SEROPREVALENCE OF *Brucella melitensis* IN SMALL RUMINANTS IN JOHOR FROM 2020 TO 2022

SHAHAZA, O.[1*], WAN MARIANI, W.H.[2], NORSHAZMIRA, S.[3] AND KAMALIAH, G.[4]

1 Southern Zone Veterinary Laboratory, 80350 Johor Bahru, Johor, Malaysia.
2 Department of Veterinary Services, Putrajaya, Malaysia.
3 Department of Veterinary Services, Johor, Malaysia.
4 Department of Veterinary Services, Pahang, Malaysia.
[*]*Corresponding author:* shahaza@dvs.gov.my

ABSTRACT. An outbreak in a flock of sheep in Johor in 1994 is the first reported case of *Brucella melitensis* in small ruminants in Malaysia. Nevertheless, there is a paucity of current information on small ruminant brucellosis seroprevalence in Johor. The objectives of this study are to describe the apparent seroprevalence at the individual-level among goats and sheep and to illustrate the spatial distribution of small ruminant brucellosis in Johor from 2020 to 2022. Serosurveillance data obtained from the Department of Veterinary Services, Putrajaya, and small ruminant population data acquired from the Department of Veterinary Services, Johor (DVSJ) were analysed. A total of 737 sera were collected involving 72 farms in ten districts in Johor between 2020 and 2022. No seropositive cases were found in goats except in 2022 where the apparent prevalence of goat brucellosis is 2.52% (95%CI: 0.00%, 5.33%) involving one farm in Muar. In sheep, the apparent prevalence is 0.85% (95%CI: 0.00%, 2.52%), 2.20% (95%CI: 0.00%, 5.20%) and 2.33% (95%CI: 0.00%, 5.20%) in 2020, 2021 and 2022, respectively. There were no seropositive cases detected among small ruminants in Kluang, Kota Tinggi, and Mersing for three consecutive years. The spatial pattern was revealed in Batu Pahat in 2020 and Pontian in 2021, while small ruminant brucellosis was detected in districts towards north peninsular Malaysia in 2022. Detection of brucellosis seropositivity in the small ruminant population in Johor signals a potential threat to animals and humans. Thus, the brucellosis control program by test-and-slaughter strategy and animal movement control should be continued along with improvement in farm practices.

Keywords: brucellosis, goat, sheep, Johor, *Brucella melitensis*

INTRODUCTION

Small ruminant brucellosis caused by *Brucella melitensis,* also known as Mediterranean fever was discovered in Malta by Lieutenant Colonel David Bruce in 1887 (Moreno & Moriyón, 2002). To date, the disease has been found in all continents. The disease is characterised by abortion in mid-third gestation in small ruminants. Occupation and drinking unpasteurised milk are significant risk factors for human brucellosis in Malaysia (Bamaiyi *et al*., 2017). In humans, most cases exhibit nonspecific clinical signs such as intermittent fever and arthralgia (Hartady *et al*., 2014). However, complications such as endocarditis have been reported in Malaysia after consumption of unpasteurised goat milk (Tay *et al*., 2017). Although the seroprevalence of brucellosis in Malaysia is low in humans (Nik Mazlan *et al*., 2022) and small ruminants (Zamri-Saad & Kamarudin, 2016), the zoonotic potential of *Brucella* spp., particularly *B. melitensis* should not be ignored. One of the cogent proofs is the outbreak of human brucellosis in Penang, Malaysia between 2011 and 2012. It involved 79 consumers of unpasteurised goat milk (Leong *et al*., 2015).

Since 1978, the National Surveillance Program for Animal Brucellosis has been

exercised by the Department of Veterinary Services (DVS), Malaysia to eradicate brucellosis in cattle, buffalo, and small ruminants (Hartady *et al*., 2014). In this program, serum samples are collected annually at registered farms by the state's DVS to detect brucellosis. The DVS policy for positive brucellosis tests includes culling the animal and compensating the affected farmer based on the number of animals culled. The remaining animals in the herd will be tested every two months until three consecutive negative test results. Six months following the last negative test, the herds will be tested and declared brucellosis-free if seronegative (Department of Veterinary Services, 2020).

Detailed information about the presence of *B. melitensis* in a specific territory is crucial in any control and prevention of brucellosis in humans and animals. Bamaiyi *et al.* (2015) reported that goat brucellosis seroprevalence in Malaysia from 2000 to 2009 was 0.91% (95% CI: 0.86-0.96). They also found that the seroprevalence of *Brucella melitensis* in goats at the animal-level in Johor was 0.56% (95% CI: 0.43-0.72) whilst in neighbouring states to Johor, which are Pahang and Melaka was 0.58% (95% CI: 0.38-0.85) and 1.69% (95% CI: 1.48-1.93), respectively (Bamaiyi *et al*., 2015). Alas, the currently published *B. melitensis* seroprevalence in small ruminants and the distribution of the disease in Johor, is sparse. Therefore, using data from the National Brucellosis Surveillance Programme from 2020 to 2022, this study's objectives are to describe the apparent seroprevalence at the individual-level among goats and sheep and to illustrate the small ruminant brucellosis spatial distribution in Johor from 2020 to 2022. Understanding seroprevalence in small ruminants is important as it plays a substantial role in transmitting the disease to humans.

MATERIALS AND METHODS

Study Area

This study involved Johor; a state located on the southernmost of Peninsular (West) Malaysia. It is divided into 10 administrative districts which are Batu Pahat, Johor Bahru, Kluang, Kota Tinggi, Kulai, Mersing, Muar, Pontian, Segamat, and Tangkak. It covers 18,987 square kilometres and has land borders with Pahang to the north and the northwest, Melaka and Negeri Sembilan.

Data Sources

There are two data sources used in this study. Those data were thoroughly checked for typing errors and accuracy in entry. Information regarding the small ruminant population and the number of farms in Johor was obtained from the Department of Veterinary Services, Johor (DVSJ). The serosurveillance data of Johor State were acquired from the Epidemiology and Surveillance Unit at the DVS, Putrajaya. The information obtained from the data includes farm names and addresses, date of sampling, locations, breed, age range, number of animals tested, the number of animals within the tested herd, testing method, and test results. It is a compilation of annual active brucellosis surveillance program data collected by the DVSJ. Sheep and goat sera were sampled from farms registered with the DVSJ. The sera were tested in the field using Rose Bengal Plate Test (Rose Bengal, CZV) where the positive RBPT sera were analysed at the Veterinary Laboratory of the Southern Zone using iELISA (PrioCHECK® *Brucella* Antibody 2.0 ELISA).

Data Analysis

The data were managed in spreadsheets (Microsoft Excel® 2016, Microsoft Corporation).

An animal was considered *Brucella* positive if the Rose Bengal Plate Test (RBPT) and the iELISA test were positive. Frequency tables were used to calculate apparent prevalence based on districts within Johor by year and species. Apparent seroprevalences were determined as the number of seropositive animals divided by the total number of animals sampled. A Pearson's Chi-squared test with Yates' continuity correction was conducted in R software (R Core Team, 2024) to examine the association between species (goats and sheep) and the presence of brucellosis-positive sera.

The spatial distribution of brucellosis seroprevalence was plotted using the GeoDa program (Anselin *et al*., 2006) and is based on digital administrative maps of Malaysia from DIVA-GIS (DIVA-GIS, 2017). In this study, the individual-level prevalence is presented as quartiles based on the apparent prevalence distribution in small ruminants for all three years across Johor in ten administrative districts which are Batu Pahat, Johor Bahru, Kluang, Kota Tinggi, Kulai, Mersing, Muar, Pontian, Segamat, and Tangkak.

RESULTS

A total of 737 sera were collected from sheep and goats between 2020 and 2022 involving 72 farms (Table 1). Samples were collected from eight, ten, and nine districts in 2020, 2021, and 2022, respectively. Four hundred sera (54%) were from goats while another 337 (45%) were from sheep. The sera collected from goats accounted for 0.005% of the total goat population in Johor in 2019 whilst another two consecutive years, only 0.003%. The sera collected from sheep each year from 2020 to 2022 were consistently 0.006% of the total sheep population in Johor. This surveillance enforced series testing, in which only the positive RBPT sera were analysed by iELISA. Nine animals and six farms were identified as seropositive during the three-year period.

The apparent seroprevalence of small ruminant brucellosis in Johor in 2020, 2021, and 2022 were 0.34% (95% CI: 0.00-0.99), 1.05% (95% CI: 0.00-2.49) and 2.42% (95% CI: 0.51-4.33), respectively. No seropositive cases were detected from goats in 2020 and 2021. However, in 2022, the apparent prevalence of goat brucellosis in Johor was 2.52% (95% CI: 0.00-5.33) involving one goat farm in Muar. The apparent prevalence of sheep brucellosis in Johor increased from 0.85% (95% CI: 0.00-2.52) in 2020 to 2.20% (95% CI: 0.00-5.20) in 2021, while in 2022, it was 2.33% (95% CI: 0.00-5.20). The seropositive cases in 2020 involved one farm with a herd size of 230 sheep in Batu Pahat. In 2021, two sheep farms were seropositive. One farm is in Batu Pahat district (herd size = 45) and another farm (herd size =15) is in Pontian. Three seropositive farms were detected in 2022, one sheep farm in Segamat (herd size = 170), one sheep farm in Muar (herd size = 70), and one farm in Muar that reared 38 goats. The Pearson's chi-squared test with Yates' continuity correction did not reveal a statistically significant association between species and brucellosis seroprevalence, $X^2(1, 737) = 0.869$, $p = 0.351$.

No brucellosis seropositive cases were detected among small ruminants in Kluang, Kota Tinggi, and Mersing for three consecutive years. The spatial distributions of small ruminant brucellosis by district are shown in Figure 1-3. The spatial pattern revealed that from Batu Pahat in 2020, the occurrence of *Brucella melitensis* was detected in the southern neighbouring district, Pontian in 2021. Interestingly, the disease was moving towards north peninsular Malaysia in 2022 while no seropositive cases were detected in Pontian and Batu Pahat.

Table 1. Number of serum and farm sampled in small ruminants for the brucellosis surveillance program in Johor from 2020 to 2022

Species	Goat			Sheep			Total
Year	2020	2021	2022	2020	2021	2022	
No. of serum tested	181	100	119	117	91	129	737
No. of brucellosis-positive sera	0	0	3	1	2	3	9
No. of farm tested	17	10	12	11	9	13	72
No. of brucellosis-positive farm	0	0	1	1	2	2	6

Table 2. The apparent seroprevalence of small ruminant brucellosis at the individual level from 2020 to 2022 by district

District	Year 2020	Year 2021	Year 2022
Batu Pahat	1.15 (0.00, 3.38)	2.50 (0.00, 7.33)	0.00 (0.00, 7.21)
Johor Bahru	NO DATA	0.00 (0.00, 25.85)	0.00 (0.00, 9.42)
Kluang	0.00 (0.00, 9.50)	0.00 (0.00-13.90)	0.00 (0.00, 9.81)
Kota Tinggi	0.00 (9.00, 25.85)	0.00 (0.00, 25.85)	0.00 (0.00, 25.85)
Kulai	NO DATA	0.00 (0.00, 25.85)	0.00 (0.00, 13.90)
Mersing	0.00 (0.00, 7.18)	0.00 (0.00, 13.90)	0.00 (0.00, 13.90)
Muar	0.00 (0.00, 5.79)	0.00 (0.00, 9.19)	10.00 (0.74, 19.26)
Pontian	0.00 (0.00-7.19)	5.00 (0.00, 14.52)	0.00 (0.00, 9.79)
Segamat	0.00 (0.00, 13.90)	0.00 (0.00, 13.90)	6.67 (0.00, 15.57)
Tangkak	0.00 (0.00, 13.28)	0.00 (0.00, 25.85)	NO DATA

Note: The prevalence was reported in percentage at 95% CI (Lower Limit, Upper Limit)

Figure 1. Spatial distribution of small ruminant *brucellosis* in Johor in 2020. Only at Batu Pahat was seropositive. No data available from Kulai and Johor Bahru

Figure 2. Spatial distribution of small ruminant brucellosis in Johor in 2021. Seropositive cases were detected in Batu Pahat and Pontian

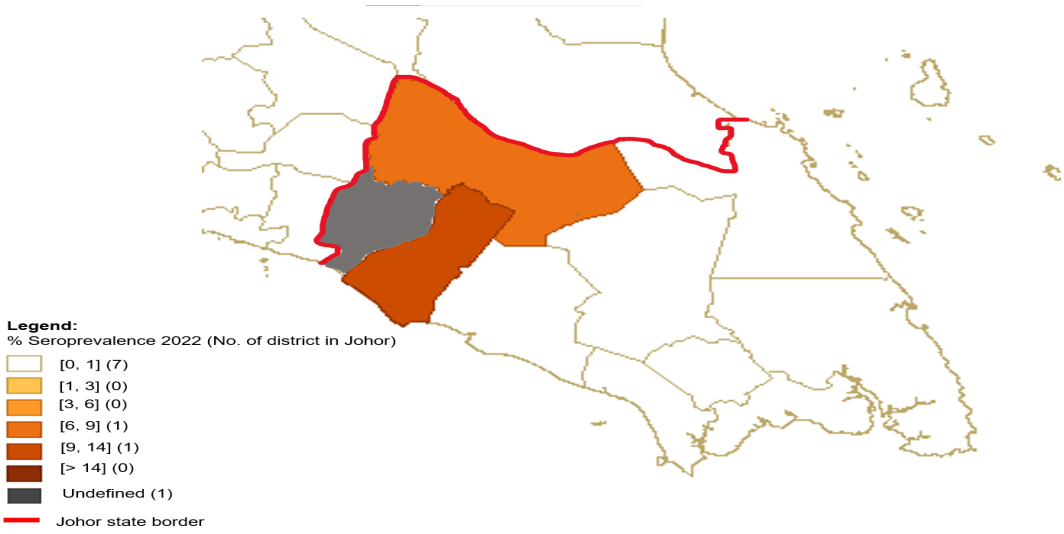

Figure 3. Spatial distribution of small ruminant brucellosis in Johor in 2022. Seropositive cases were detected in Muar and Segamat. No data (undefined) is available from Tangkak

DISCUSSION

This study reported the apparent seroprevalence at the individual-level among goats and sheep in Johor using the brucellosis surveillance data from 2020 to 2022. Using the GeoDa program (Anselin *et al.*, 2006), the spatial distribution of small ruminant brucellosis for the aforementioned period was demonstrated by districts in Johor. This study detected that brucellosis seroprevalence from 2020 to 2022 in small ruminants was increasing in Johor. The overall seroprevalence of brucellosis was found to be higher in sheep than in goats. This finding is consistent with Gompo *et al.* (2021). The seroprevalence in goats was higher than reported by Bamaiyi *et al*. (2015). In 2022, seroprevalence of *B. melitensis* was slightly higher in goats compared to sheep. The lower sample size of goats compared to sheep whilst the number of positive sera is similar in each species might account for this observation (Table 1). However, the results of the Pearson's chi-square test indicate that there is no statistically significant difference in brucellosis seroprevalence between goats and sheep. Therefore, the seroprevalence of brucellosis does not significantly differ between the two species.

This study spotted brucellosis seropositive cases scattered from a small herd size farm with fifteen heads of animal to a large farm with a herd size of 230 animals. Transmission between animals is commonly through ingestion of the organism and doe or ewe at the third trimester of gestation is more susceptible to infection (Poester *et al*., 2013). An introduction of pregnant and infected animals into the farm might expose the herd to brucellosis. Those animals could shed the organism through vaginal discharges. Ewe might shed the organism in vaginal discharge for about a few weeks postpartum (Givens & Marley, 2008). However, shedding in vaginal discharges persists for at least two to three months in goats (Rovid-Spickler, 2007). During the investigative years, all positive cases involved less than four

animals per farm. The source of infection was not established.

There was variability in the distribution of the disease with one or two districts experiencing significantly higher seroprevalence of brucellosis than others**.** This pattern resembled a study in the goat population in Negeri Sembilan reported in 2012. The team found that, out of seven districts, only two districts (Rembau and Kuala Pilah) have farms that were seropositive for *B. melitensis* (Siti Sumaiyah *et al*., 2012). In 2022, no seropositive cases of brucellosis were detected in Batu Pahat, the district that had the largest small ruminant population in Johor. Effective brucellosis control program by test-and-slaughter strategy, animal movement control, and improvement in farm management practices could have contributed to the absence of brucellosis in 2022. Those control measures are proven effectual in New Zealand, which is now free of brucellosis for the past 28 years (WOAH, 2021).

The results of this study should be considered with limitations in mind. There was a potential for bias in using data collected for the surveillance system. Farms taking part in the surveillance system actively seek out registration with the DVSJ and may not be entirely representative of farms at the state level. Moreover, the sampling did not include animals in the Mersing archipelago. An additional limitation of this data was in 2020, where there were no small ruminant population from Johor Bahru and Kulai involved in the surveillance. From 2020 to 2022, data on sheep brucellosis in Kota Tinggi were not available. In 2021, although all districts were involved in the brucellosis surveillance, no sheep were sampled in Johor Bahru, Kluang, Kota Tinggi, and Kulai. In addition, in the same year, goats were not sampled in Tangkak. In 2022, no serosurveillance data were gathered from Tangkak. No specific reasons could be offered for the imperfection. Consequently, interpretation of disease distribution across species and districts should be exercised with caution. To improve the data quality, it is crucial to ensure the data on brucellosis seropositive cases are complete and consistently collected across all districts.

Another point to be considered is this study utilised serological tests that were unable to differentiate *Brucella* spp. (Applied Biosystem, 2019; CZ Vaccines, 2023; WOAH, 2022). We presumed that a seropositive small ruminant was infected with *B. melitensis*. The small ruminant could be seropositive while being infected by other smooth strains of *Brucella* spp such as *B. abortus*. In the National Brucellosis Surveillance Programme, Malaysia deployed serial testing instead of parallel testing. The latter will reduce the likelihood of false negative cases (Paucar *et al*., 2021) and is recommended by the World Organisation of Animal Health in eradication policy (WOAH, 2022). To address the cost-effectiveness issue, which might be one of the hindrances in using two tests simultaneously, DVS might consider a combination of serial and parallel testing. Districts with low to moderate seroprevalence i.e., less than 5% (European Commission, 2009) might continue with series testing as it reduces false positive occurrence. Meanwhile, districts with high seroprevalence (>5%), for instance, Muar (10.0%) and Segamat (6.67%) should implement parallel testing to accelerate the process of reducing the number of infected animals that could linger on the farm.

CONCLUSION

The presence of brucellosis seropositive animals along with the increasing small ruminant population, particularly in Johor, signals a potential threat for animals and humans. Serological test is a more convenient tool for brucellosis detection compared to the isolation

of the *Brucella melitensis*. Seroprevalence data provide evidence of previous exposure to the bacteria. It also enables monitoring of trends and facilitates the control of small ruminant brucellosis in Johor. Thus, this leads to a well-informed decision and ensures appropriate resource allocation for active surveillance that certainly should be concurrently running with other existing components such as brucellosis-free farm certification programs, education and awareness campaigns, and control of animal movement.

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