Identification of biovars of *Brucella abortus* in aborted cattle and buffaloes herd in Sri Lanka

Priyantha M.A.R.

Veterinary Research Institute,
PO Box 28, Peradeniya, Sri Lanka
E-mail: appuhami1974@yahoo.com
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Abstract

Bovine brucellosis is an endemic disease in Sri Lanka, caused by *Brucella abortus* and had been reported all part of the country for last six decades. Since available biovar is still unknown, the objective of the study was to identify the biovar of *B.abortus* from sporadically aborted cattle and buffaloes. Samples were collected from 18 aborted herds out of 19 herds of Cattle and Buffaloes in the year 2010. Rose Bengal plate test and Compliment Fixation test were carried out. Milk, vaginal swabs, placental contents and aborted fetus were collected and cultured by conventional bacteriological methods. The detection of biovars were based on growth on Thionin and Bacto fuschin,CO_2 requirement, H_2S production, serum agglutination with *Brucella* negative, A,M and R reference antiserum. Eighteen herds investigated out of 19 herds reported, 11 herds were serologically positive for brucellosis (61.11%) and only *Brucella abortus* were isolated from 8 individuals from six herds. All were identified as Biovar 3 of *Brucella abortus*.
Key words: Bovine, Brucellosis, Sri Lanka, Endemic disease, Occupational health Hazard, Zoonoses, Laboratory Techniques, Biovars.

Introduction

Bovine brucellosis, caused by bacterium *Brucella abortus* is an important zoonotic disease in many developing countries (Smits, H.L. and Kadri, S.M.,2005). The disease has been reported in different provinces in India, Pakistan and Bangladesh (Abubakar, M. *et al*, 2010., Mantur, B.G., and Amaranth, S.K., 2008., Rahman,*et al*, 2006). Brucellosis is considered an threatening disease in Sri Lanka (Priyantha *et al*,2008) and had been introduced through importation of cattle during the Second World War (De Alwis,1993). In Sri Lanka, first Clinical outbreak was reported in 1956 and subsequently, bovine brucellosis has become an endemic disease in the country, causing severe economical impact to current livestock industry (Gajanayake *et al*, 2000). In the year 2010, 19 herds were reported with abortion and 18 herds were investigated for the present study. However, isolated clinical cases had reported all over the dry zone in year 2010 although laboratory confirmation was not carried out (Veterinary Epidemiological Bulletin, 2010).

Objective of this study was to identify available biovars of Species *Brucella abortus* from aborted herds and to understand different livestock hosts in which *Brucella abortus* survives in the environment (Qiunn *et al*, 1994).

Materials and methods

Eighteen herds out of 19 herds in the Sri Lanka were investigated and samples were collected from cattle and buffaloes with the history of abortion nationwide. Information were received from regional Government Veterinary offices when sporadic abortions continuing in particular regions, is the routine disease reporting system established in the country. Samples were collected 1-14 days from day of abortion up to less than 6 weeks (Nielsen &Duncan, 1990). Serum, milk, vaginal swabs and aborted fetus were collected from individual animal.

Serology: Complement Fixation Test (CFT) is the gold standard test for serological diagnosis of brucellosis in cattle (OIE, 2009). Serums were first tested by the Rose Bengal Plate test and confirmation was done by the CFT according to method described by Alton *et al*, 1988. RBPT antigen derived from S 99 imported from Weighbridge laboratory, UK produced in the Veterinary Research Institute, Sri Lanka according to the methods described in the OIE Manual of Standards for diagnostic tests and vaccines (2008).

Table-1. The summary of data collected from different herds' investigation

Location	Type of animals	Herd size	Management system	Age	Isolation of Brucella organism
Ambilipitiya	Sahiwal and Jursey crosses	< 25	Extensive and semi extensive	3-12	-
Dambulla	Sahiwal Jursey crosses	< 25	extensive	2-9	+
Anamaduwa	Jursey crosses, Frersein crosses	< 25	extensive	3-8	-
Anamaduwa (Herd with stud)	Sahiwal crosses	25-50	extensive	2-7	-
Mininthale	Sahiwal crosses	< 25	extensive	2-10	-
Sippukulam	Indigenous	< 25	extensive	2-9	+
Buttala	Indegenois	25-50	extensive	2-8	
Wellawaya	Indegenoius	25-50			+
Parasangaswewa	Sahiwal and sahiwal crosses	< 25	Extensive (S 19 vaccinated)	2-8	
Oyamaduwa	Sahiwal crosse and sahiwal	25-50	extensive	2-8	
Karuwalagaswewa	Indigenous, Sahival crosses, Jursey crosses	25-50	extensive	2-9	+
Karuwaklagaswewa	Sahiwal crosess	<25	extensive	2-9	
Mundalama	Local crosses	<25	Semi intensive	3-7	+
					+
					+
					+
Pannala	-	-	-	-	+
Murukkan	Local crosses	25-50	extensive	3-11	-
Mannar 1	Local crosses	25-50	extensive	3-9	
Mannar 2	Local crosses	<25	extensive	3-9	

Bacterial isolation: *B.abortus* was isolated from vaginal swabs, milk and aborted fetus by conventional methods described by Alton *et al*, (1988). Commercial Brucella agar (Oxoid CMO169) was used as base for both selective and non selective medium with 4% sheep blood. The OXOID CMO 169 selective supplement was added only for selective media to inhibit contamination. The isolated organisms were tested by biochemical tests and serum agglutination test with Brucella reference antiserum brought from International reference laboratory, France. Milk samples were collected directly to sterile bottles (20 ml each) from each teat of aborted cows aseptically. The samples were centrifuged at 6000 g for 15 minutes. Both deposited and cream layer were cultured on selective and non selective media, were incubated at 10% CO_2 and without CO_2 as described by Alton *et al*, 1988. Sterile, 10 cm long swabs were used for this purpose. Samples were (Five samples from each and pooled) collected directly from the vaginal wall, cultured on Brucella specific medium and incubated at 37^0C with and without 10% CO_2 incubation as described by Alton *et al*,1988. The aborted fetus is considered as one of the best sample to isolates *Brucella* in cattle and Buffalo (Nielsen & Duncan,1990). Inoculations were made from aborted fetuses specially from stomach contents, lungs, spleen, liver, on *Brucella* specific and nonspecific medium as described by Alton *et al*, 1988. However, collection of fetuses from extensive herds were difficult as fetuses were eaten by other animals.

The identification of biovar of *Brucella* was done by the method described by Alton *et al*,1988, based on growth on Thionin and Bacto fuschin,CO_2 requirement, H_2S production, reaction with *Brucella* negative anti-serum and A,M and R reference antisera.

Results

Eighteen aborted herd were investigated (Out of 19 reported in 2010), 11 herds were identified as serologically positive for brucellosis (61.11%). The seropositive animals were detected in herds at Dambulla, Anamaduwa, Mihintale, Sippukulam, Buttala, Mannar and Bingiriya and their Complement Fixation Test (CFT) titers were varied from 1:16 to 1:2018. There were nine *Brucella abortus* organism isolated from six different herds and biovar 3 was isolated from milk, aborted fetus and vaginal swabs.

Discussion

Brucellosis is an endemic disease specially in dry zone (Priyantha *et al*,2008, De Alwis *et al*,1989), where the highest density of cattle reported in the country. The serological brucellosis in local herds has been studied at regional level, although surveillance on brucellosis is still a subject area of priority. It was apparent that brucellosis is the main cause of sporadic abortion in cattle at last trimester of pregnancy and 61.11% of aborted herds examined were found positive serologically. Similar information was published by Priyantha *et al* in 2010, indicated that *Brucella abortus* was the main cause of abortion in water buffaloes. Furthermore, Brucellosis is considered major reproductive disease of cattle in Dry zone of Sri Lanka

Table-2. Biochemical and serological characters of the isolated Brucella cultures.

Brucella Isolates	CO2 requirement	H2S production	Growth on Thionin	Growth on Basic Fuschin	Agglutination in SeraA	Agglutination in SeraM	Agglutination in SeraR
2010/Anu/01	+	+	+	+	+	-	-
2010/Bad/02	+	+	+	+	+	-	-
2010/Mat/03	+	+	+	+	+	-	-
2010/Put/04	+	+	+	+	+	-	-
2010/Put/05	+	+	+	+	+	-	-
2010/Put/06	+	+	+	+	+	-	-
2010/Put/07	+	+	+	+	+	-	-
2010/Put/08	+	+	+	+	+	-	-
2010/Kur/09	+	+	+	+	+	-	-
S 19 control	-	+	-	+	+	-	-

for long time, (Priyantha *et al*, 2008, Kumaraswami, 1971).

The isolation of *Brucella abortus* were from nine individuals, representing six different herds (Table-3). Most isolates were from indigenous and extensive or semi extensive herds of dry zone and only one isolate from Bingiriya. The rearing indigenous animals are found in dry zone (Priyantha *et al*, 2008). Those herds are sent to jungle or pasture land around big tanks in the region, shearing same pasture by different herd and cross contamination is common (Priyantha *et al*, 2008). The oral contamination which is the most common method of transmission in an extensive herd (Abubakar, M.*et al*, 2010. Nielsen & Duncan, 1990). Higher stocking density has been cited as an important factor influencing transmission and persistence of infection (Nielsen &Duncan,1990).

The important finding of this study was detection of latent carriers of *Brucella abortus*. There were three animals detected at Mundalama and one detected at Pannala. They were excreted bacteria through milk and reproductive secretion although not detected by RBP test. The latent carrier may cause detrimental results in control program (Nielsen &Duncan, 1990., Dolan L.A.*et al*, 1980), reported by different authors in different hosts (Huurne *et al*, 1993., Verma *et al*, 2000.,Dolan,L.A.,1980). Meanwhile calves infected by *B.abortus* in utero or after ingestion of infected milk may acquire a persistent infection, remain negative until calving or abortion (Huurne *et al*, 1993). However, those in this study were remain negative for serologically even at calving and excreting organism with milk and reproductive secretion, which needs to be study further.

The causative bacteria, *Brucella abortus* has been isolated from at least nine species of domestic livestock (Nielsen &Duncan, 1990). All isolated were as biovar 3 by biochemical and agglutination tests described by Alton *et al*, 1988, (Table-3) out of 7 biovars identified from *B.abortus* (OIE, 2009). *Brucella abortus* biovar 3, only biovar was given positive for CO_2 requirement for growth, H_2S production, growth on Thionine and basic fuschin reagent. It was given positive results for Brucella type A antisera and negative for M and R antisera. All isolates required CO_2 for growth on artificial media and produced H_2S, described as biovar1,2, 3,4 or 9 of *B.abortus*. Also all nine Isolates were grown on Thionin and basic fuchin given concentration by Alton *et al*,1988 and categorized under biovar 3 after the agglutination test of A.M and R. Biovar 1 of *Brucella abortus* is appeared as most common and widely distributed in the world (Nielsen &Duncan, 1990). The distribution is varied with one country to the other and Only 1.2 and 4 are reported in USA while biovar 3 and 7 are not yet reported in England (Nielsen &Duncan, 1990). In the literature published, Only biovar 1,3,5 of *Brucella abortus* have been reported in India, which is considered as the source of brucellosis in Sri Lanka during the period of second world war (Perumal

Table-3. Details of origin of different isolates of *Brucella abortus* from the field.

Isolate	Location	Type of Sample	CFT titer of individual animal
2010/Anu/01	Sippukulam	Aborted fetus	1:1024
2010/Bad/02	Wellawaya	Aborted fetus	Hemolysed
2010/Mat/03	Dambulla	Milk	1:1024
2010/Put/04	Karuwalagaswewa	Milk	1:2048
2010/Put/05	Mundalama	Milk, Vaginal swabs	Negative
2010/Put/06	Mundalama	Milk, Vaginal swabs	Negative
2010/Put/07	Mundalama	Milk, Vaginal swabs	1:2048
2010/Put/08	Mundalama	Milk	Negative
2010/Kur/09	Pannala	Milk	Negative

Pillai,1957). However, in the taxonomy sequential flow of *Brucella abortus* biovar 3 is found at the mid level. In contrast, biotype 2 has the greatest environment demand, considered possible progenitor of the biotype while biovar 5 is at the end of the line (Nielsen &Duncan, 1990). The biovar 3 is mainly associated with cattle and buffaloes (Nielsen & Duncan, 1990, Alton *et al*, 1988). Eradication must be emphasized on those specific host than rest of the range of hosts of *Brucella abortus* which survives in the environment. *Brucella* infected milk may cause negative effect on human health (Emerging Infectious Disease,1997) though human brucellosis had not been reported in Sri Lanka. However, biovar 3 had been isolated from human cases (L.Valdezate, 2009) and risk has not been evaluated locally.

Conclusion

The study concluded that biovar 3 of *Brucella abortus* was identified in local cattle of Sri Lanka mainly in extensively reared cattle in 2010, provided basic information on eradication of Brucella in future. Further studies are necessary about the epidemiology, pathogenesis and molecule characterization of the organisms to eradicate the disease from the certain region or country.

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