolaou, E., Stournara, A., Giakkoupi, P., Papadogiannakis, E., Zdragas, A., Giadinis, N. D., & Petridou, E. (2022). Molecular detection of *Brucella* spp. in ruminant herds in Greece. *Tropical Animal Health and Production*, *54*, Article 173. https://doi.org/10.1007/s11250-022-03175-x

Küplülü, Ö., & Sarımehmetoğlu, B. (2004). Isolation and identification of *Brucella* spp. in ice cream. *Food Control*, *15*(7), 511-514. https://doi.org/10.1016/j.foodcont.2003.08.002

Legesse, A., Mekuriaw, A., Gelaye, E., Abayneh, T., Getachew, B., Weldemedhin, W., Tesgera, T., Deresse, G., & Birhanu, K. (2023). Comparative evaluation of RBPT, I-ELISA, and CFT for diagnosing brucellosis and PCR detection of *Brucella* species from Ethiopian sheep, goats, and cattle sera. *BMC Microbiology*, *23*, Article 216. https://doi.org/10.1186/s12866-023-02962-2

Lekko, Y. M., Che-Amat, A., Ooi, P. T., Omar, S., Mohd-Hamdan, D. T., Linazah, L. S., Zakaria, Z., Ramanoon, S. Z., Mazlan, M., Jesse, F. F. A., Abdul-Razak, M. F. A., Jasni, S., & Abdul-Hamid, N. (2021). Detection of *Mycobacterium tuberculosis* complex antibodies in free-ranged wild boar and wild macaques in selected districts in Selangor and reevaluation of tuberculosis serodetection in captive Asian elephants in Pahang, Peninsular Malaysia. *Journal of Veterinary Medical Science*, *83*(11), 1702-1707. https://doi.org/10.1292/jvms.21-0144

Liedigk, R., Kolleck, J., Böker, K. O., Meijaard, E., Md-Zain, B. M., Abdul-Latiff, M. A. B., Ampeng, A., Lakim, M., Abdul-Patah, P., Tosi, A. J., Brameier, M., Zinner, D., & Roos, C. (2015). Mitogenomic phylogeny of the common long-tailed macaque (*Macaca fascicularis fascicularis*). *BMC Genomics*, *16*, Article 222. https://doi.org/10.1186/s12864-015-1437-0

Lighter, A. G. (1953). Observations on anticomplementary reactions. *JAMA Dermatology*, *67*(4), 362-368. https://doi.org/10.1001/archderm.1953.01540040020004

Magden, E. R., Mansfield, K. G., Simmons, J. H., & Abee, C. R. (2015). Nonhuman primates. In J. G. Fox, L. C. Anderson, G. M. Otto, K. R. Pritchett-Corning, & M. T. Whary (Eds.), *Laboratory animal medicine* (3rd ed., pp. 771-930). Academic Press. https://doi.org/10.1016/B978-0-12-409527-4.00017-1

Magouras, I., Brookes, V. J., Jori, F., Martin, A., Pfeiffer, D. U., & Dürr, S. (2020). Emerging zoonotic diseases: Should we rethink the animal-human interface? *Frontiers in Veterinary Science*, *7*, Article 582743. https://doi.org/10.3389/fvets.2020.582743

Manivannan, K., Mahmoud, S. M., Ramasamy, M., Shehata, A. A. E., Ahmed, H., Solaimuthu, C., & Dhandapani, K. (2021). Molecular detection of brucellosis in dromedary camels of Qatar by real-time PCR technique. *Comparative Immunology, Microbiology and Infectious Diseases*, *78*, Article 101690. https://doi.org/10.1016/j.cimid.2021.101690

Masjedian Jazi, F., Mirnejad, R., Piranfar, V., Amir Mozafari, N., Zahraei Salehi, T., Khormali, M., Sedighi, M., & Irajian, G. (2017). Real-time PCR and high-resolution melt analysis methods for detection of pathogenic species of *Brucella*. *LaboratoriumsMedizin*, *41*(6), 325-331. https://doi.org/10.1515/labmed-2017-0030

Mazlan, M. (2016). *Pathogenesis of Brucella melitensis infection in female goats* [Doctoral dissertation, Universiti Putra Malaysia]. Institutional Repository UPM. http://psasir.upm.edu.my/id/eprint/92205/

Mense, M. G., Borschel, R. H., Wilhelmsen, C. L., Pitt, M. L., & Hoover, D. L. (2004). Pathologic changes associated with brucellosis experimentally induced by aerosol exposure in rhesus macaques (*Macaca mulatta*). *American Journal of Veterinary Research*, *65*(5), 644-652. https://doi.org/10.2460/ajvr.2004.65.644

Monlab. (2013). *Rose Bengal Plate Test* (Ref: MO-165016) [Package insert]. Monlab S.L. https://www.monlab.es/descargas/microbiologia/SUSPENSIONES%20BACTERIANAS%20y%20rosa%20bengala/IFU%20rosa%20bengala%20monlabtest%20english.pdf

Nyarku, R., Hassim, A., Jonker, A., & Quan, M. (2020). Development of a genus-specific *Brucella* real-time PCR assay targeting the 16S-23S rDNA internal transcribed spacer from different specimen types. *Veterinary Sciences*, *7*(4), Article 175. https://doi.org/10.3390/vetsci7040175

Percy, D. H., Egwu, I. N., & Jonas, A. M. (1972). Experimental *Brucella canis* infection in the monkey (*Macaca arctoides*). *Canadian Journal of Comparative Medicine*, *36*(3), 221-225. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1319672/

Pereira, C. R., Cotrim de Almeida, J. V. F., Cardoso de Oliveira, I. R., Faria de Oliveira, L., Pereira, L. J., Zangerônimo, M. G., Lage, A. P., & Dorneles, E. M. S. (2020). Occupational exposure to *Brucella* spp.: A systematic review and meta-analysis. *PLoS Neglected Tropical Diseases*, *14*(5), Article e0008164. https://doi.org/10.1371/journal.pntd.0008164

Perhilitan. (2018). *Annual report 2018*. Department of Wildlife and National Parks Peninsular Malaysia. https://files.wildlife.gov.my/wildlife-wp-media/2025/09/13094802/LT2018.pdf

Piao, D., Li, Z., Yang, X., Tian, G., Zhao, H., & Jiang, H. (2020). Emerging brucellosis outbreaks associated with unpasteurized milk in China. *China CDC Weekly*, *2*(46), 888-890. https://doi.org/10.46234/ccdcw2020.242

Pradeepkiran, J. A., Bhaskar, M., Shrikanya, K. V. L., Krishna, P. G., Reddy, M. H., Venkatrayulu, C., & Sainath, S. B. (2021). Chapter 1 - Introduction to brucellosis. In S. B. Sainath (Ed.), *Brucella melitensis* (pp. 1-23). Academic Press. https://doi.org/10.1016/B978-0-323-85681-2.00007-0

Preena, P., Ronald, B. S. M., Balakrishnan, S., Murugan, M., Anbu Kumar, K., & Ganesan, P. I. (2024). Serological, bacteriological, and molecular detection of brucellosis in pigs of Tamil Nadu, India. *Emerging Animal Species*, *10*, Article 100041. https://doi.org/10.1016/j.eas.2024.100041

Probert, W. S., Schrader, K. N., Khuong, N. Y., Bystrom, S. L., & Graves, M. H. (2004). Real-time multiplex PCR assay for detection of *Brucella* spp., *B. abortus*, and *B. melitensis*. *Journal of Clinical Microbiology*, *42*(3). https://doi.org/10.1128/JCM.42.3.1290-1293.2004

Sack, A., Daramragchaa, U., Chuluunbaatar, M., Gonchigoo, B., & Gray, G. C. (2018). Potential risk factors for zoonotic disease transmission among Mongolian herder households caring for horses and camels. *Pastoralism*, *8*, Article 2. https://doi.org/10.1186/s13570-017-0109-x

Saechan, V., Tongthainan, D., Fungfuang, W., Tulayakul, P., Ieamsaard, G., & Ngasaman, R. (2022). Natural infection of leptospirosis and melioidosis in long-tailed macaques (*Macaca fascicularis*) in Thailand. *Journal of Veterinary Medical Science*, *84*(5), 700-706. https://doi.org/10.1292/jvms.21-0514

Saytekin, A. M., & Ak, S. (2018). Direct diagnosis of *Brucella* species through multiplex PCR formed by a new method. *Journal of Microbiological Methods*, *154*, 86-94. https://doi.org/10.1016/j.mimet.2018.10.011

Schlabritz-Loutsevitch, N. E., Whatmore, A. M., Quance, C. R., Koylass, M. S., Cummins, L. B., Dick Jr, E. J., Snider, C. L., Cappelli, D., Ebersole, J. L., Nathanielsz, P. W., & Hubbard, G. B. (2009). A novel *Brucella* isolate in association with two cases of stillbirth in non-human primates - first report. *Journal of Medical Primatology*, *38*(1), 70-73. https://doi.org/10.1111/j.1600-0684.2008.00314.x

Song, L., Gao, J., & Wu, Z. (2021). Laboratory-acquired infections with *Brucella* bacteria in China. *Biosafety and Health*, *3*(2), 101-104. https://doi.org/10.1016/j.bsheal.2020.07.010

Sun, S., Jiang, H., Li, Q., Liu, Y., Gao, Q., Liu, W., Qin, Y., Feng, Y., Peng, X., Xu, G., Shen, Q., Fan, X., Ding, J., & Zhu, L. (2021). Safety and transcriptome analysis of live attenuated *Brucella* vaccine strain S2 on non-pregnant cynomolgus monkeys without abortive effect on pregnant cynomolgus monkeys. *Frontiers in Veterinary Science*, *8*, Article 641022. https://doi.org/10.3389/fvets.2021.641022

Teng, Y. -H., Teng, J. -J., Chao, S., Chao, H., & Waghela, S. D. (2017). Comparison of the Rose Bengal plate and the complement fixation tests with the tube agglutination test for diagnosis of human Brucellosis. *Open Journal of Clinical Diagnostics*, *7*(3), 73-82. https://doi.org/10.4236/ojcd.2017.73008

Traxler, R. M., Lehman, M. W., Bosserman, E. A., Guerra, M. A., & Smith, T. L. (2013). A literature review of laboratory-acquired brucellosis. *Journal of Clinical Microbiology*, *51*(9). https://doi.org/10.1128/JCM.00135-13

Vassallo, D. J. (1992). The corps disease: Brucellosis and its historical association with the Royal Army Medical Corps. *BMJ Military Health*, *138*(3), 140-150. https://doi.org/10.1136/jramc-138-03-09

Whatmore, A. M., Davison, N., Cloeckaert, A., Al Dahouk, S., Zygmunt, M. S., Brew, S. D., Perrett, L. L., Koylass, M. S., Vergnaud, G., Quance, C., Scholz, H. C., Dick Jr, E. J., Hubbard, G., & Schlabritz-Loutsevitch, N. E. (2014). *Brucella papionis* sp. nov., isolated from baboons (*Papio* spp.). *International Journal of Systematic and Evolutionary Microbiology*, *64*(12), 4120-4128. https://doi.org/10.1099/ijs.0.065482-0

World Organisation for Animal Health (WOAH). (2022). Chapter 3.1.4. – Brucellosis (infection with Brucella abortus, B. melitensis and B. suis). In *WOAH Terrestrial Manual 2022* (pp. 1-47). https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.01.04_BRUCELLOSIS.pdf.

Wyatt, H. V. (2013). Lessons from the history of brucellosis. *Revue Scientifique et Technique (International Office of Epizootics)*, *32*(1), 17-25. https://doi.org/10.20506/rst.32.1.2181

Yagupsky, P., Morata, P., & Colmenero, J. D. (2019). Laboratory diagnosis of human Brucellosis. *Clinical Microbiology Reviews*, *33*(1), Article e00073-19. https://doi.org/10.1128/CMR.00073-19

Yingst, S. L., Huzella, L. M., Chuvala, L., & Wolcott, M. (2010). A rhesus macaque (*Macaca mulatta*) model of aerosol-exposure brucellosis (*Brucella suis*): Pathology and diagnostic implications. *Journal of Medical Microbiology*, *59*(Pt 6), 724-730. https://doi.org/10.1099/jmm.0.017285-0

Yong, S. -B. C. (2017). *Seroprevalence of leptospirosis and brucellosis in long-tailed macaques (Macaca fascicularis) of Peninsular Malaysia* [Unpublished final year project report]. Universiti Putra Malaysia. http://psasir.upm.edu.my/id/eprint/78261/

Yu, W. L., & Nielsen, K. (2010). Review of detection of *Brucella* sp. by polymerase chain reaction. *Croatian Medical Journal*, *51*(4), 306-313. https://doi.org/10.3325/cmj.2010.51.306

Zakaria, A. M., Ahmed, S. F., & Motawae, M. S. (2019). Seroprevalence and real-time PCR detection of brucellosis in abattoirs animals as a potential route of infection in Egypt. *European Journal of Medical and Health Sciences*, *1*(5). https://doi.org/10.24018/ejmed.2019.1.5.105

Zulhisam, A., Che-Amat, A., Bejo, S. K., & Omar, A. R. (2026). Serological detection of *Brucella* spp. in wildlife from selected areas in Selangor and Pahang. *Journal of Wildlife and Parks*, *40*. https://doi.org/10.64291/xx33xa07