

STATUS OF BOVINE BRUCELLOSIS IN THE SUBMITTED SAMPLES AT VETERINARY RESEARCH INSTITUTE FROM 2019 TO 2021

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ABSTRACT

Bovine brucellosis is an economically significant disease in Sri Lanka due to reducing calves with abortion, reproductive disorders, reduced milk production in cattle and buffaloes. *Brucella* is a Gram-negative, facultative intracellular bacterium and appears as a small coccobacillus of 0.6-1.5µm long and 0.5-0.7µm wide. Brucellosis is an endemic disease in Sri Lanka with multiple hosts, including cattle, buffaloes, goats, sheep and pigs. Rose Bengal Plate Test (RBPT) is the standard screening test for serological diagnosis of brucellosis in ruminants and confirmation is done by the Complement Fixation Test (CFT), which is the gold standard test for serological diagnosis of brucellosis in ruminants. In Sri Lanka, the CFT confirmation is only done at Veterinary Research Institute, Peradeniya, as the national reference laboratory for brucellosis in livestock. The objective of this study was to determine the status of bovine brucellosis in the submitted sample to the Veterinary Research Institute during the period 2019 to 2021. The samples were received from herds with a recent history of abortion from 2019 to 2021. The serum samples were tested by RBPT and confirmation was done by CFT. Conventional bacteriological methods carried out the isolation and identification of *Brucella abortus*. PCR was carried out only for the confirmed isolates. The average annual percentage of bovine brucellosis in the herd with a history of abortions was varied from 19.4 - 38.5, 1/3 of cattle and buffaloes with a history of abortion were diagnosed as brucellosis serologically. More than 60% of CFT positive samples each year were shown high antibody titre as 1:32, indicating actual incidence of disease in a suspected herd. Serologically positive animals were found in several administrative districts in the country, including dry zone and wet zone. Brucellosis was the main cause of abortion in cattle and buffaloes in Sri Lanka for the samples that were submitted during 2019 – 2021 to the VRI. The current prevalence of bovine brucellosis is unknown since no countrywide surveillance is established. The sample submission had been badly affected by ongoing COVID 19 pandemics in the country. In summary, brucellosis is an endemic disease in the dry zone of the country, although single outbreaks have been reported in the upcountry during recent years. Bovine brucellosis was one of the most common causes of bovine abortion from 2019 to 2021 in Sri Lanka.

Keywords: Brucellosis, Cattle, Complement Fixation Test, Rose Bengal Plate Test

1. INTRODUCTION

Brucellosis is an old disease in livestock and human (Bandara and Mahipala, 2002) Karunanayake et al., 2019; Rossetti et al., 2017). *Brucella* was reported by David Bruce from the spleen of a British soldier who died from Malta fever in Malta in 1887 (Rossetti et al., 2017). The organism was named *Brucella* in honour of David Bruce by Alice Evans (Rossetti et al., 2017). It is a Gram-negative, facultative intracellular bacterium and appears as a small coccobacillus 0.6-1.5µm long and 0.5-0.7µm wide (Quinn, 1994). Importantly, all the species under *Brucella* are closely related phylogenetically, while over 90% are similar genetically (Li et al., 2019; Quinn, 1994; Rossetti et al., 2017). As these species are closely related, the

brucella genome consists of highly conserved genetic material at the genus level (Khurana et al., 2021). There are two independent chromosomes in a cell, a size of 2.10 and 1.15 Mb in size (Kim et al., 2012; Li et al., 2019). The GC content is an average of 57%, while the single genome comprises 3200-3400 opening frames (Kim et al., 2012; Li et al., 2019).

More than 13 species have been reported under Genus *Brucella*. *Brucella abortus* is found commonly in cattle & buffaloes mainly, while *Brucella melitensis* in sheep and goats (Khurana et al., 2021; Li et al., 2019). *Brucella* species are also found in other species of livestock; multiple hosts are a unique characteristic of Genus *Brucella* (Khurana et al., 2021; Li et al., 2019). Brucellosis is an economically important disease in

most low-income countries in the world (Maryam Dadar, 2021). Bovine Brucellosis is a common problem in South Asia and South-East Asia. Prevalence of Brucellosis is 1%, 2%, 4%, 5% in Thailand, Indonesia, Malaysia and Myanmar, respectively (Maryam Dadar, 2021). In summary, the prevalence of brucellosis is varied from 1-60% based on sampling techniques and a number of risk factors have been identified for bovine brucellosis as host factors, farmer's factor, management factors and agro-ecological factors (Maryam Dadar, 2021).

Brucellosis is an endemic disease in cattle, buffaloes, goats, sheep and pigs in Sri Lanka (Priyantha, 2008; Priyantha, 2010; Priyantha, 2009). *Brucella abortus* is the dominant species in livestock now, while only one report of *B. melitensis* and no case of *B. ovis* were reported in livestock recently (Fernando, 2017; Priyantha, 2009). The disease was first introduced into Sri Lanka by the British Army during the second world war and the first clinical case was found in the Livestock Research station at Polonnaruwa in 1956 (Bandara and Mahipala, 2002). It has been reported that Biotype 3 was the main organism of *Brucella abortus* in Sri Lanka in 1971 (Bandara and Mahipala, 2002; Priyantha, 2011). The overall prevalence of Brucellosis was 4.7% and 4.2% in cattle and buffaloes in 1992-95, respectively (Silva et al., 2000). A similar percentage of prevalence was reported from late 1990 to 2006 (Priyantha, 2008). The disease is endemic dry zone and over 75% of seropositive males were found in that endemic zone of the country from 1992-95 (Silva et al., 2000). In a recent study, animal level seroprevalence and herd-level seroprevalence were reported as 2.7%, 9.6% in selected Veterinary ranges in the Northern Province of the country, respectively (Kothalawala et al., 2017).

Bovine brucellosis is an economically important disease in Sri Lanka due to reducing calves with abortion, reproductive disorders, reduced milk production and other systemic diseases in cattle and buffaloes (Priyantha, 2008). In the livestock sector, diagnosis of brucellosis is done by the regional veterinary investigation centers in the country by Rose Bengal Plate test (Priyantha, 2008). In addition, Milk ring test is also performed for the bulk milk samples at milk collection centres (Priyantha, 2008). The confirmation of RBPT positive samples needs to be sent to the bacteriology division, the Veterinary Research Institute being the national reference laboratory for diagnosis of Brucellosis in the country in livestock (Priyantha, 2008). The complement fixation test is performed for the RBPT positive sample for confirmation in livestock (Priyantha, 2008). Only a limited sample from companion animals under the special request is done at Veterinary Research Institute. However, samples from companion animals are not tested for brucellosis routinely. In addition, isolation and identification of *Brucella abortus* is also performed in suspected milk, aborted fetus and vaginal swabs in infected animal at the central facility only since special requirement are needed for *Brucella abortus* as CO₂, 42°C and long

incubation period as 72 hours. All the testing agents, such as RBPT antigen, MRT antigen, are locally prepared by the Veterinary Research Institute from S99 strain of *Brucella abortus* (Priyantha, 2008).

There is no fixed document for Brucellosis control programme in the country, although an official document is found only for S19 vaccination. Only 6-14 months old female calves at endemic region are vaccinated (Priyantha, 2009). Since culling of animals is complicated with the government slaughter policies, existing laws, and regulation as well as religious taboos in the country. In addition, no ongoing surveillance programme for brucellosis has existed, although suspected animals are screened for brucellosis. The vaccination is not properly organized due to the lack of national policies. Only 7500-15000 doses of vaccine are used in the national programme to control brucellosis in the country. In summary, the exact prevalence of brucellosis in the country is not known due to the lack of surveillance mechanism, extensive studies in livestock now. However, human infection is considered emerging in Sri Lanka. The objective of this study was to determine the status of bovine brucellosis in the submitted sample at Veterinary Research Institute in the period of 2019-2021. This is the only alternative to understand the status of the disease in livestock since no ongoing countrywide brucellosis surveillance is established.

2. MATERIALS AND METHODS

2.1. Sample collection

The sample collections were done in the suspected herds for brucellosis with clinical signs (history of abortion at the third trimester, other reproductive disorders such as repeat breeding, retained placenta and infertility) in a herd, farm. The suspected herds were initially identified by regional government veterinary surgeon and district veterinary Investigation officer were informed to collect samples for brucellosis. The collected blood samples were separated into serum and stored at -20°C. The RBPT was done at VICs, only positive samples were sent to the reference laboratory for the confirmation

2.2 Laboratory test:

All the laboratory tests were done in the national reference laboratory for diagnosis of brucellosis in animals, Veterinary Research Institute, Peradeniya, Sri Lanka. The serological diagnosis of brucellosis is carried out with Rose Bengal Plate test (RBPT) and confirmed by complement fixation test (CFT) as described by Alton et al 1988 (Alton et al., 1988). The RBPT antigen is made in house in the division as described previously (Alton et al., 1988). The CFT is carried out according to the methods described by Alton et al, 1988 (Alton et al., 1988). Equivalent and greater than 20 ICFTU (International unit of complement fixing antibodies) was considered as a positive serum as described previously (Królak and Błaszczuk, 1986). The conventional isolation identification of *Brucella* species was carried out with

submitted samples as described by Alton et al, 1988 for placental tissue, aborted fetuses, milk and vaginal swabs (Alton et al., 1988). The isolates of *Brucella* were confirmed by conventional PCR test as described (Alamian et al., 2017). PCR conditions were one initial denaturation at 95°C for 4 minutes followed by 30 cycles of 94°C for 30 seconds, 52°C for 30 seconds, and 72°C for 45 seconds. The final extension step was performed at 72°C for 5 minutes (Primer sequences were 5'-GGC TAT CGG CTG GGA AAG G-3' and 5'-CCT TCC GAA GAA AAT ACC CCT-3'). Both known positive control and negative control are used in every test of CFT. All samples were tested for *Leptospira* by Micro agglutination test for common serovars found in the country as described methods (Chirathaworn et al., 2014).

Isolation of *Brucella abortus* was minimum in the study period. Only isolate in 2020 was identified as *Brucella abortus* by conventional biochemical test and confirmed by PCR method described previously. The submitted samples were serologically negative for common for *Leptospira* serovars tested for in the study.

3. RESULTS

According to the clinical submission to the division of bacteriology which is the national reference laboratory for diagnosis of brucellosis in livestock, the average annual percentage of bovine brucellosis was varied 19.4-38.5% in herds of the history of abortions in 2019 - 2021 (Table: 1). The samples were collected from regional veterinary investigation centres (VICs) at district level, those samples were sent to the national laboratory for the confirmation. Serologically positive animals were found from several administrative districts such as Trincomalee, Ampara, Polonnaruwa, Kilinochchi, Mullaitivu, Vavuniya,

Jaffna, Batticaloa, Monaragala, Kurunegala in 2019. The serologically positive animals were identified in Anuradhapura, Polonnaruwa, Mullaitivu, Batticaloa, Vavuniya, Kurunegala, Nuwara Eliya Trincomalee, Kilinochchi, Monaragala, Ampara, Puttalam, in 2020 and in Nuwara Eliya, Polonnaruwa, Trincomalee, Vavuniya, Mannar, Kurunegala, Mullaitivu, Puttalam in 2021. More than 60% of CFT positive samples each year were shown a high antibody titre of 1:32, indicating a recent incidence of disease in a suspected herd (Table:2). In 2019, 66% of samples were shown high than 1:32 and out of 66%, 28% of animals were shown higher CFT as 1:512 or more. In addition, 63% and 78% of high CFT titres were observed in 2020 and the first four-month of 2021, respectively.

4. DISCUSSION

As a trend, the average annual positive percentage of submission has been increased from 2019 - 2021 in herds with a history of abortion of cattle and buffaloes. The percentages were 19.4%, 30% to 38.5% during 2019 -2021 (Table: 1). The greatest number of cases were reported in the dry zone of the country, where animals are reared extensively in natural pasture. A similar finding has been shown in India. Brucellosis is an endemic disease in free-grazing animals Panjab, India (Deka et al., 2018). Brucellosis is an endemic disease in the dry zone of the country and very few cases were reported in wet zone and upcountry (Priyantha, 2008). Importantly, the disease was reported in Northern, Eastern, North Central, Northwestern, Uva and Central provinces in the country. A considerable number of samples have not been submitted from the rest of the two provinces where the dry zone encountered as Southern and Sabaragamuwa in this study. Those two provinces were shown positive for bovine brucellosis previously (Priyantha 2011; Priyantha, 2008). Therefore, bovine

Table 1. The percentage of *Bovine brucellosis* in cattle and buffaloes from 2019-2021

Year	Number of neat cattle/Buffaloes tested for brucellosis	Number of Positive	Positive %
2019	789	153	19.4%
2020	377	113	30.0%
2021 until April	278	107	38.5%

Table 2. CFT titre and percentage distribution of each titre in 2019 - 2021 April

Year	Number of animals in specific CFT titre											Total
	4	8	16	32	64	128	256	512	1024	2048	4096	
2019	5	14	12	19	16	17	25	22	12	7	2	153
%				32.5			37.9					28.1
2020	2	2	8	21	16	25	21	6	03	0	0	113
%				29.0			54.8					8.0
2021: April	7	6	11	10	25	25	15	11	07			107
%				31.6			60.8					16.8

brucellosis is an endemic disease in the dry zone of the country and over one third of abortions were caused by *Brucella abortus*. However, one outbreak in the upcountry was serologically positive at the end of 2020 and the beginning of 2021 in this study. The upcountry was free of brucellosis till 2006, the first outbreak of brucellosis was reported on the government farm due to the transportation of animals from the dry zone (Priyantha, 2008). A wave of abortion was observed in privately owned farms in upcountry between 2006-2011 resulting transportation of animals from dry zone of the country where brucellosis is endemic (Priyantha 2011; Priyantha, 2010). However, bovine brucellosis is found in selected pockets of cattle farms and very few cases were reported in small, scaled farms in upcountry. In contrast, sample submission and testing were badly affected by COVID 19 pandemic in the country in 2020 and 2021. In addition, a single outbreak in up country was dominant with increased sample submission in 2021.

CFT is the gold standard for the diagnosis of brucellosis in cattle and buffaloes serologically (Chisi et al., 2017). High CFT titres (>1:32) observed in the study indicated an ongoing infection in sampled herds of cattle and buffaloes. The maximum CFT titers of a vaccinated animal were 60 ICFTU. Therefore, antibody titers derived from an infection are high in CFT. However, adult vaccination by S 19 is rarely done in Sri Lanka (Only during an outbreak of bovine brucellosis) (Królak and Błaszczuk, 1986; Priyantha, 2008). The routine S 19 vaccination is carried out in 10-14 months of old female calves only. However, infected cows are vaccinated in the outbreak risk with a reduced dose with the precaution of minimizing excretion of the vaccine strain in milk. Milk from vaccinated animals need to be pasteurized and extra precaution must be taken to minimize zoonotic risk to human. Therefore, high ICFTU in CFT indicated a true infection of bovine brucellosis in the sampled herd of cattle and buffaloes. All three years, low CFT titres (<1:32) were reported as 30% in three consecutive years, although medium and high antibody titers were reported differently in each year (Table: 2). Importantly over 60% of antibody titers reported in the first four months of 2021 were shown medium-high titres. The very high antibody titres such as 1:1024, 1: 2048 and 1: 4096 also were reported during the period. Although the exact reason cannot be explained in the distribution of CFT titres in three years considered in the study,. Quantitative data were suggestive of an ongoing infection of brucellosis in sampled herds of cattle in the country. In addition, the duration of sample collection after infection of brucellosis may be the possible reason for antibody titres in CFT.

The prevalence of bovine brucellosis is not known since systematic national surveillance has not been established in the country. The basic epidemiological information, such as the prevalence of the disease is vitally important in order to make the decision by policy makers to control or eradicate of bovine

brucellosis in the country. A limited study was done from time to time and no island-wide study was done after the study of Silva et al., 2000 (Bandara and Mahipala, 2002; Fernando, 2017; Kothalawala et al., 2017; Priyantha 2011; Priyantha, 2008; Silva et al., 2000). Furthermore, cattle and buffaloes in South Asia are endemic for brucellosis (Priyantha 2011). In neighbouring India, the prevalence of brucellosis is 0.9-18.1% in animals, including livestock and wild animals (Kang et al., 2014; Smits and Kadri, 2005). The overall seroprevalence of bovine brucellosis in cattle and buffaloes was 5% and 3% in India, respectively (Kang et al., 2014). In contrast, the recent finding suggested a high seroprevalence of 15.1 % in Panjab state, India (Holt HR, 2021). Therefore, continued surveillance is required to understand the real picture of a disease in a country and snapshot prevalence of a disease is not capable of understanding the trends and pattern of disease in livestock.

The results suggested that 1/3 of cattle with a history of abortion were diagnosed for brucellosis. Importantly, Brucellosis is an economically important disease since losses of calves in bovine husbandry. Reproductive disorders have a significant effect on the farmer's budget. In addition, the zoonotic risk of consumption of livestock products and the professional risk of handling animals is vitally important. Clinical brucellosis has been found in the country (Karunanayake et al., 2019). Furthermore, brucellosis is a critically important disease in the export market and animal movement locally (Priyantha, 2008). Therefore, control of brucellosis will gain a significant economic benefit to the industry. Surveillance, improving diagnostic facilities, improving biosecurity, removing risk factors for brucellosis, slaughtering positive animals, and vaccination is collective strategies for controlling bovine brucellosis in a country (Deka et al., 2018).

Leptospira has not been diagnosed as a cause of abortion in the study serologically. Although no information has been found, Leptospira is considered one of the main courses of abortion in cattle in tropical water logging environments (Higgins et al, 1988). In addition, abortion caused by a mixed infection of brucellosis and leptospirosis had been reported in water buffaloes in Sri Lanka previously (Priyantha, 2010).

5. CONCLUSION

The status of brucellosis in herds with abortions has been analyzed in the study. Importantly, over 1/3 of animals showing abortion were diagnosed for brucellosis. Brucellosis is considered as the most common course of abortion in cattle. The frequency of serological diagnosis was varied from 19.4% to 38.5% in 2019, 2020 and 2021, respectively. The high CFT indicated ongoing infection in different herds of cattle in the country from 2019 to 2021. The role of Leptospira causing abortion was reported to be low in cattle and buffaloes in the country.

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