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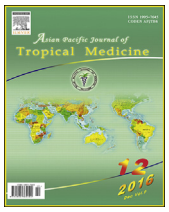

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Control of animal brucellosis: The Malaysian experience

M. Zamri-Saad[1✉], M.I. Kamarudin[2]

[1]*Research Centre for Ruminant Diseases, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Malaysia*

[2]*Department of Veterinary Services Malaysia, Ministry of Agriculture and Agro-based Industry, 62250 Putrajaya, Malaysia*

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ABSTRACT

Brucellosis is a zoonotic disease characterized by reproductive failure in animals and undulent fever in humans. In cattle, it is caused by *Brucella abortus* while in goats by *Brucella melitensis*, the main cause of brucellosis in humans. Brucellosis in livestock has been associated with importation of animals from breeder herd of unknown disease status. The prevalence of bovine brucellosis *Brucella abortus* in 2014 ranged between 1% and 2% in Thailand and Indonesia, and 4%–5% in Malaysia and Myanmar. Prevalence of goat brucellosis *Brucella melitensis* is approximately 1% in Malaysia and Thailand. 'Test-and-slaughter' is the general policy against brucellosis adopted by most ASEAN countries to eradicate the disease. Under this program, the Rose Bengal Plate Test (RBPT) is used as the screening test to identify infected farm/herd while the complement fixation test (CFT) is the confirmatory test. The test-and-slaughter eradication strategy that was implemented since 1979 had managed to keep the prevalence rate to less than 5%, from 3.3% in 1979, 0.23% in 1988, 1% in 1998 and 5% in 2016. The test-and-slaughter program seemed effective in reducing the prevalence of brucellosis but was unable to eradicate the disease due to several factors, which include failure to locate and identify the remaining affected animals and to control their movement, importation of breeder animals from non-brucellosis free countries and lack of participation by the farmers following unreliable test results. To support the eradication policy, research activities since 1980s have suggested combinations of serological tests to improve diagnosis while surveillance should be focused on hotspots areas. The prevalence can be further reduced by strictly sourcing breeder animals from brucella-free areas or countries.

1. Introduction

Brucellosis is one of the most important zoonotic diseases of ruminant in the Southeast Asia. In large ruminant, it is caused by *Brucella abortus* (*B. abortus*) [1] while *Brucella melitensis* (*B. melitensis*) infects mainly small ruminant, particularly goats [2]. The disease has been associated with importation of breeder cattle and goats from various ruminant-producing countries of unknown disease status. With the importation of animals, diseases are likely to accompany [3].

Most ASEAN countries use the 'test-and-slaughter' program to eradicate brucellosis. This requires an efficient surveillance program and a quick and reliable test protocol. The current use of Rose Bengal Plate test (RBPT) for screening of infected herd followed by the complement fixation test (CFT) on RBPT-positive sera is believed to lead to culling of many uninfected breeders [4]. This paper reviews the status, control policy and research activity on animal brucellosis in Malaysia.

2. Prevalence of brucellosis in Southeast Asia

Indonesia records the most numbers of livestock in the Southeast Asia, followed by Vietnam and Thailand (Table 1). Malaysia recorded the least. Animal brucellosis is considered endemic in Southeast Asia [11]. The prevalence of bovine brucellosis (*B. abortus*) in 2013 ranged between 1% and 2% in Thailand and Indonesia, and 4%–5% in Malaysia and Myanmar (Table 1). The prevalence of goat brucellosis

✉First and corresponding author: M. Zamri-Saad, Research Centre for Ruminant Diseases, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Malaysia.
Tel: +603 86093453
Fax: +603 89471971
E-mail: mzamri@upm.edu.my
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Table 1
Livestock population and prevalence of *Brucella abortus* and *Brucella melitensis* among livestock in the Southeast Asia (2013).

Country	Large ruminants	Small ruminants	Pigs	*B. abortus* (%)	*B. melitensis* (%)	References
Indonesia	18 727 000	33 136 000	NA	2.7	NA	[5]
Lao PDR	3 641 858	603 078	2 964 762	0.0	0.0	[6]
Malaysia	869 826	612 130	1 816 661	4.8	0.8	[7]
Myanmar	14 200 000	2 000 000	4 500 000	0.5	NA	[8]
Thailand	5 920 484	482 317	9 511 389	1.1	1.0	[9]
Vietnam	7 316 266	1 345 421	26 261 408	0.0	0.0	[10]

(*B. melitensis*) is approximately 1% in Malaysia and Thailand (Table 1). The high prevalence in Malaysia has been associated with importation of breeder animals [12], herd size and farm biosecurity [8] while the unknown prevalence in Vietnam and the Philippine might be due to the different focus of the disease surveillance activity [8] and brucellosis eradication program is not in place. However, the most important factor that influences prevalence rate of brucellosis is the uncontrolled animal movements. Stable animal populations in Indonesia and Thailand maintained low rate of prevalence because of less animal movement [5,9] while unstable population in Malaysia and Myanmar due to livestock importation and export resulted in active animal movement that spread the disease. Singapore and the Philippine had eradicated the disease earlier but brucellosis has recently re-emerged [13].

Brucellosis caused by *B. abortus* is an old disease in most Southeast Asian countries, especially Malaysia that recorded the disease since 1950 and Thailand since 1956 [14,15]. In Malaysia, it has been reported to be high among cattle population under integration with plantation i.e. cattle raised extensively, utilizing local grass and shrub grown underneath the main trees such as oil palm with 6.9% prevalence [16]. This is particularly high in the states of Pahang and Johor following importation of infected cattle (Table 2) from Thailand [18]. A recent study in Malaysia revealed the overall prevalence of bovine brucellosis at 2.5%, involving the central states of Peninsular Malaysia and the state of Pahang remained high [15]. Herd size, breed, animal movement and interaction with other species of animal particularly wildlife and small ruminants have been associated with the high prevalence [19]. Thus, the odd of brucellosis in large ruminants is 1.6 times more compared to small ruminants [20].

Estimated total economic losses due to brucellosis in Malaysia is approximately RM200 607 946.80 a year [20]. Nevertheless, there are several hotspots for bovine brucellosis that have been identified in Peninsular Malaysia [19]. The first involves states of Johor and Pahang, covering 68.14 km radius mostly due to concentration of cattle rearing within FELDA Palm Oil Plantation in these two states. The second hotspot involves 51 districts in Kelantan, Perak, Terengganu, Selangor and Pahang covering a total area of 229.98 km radius due to the importation activities and presence of private quarantine station. The third hotspot covers an area of 34.39 km radius in Selangor and Federal Territory.

Table 2
Reactor rates (%) for brucellosis among imported cattle [17].

Quarantine station	1994	1995	1996	1997	1998	Average
Rantau Panjang [Kelantan]	8.07	0.00	5.30	7.52	5.30	5.24
Padang Besar [Perlis]	2.60	6.10	9.70	2.80	1.30	4.50

There was no sero-positive reactor among sheep between 1981 and 1986 when a total of 1436 sheep sera were tested. However, following massive importation of sheep in 1986, there were 2 reactors in 1987 and since then sero-positive had been recorded among sheep in Terengganu, Kedah, Perlis and Pahang [21]. The prevalence of ovine brucellosis in Malaysia was 0.02% in 1987, 0.11% in 1988, 0.05% in 1989, 0.03% in 1990 and 0.13% in 1991 with Terengganu showing higher percentage. *Brucella ovis* was eventually isolated for the first time from sheep in Malaysia in 1991 [21] that prompted a call for control measure among sheep.

Infection by *B. melitensis* among goats in Asia is quite recent [22]. In Malaysia, it coincides with the extensive importation of breeder goats since 2006 [23]. The prevalence among goats and sheep in Malaysia was reported at 10.7% and 14.5% in 2009, respectively [24]. This highlighted the need for a serious attention also extended for control measure against *B. melitensis* infection in Malaysia. A later study revealed that the prevalence of brucellosis among sheep and goats in Malaysia in 1994 was 16.84% but reduced to approximately 1% between 1997 and 2008. Between 2009 and 2011, the prevalence was 1.5% [17]. Recent study revealed that the seroprevalence of brucellosis among goats was 0.91% (95% *CI* = 0.86–0.96) and among farms was 7.09% with states like Perlis that border neighbouring countries showing higher seroprevalence [25].

3. Human brucellosis in Southeast Asia

Human brucellosis has been reported in Southeast Asia, particularly Indonesia [26], Malaysia [27], Singapore and Myanmar [28] and Thailand [29]. It is recognised as an emerging zoonotic disease in Southeast Asia and is frequently caused by *B. melitensis* [30]. Human infections are usually associated with handling of infected animals and/or consumption of contaminated milk, particularly goats [31,32]. Therefore, failure to control animal brucellosis might lead to increasing incidence of human brucellosis [31]. Most isolates of *B. melitensis* are susceptible to most antibiotics except rifampicin [33]. Nevertheless, prescription of rifampicine *p.o.* once a day combined with doxycycline *p.o.* twice a day for 6 consecutive weeks has been proven effective [27].

4. Government policy on brucellosis

'Test-and-slaughter' is the current method used to control brucellosis in many ASEAN countries. This method is suitable for eradicating all emerging and re-emerging zoonotic livestock diseases when the disease prevalence is low [34]. In Malaysia, the national program for 'Area-Wise Eradication of Bovine Brucellosis' was implemented in 1979 that was based on the

'test-and-slaughter' protocol. The Rose Bengal Plate Test (RBPT) is used as the screening test to identify infected farm or herd while the complement fixation test (CFT) is the confirmation test to identify infected individuals [35]. Farms/herds tested positive on RBPT are recognized as infected farm/herd before sero-positive animals in the infected farms/herd are subjected to CFT. Subsequently, animals tested positive on CFT are culled with compensation. A 4-year study in the state of Melaka, one of the identified areas for eradication of animal brucellosis revealed a total compensation of RM146, 986.50 [USD45, 865.24] was paid under the brucellosis eradication program [26].

The 'test-and-slaughter' method is expensive and only recommended and feasible in countries where prevalence rate is not exceeding 2% [2] while surveillance and laboratory facilities are excellent. At 5% prevalence, the 'test-and-slaughter' seemed unsuitable for Malaysia but the small number of cattle in Malaysia of approximately 800 000 head makes the program less expensive and feasible. Nevertheless, the national bovine brucellosis eradication program in Malaysia had successfully reduced the prevalence from 3.3% in 1979 to 0.23% in 1988 to approximately 1% in 1998 [36] but eventually increased to the current 5% [7]. However, the 'test-and-slaughter' program remains unpopular among farmers who claim that the program eliminated uninfected breeder animals. It was claimed that only 10% of goats that were sero-positive to *B. melitensis* in Malaysia that were culled were infected [4] while 21%–23% of the infected animals were correctly removed from the herds in Macedonia [37].

Since brucellosis is endemic in most ASEAN countries with prevalence rates range between 1% and 4%, implementation of brucellosis control policy needs to be reviewed. Furthermore, ASEAN region has been living with this disease particularly *B. abortus* for more than 30 years and the current 'test-and-slaughter' with compensation policy as practiced in Malaysia had proven to be successful in reducing the prevalence at the initial stage [36]. However, the subsequent increase in prevalence [11] was due to the difficulties in detecting infected animals in each herd since the prevalence was extremely low [36]. It, therefore, becomes necessary to review the current 'Area-Wise Eradication of Bovine Brucellosis' by enhancing the approaches and reducing time required from serum collection to compensation payment. This enhanced herd-wise eradication program requires extensive surveillance program that is supported by an efficient laboratory, particularly speedy testing turn-around time and sufficient compensation to ensure the success of 'test-and-slaughter' eradication program. Generally, for developing countries, elimination of *Brucella*-infected animals is not affordable [38].

5. Control by vaccination

Vaccination is aimed at reducing the amount of the agent produced by infected herd with the hope of reducing the incidence [39]. Therefore, it is only practiced for diseases that have become endemic in a country or herd [40] because it significantly reduces excretion of microorganisms [41] although the organism remains in the country. The current available vaccines for both bovine and caprine brucellosis contain live attenuated organisms. Cattle vaccines contain either the smooth strain *B. abortus* S-19 or the rough strain RB-51 [42,43] while caprine vaccine contains attenuated *B. melitensis* vaccine strain Rev-1 [38]. These live attenuated vaccines were shown to be effective in preventing abortion and transmission of brucellosis, but poor at preventing infection or sero-conversion [44].

In principle, vaccination against brucellosis is practiced in countries with high prevalence of more than 5%, particularly in developing countries since vaccination is relatively cheap and readily acceptable by the farmers [45]. In fact, regions with high prevalences of brucellosis have been shown to experience significant economic advantages when implementing a vaccination strategy to control the disease [46], but vaccination alone does not allow control of brucellosis. Health management and timely diagnosis are also needed to control the disease [47]. Despite these, vaccination against brucellosis is not practiced in Malaysia. Since vaccination is not going to eliminate brucellosis [48,49] while the immune response following vaccination might interfere with sero-diagnosis [50], vaccination is not recommended in countries practicing 'test-and-slaughter' program.

6. Research activity

In line with the policy of test-and-slaughter that was started in 1979, initial research activities on ruminant brucellosis in Malaysia were focused on improvement of diagnosis. This is because quick and accurate diagnostic procedure is important when the 'test-and-slaughter' policy is to be used to control the disease [51] and since there are no accurate serological tests available, sero-diagnosis is achieved based on the results of two or more tests [52]. Quick and accurate identification of infected individuals in infected farms/herds is the key objective in this policy.

The serology gold standard for brucellosis, the complement fixation test (CFT) was compared with indirect ELISA and competitive ELISA as confirmatory serological diagnosis of brucellosis in goats [35]. The outcomes revealed strong correlations between the results of CFT and indirect ELISA (R^2 = 0.96), and competitive ELISA (R^2 = 0.91). Similarly, Fikri [53] developed a *B. melitensis* ELISA kit and found 98% comparable to CFT. Thus, the CFT was recommended as confirmatory test for goat brucellosis in Malaysia. Recently, however, an in-house RBPT was developed using local isolate of *B. melitensis* [53]. The subsequent comparison on the sensitivity and specificity with the commercially available RBPT that uses *B. abortus* as antigen revealed that the in-house RBPT was more sensitive (93.2%) than the commercial RBPT (89.0%) while specificity was lower (95.8%) than commercial RBPT (99.1%). Eventually, it was recommended that the commercial RBPT being replaced by the in-house RBPT for screening of goat brucellosis in Malaysia [54].

Subsequently, organ samples from culled goats that were sero-positive on RBPT and CFT were subjected for isolation of *B. melitensis* and PCR and were found that there was no correlation between serological test positive results and the presence of *B. melitensis* in goats [55]. Similarly, the organs of goats that was sero-positive on RBPT were examined for lesions and was found that only goats that were sero-positive with RBPT titre of $\geq$4 showed pathology lesions, particularly in the uterus and mammary glands and likely to be infected [56].

In recent years, research activities on animal brucellosis in Malaysia have shifted to epidemiological issues, particularly understanding the prevalence [11], pattern of incidence [23], risk factors [57] and identification of hotspots for brucellosis

occurrence [25]. Furthermore, research on the pathogenesis and immune response against *B. melitensis* are gaining momentum in trying to understand this newly emerging zoonotic disease in Malaysia [58,59].

7. Conclusion

The prevalence rate, endemic status of the disease and the non-effective 'test-and-slaughter' policy after 30 years of implementation require a thorough review of brucellosis control policy. However, alternative control measure of using vaccine has its drawbacks. Therefore, in reviewing the policy considerations must be used. Review might involve identifying gaps on each activity, addressing each gap, and plotting the timeline for freedom to be achieved.

Conflict of interest statement

We declare that we have no conflict of interest.

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