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Control of brucellosis at an endemically infected sheep farm in Haryana (India)

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ABSTRACT

Brucellosis in small ruminants is caused by *Brucella melitensis* and appears to have established on many sheep or goat farms in the country where losses occur in the form of abortion, premature or stillbirth, birth of weak lamb from females and epididymo-orchitis in males, year after year. Vaccination of small ruminants against brucellosis to control the disease is not practiced in India. A sheep breeding farm in Haryana was established in 1969, and brucellosis at this farm is prevalent for more than 3 decades. An epidemiological study of more than 10 years revealed continuous presence of the disease at the farm. Culling of positive animals did not help in controlling the disease and reducing the losses. A strategy of vaccination of serologically negative population with *Brucella melitensis* Rev.1 vaccine coupled with segregation and culling of positive animals was implemented to control the disease at the farm. Vaccination of all the serologically negative animals was carried out in 2007 with simultaneous culling of positive population. More than 3 years have passed but the cases of abortion in ewes and epididymo-orchitis in rams have not been recorded in animals subsequent to vaccination, which otherwise were noticed every year at the farm prior to vaccination. This study could serve as an effective model for many sheep or goat farms in the country where the disease is endemic.

Key words: Brucellosis, Haryana, Sheep, Vaccination

Brucellosis in sheep is a serious problem in many parts of the world particularly in developing countries where the disease is prevalent but vaccination is not practiced. *Brucella melitensis*, the causative agent of brucellosis in small ruminants, causes abortion in large numbers of animals in advance pregnancy when introduced in an uninfected flock. Subsequently, the disease continued in the flock resulting into continuous losses due to abortion, premature birth, stillbirth, birth of weak lamb/kid. Once disease get established in the flock and attains endemic situation the cases of abortion decreases, however, the disease persisted and infected animals contaminate the environment particularly during parturition and infection to uninfected population (Radostits *et al*. 2000). Moreover, a high antigenic load in the environment also causes epididymo-orchitis in rams (Chand *et al.* 2002) which otherwise is not common on lightly infected farms. The disease causes considerable economic loss to the sheep breeders and also leads to restrictions in trade of animals and their products. In India, the disease is endemic and there is no policy for controlling it in sheep and goats (Renukaradhya *et al.* 2002). Vaccination of small ruminants is not practiced, neither the vaccine is manufactured nor is available in the country. Hence, in many parts of the country particularly at farms the disease is endemic. The present communication describes control of brucellosis at the sheep farm in Haryana on which more than 6000 animals were maintained and the disease was endemic for more than 3 decades. To achieve this goal at the farm the strategy of vaccination of serologically negative population with *B. melitensis* Rev.1 vaccine coupled with culling of positive animals was implemented. The epidemiological data of more than 10 years along with the steps for testing, culling and vaccination of animals, being taken to contain and control the disease, are presented.

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MATERIALS AND METHODS

Sheep farm: The Central Sheep Breeding Farm, Hisar was established in 1969 in collaboration with Australia. This farm maintains pure as well as crossbred sheep of Rambouilet, Corriedale and Sonadi breeds. The strength of the farm ranged between 6000 and 7000 animals. The disease in animals was recorded by Kulshereshta *et al.* (1978).

Samples: The samples included blood from ewes and rams, milk from lactating ewes, uterine discharge from ewes

and stomach contents from aborted foetuses. The breeding rams exhibiting clinical signs of enlargement of genital organs (i.e. testis and epididymis) were euthanized. From these animals, the pieces of tissues and swabs were collected from testes and epididymes at the time of postmortem. Serum from blood samples was separated, stored at –20°C and used for serological examination. The stomach contents from aborted foetus, milk and uterine discharge from ewes and materials from rams were subjected for isolation of brucellae.

Isolation of brucellae: Isolation of *Brucella* organism was attempted from materials collected from ewes, rams and aborted foetuses (Alton *et al.* 1988). The stomach contents, uterine discharge, swabs from cut surface of testis and grinded tissues of epididymis and testis were directly inoculated onto trypticase soya agar (TSA) containing antibiotics (Farrell 1974). The milk samples were collected in sterile vials and processed on the day of collection for isolation of brucellae. Approximately 5 ml of milk sample was centrifuged at 6000 ×g for 15 min in a refrigerated centrifuge and the cream layer and deposit, if any, were inoculated onto TSA. The TSA plates were incubated at 37°C for several days and inspected daily after 3 days of incubation for the development of *Brucella* like colonies. Preliminary examination of suspected colonies with respect to growth characteristics, gram's staining of the isolates, agglutination reaction with the anti-*Brucella* polyclonal serum and monospecific A, M and R sera obtained from the OIE Reference Laboratory, Weybridge, Surrey, UK was carried out as per Alton *et al.* (1988). Some isolates were also submitted to the OIE/FAO Brucellosis Reference Laboratory, Maisson-AlfortCedex, France, for confirmation and biovar typing.

Serological assays

The Rose Bengal test (RBT) and enzyme linked immunosorbent assay (ELISA) were conducted to determine serological status of animals for brucellosis.

Rose Bengal plate test (RBT): The antigen for RBT was procured from the Indian Veterinary Research Institute, Izatnagar, and conducted according to Alton *et al.* (1988). Briefly, 35µl of coloured antigen and equal amounts of serum sample were taken on a clean glass plate and mixed with the help of a wooden stick. The plate was rotated clock-wise and anti-clock wise and development of any degree of agglutination within 4 min was considered positive reaction.

A modified Rose Bengal plate agglutination test (mRBT) was also performed in which amounts of antigen reduced and ratio of antigen to serum was used at 1:4 (Blasco *et al.* 1994a, Ferreira *et al.* 2003). For this 20µl of antigen was mixed with 60µl of serum and plate was shaken for 4 min and any agglutination appeared within this time was recorded as positive.

Enzyme linked immunosorbent assay (ELISA): Brucella melitensis biovar-1 recovered from an aborted sheep foetus was used to prepare the lipopolysaccharide (LPS) as described by Jimenez de Bagues *et al.* (1992) and used as antigen in ELISA. Prior to preparation of LPS antigen the isolate was characterized and was got confirmed from the OIE/FAO Brucellosis Reference Laboratory, Maisson-AlfortCedex, France. The ELISA was performed as described by Jimenez de Bagues *et al*. (1992) with some modifications (Chand *et al.* 2005). The criterion for considering a sample positive or negative was based on % positivity calculated as follows:

$$\text{Per cent positivity (\% P)} = \frac{\text{OD value of test sample}}{\text{Average OD value of positive controls}} \times 100$$

Samples with equal or above 25% positivity were considered positive (Chand *et al.* 2005).

Vaccination of animals: The *B. melitensis* Rev. 1 is a reference vaccine against brucellosis for small ruminants (OIE 2004), however, this vaccine is neither available nor is manufactured by any Government or private agency in India. This vaccine is used in small ruminants for last several decades (Elberg 1996) and recommended by OIE (OIE 2004). In freeze dried form, the vaccine was imported from Spain and was used as per the manufacturer instructions.

RESULTS AND DISCUSSION

Abortions in ewes and cases of epididymo-orchitis in breeding rams occurred every year at the farm. *Brucella melitensis* biovar-1 was cultured from many kinds of clinical samples, viz. aborted foetuses, uterine discharge, milk from lactating ewes, testis and epididymis from rams during the study period from 1996 to 2006 except in 1997 and 2000 when samples were not processed (Table 1). Since cases of abortion and epididymo-orchitis were not found following vaccination in 2007 till date in 2010 isolation of brucellae could not be attempted during this period.

Table 1. Isolation of *Brucella melitensis* from various kinds of clinical samples belonging to the Central Sheep Breeding Farm, Hisar during the period of study from 1996 to 2010

Year	Clinical sample	Number examined	*B. melitensis* isolated
1996	Aborted foetus	8	2
1998	Aborted foetus	4	2
1999	Genital Organs	3	3
2001	Genital Organs	9	6
2002	Milk	175	45
2003	Milk	75	4
2004	Milk	200	53
	Aborted foetus	2	2
2005	Uterine discharge	48	21
2006	Milk	25	3
	Aborted foetus	1	1
2007 to 2010	None*	Nil	Nil

*In 2007 positive animals were culled and negative animals above 4 months of age were vaccinated.

Table 2. Serological prevalence of brucellosis in animals at the Central Sheep Breeding Farm, Hisar prior to and after vaccination

Year	Rams			Ewes			Total	Positive	Percentage
	Tested	Positive	Percentage	Tested	Positive	Percentage			
1996	107	13	12.1	232	29	12.5	339	42	12.4
1997	154	28	18.1	24	23	95.8	178	51	28.6
1998	Nil	-	-	4	4	100	4	4	100
1999	86	10	11.6	Nil	-	-	86	10	11.6
2000	297	42	14.1	Nil	-	-	297	42	14.1
2001	444	58	13.0	Nil	-	-	444	58	13.0
2002	Nil	-	-	175	39	22.3	175	39	22.3
2003	11	11	100	75	23	30.6	86	34	39.5
2004	327	53	16.2	200	47	23.5	452	87	19.2
2005	-	-	-	-	-	-	6742	2412	35.7
2006	Nil	-	-	Nil	-	-	Nil	-	-
2007	-	-	-	-	-	-	4840	474	9.7
Dec. 2007	-	-	-	-	-	-	320	295*	92.1
Feb. 2008	-	-	-	-	-	-	180	142*	78.8
2009-2010	-	-	-	-	-	-	Nil	-	-

* Animals exhibiting antibodies following vaccination.

The results of serological screening of animals conducted in different years are presented in Table 2. Some animals were screened almost every year since 1996 and a percentage of animals each year were positive. In the year 2005 all the 6742 animals above 4 months of age including lambs, hogget, ewes and rams were screened, therefore, animals were not divided in categories of ewes and rams. Serological prevalence of brucellosis at the farm was 35.77% in 2005. Serological screening, after a period of about 2 years and just prior to vaccination, was also conducted in 2007. In this screening 9.79% animals were found serologically positive (Table 2).

For routine testing, conventional RBT was used up to the year 2004, however, in 2005 when whole farm was screened to identify all positive animals the RBT, mRBT and ELISA were used. The results of testing are presented in Table 3. It was noticed that 18.27%, 25.16% and 35.77% animals were positive in RBT, mRBT and ELISA, respectively.

Approximately 10% (N, 500) of the vaccinated animals, selected randomly, were tested by RBT during 2007–08 at various post vaccination periods. Testing of 320 animals after about 2 months of vaccination revealed 295 (92.18%) of them RBT positive and testing of 180 animals after about 4 months yielded 142 (78.88%) positive (Table 2). Overall 437 (87.4%) animals were positive for agglutinins in RBT following vaccination which indicated that induction of immune response had occurred almost in all the vaccinated animals.

Data on isolation of *B. melitensis* biovar-1 from clinical cases on many occasions in different years and continuous presence of serologically positive animals from 1996 to 2007 explained endemic situation of brucellosis at the farm. In 2005, screening of all the animals revealed high prevalence of brucellosis. The positive animals (35.77%) were culled. In 2007, when vaccination with *B. melitensis* Rev.1 vaccine was implemented, retesting of animals was done in which 9.74% animals were positive which were also culled (Table 2). These findings clearly established that culling of positive animals from the farm was not effective in controlling brucellosis. The simple reason for this was that in endemic situation the contaminated surroundings, housing, premises etc. remained constant source of infection to uninfected population. Hence, culling of positive population coupled with vaccination of negative animals would be an effective strategy to achieve control of brucellosis.

Table 3. Results of testing of animals belonging to the Central Sheep Breeding Farm, Hisar by RBT, modified RBT and ELISA

Assay	No. of animals	Positive	Negative	Per cent (%)
Positive				
RBT	6742	1232	5510	18.27%
mRBPT	6742	1694	5048	25.16%
ELISA	6742	2412	4330	35.77%

RBT, Rose bengal plate test; mRBT, modified rose bengal plate test; ELISA, enzyme linked immunosorbent assay.

The disease seemed to have introduced in animals subsequent to establishment of the farm in 1969 and presence of the disease was detected in 1978 (Kulshereshta *et al.* 1978). How the disease was introduced at the farm is not known but was likely from surroundings as brucellosis in sheep and goats was prevalent in the area (Mathur 1966).

In rams the disease did not appear as an outbreak. Many of the affected animals exhibited enlargements of genital

organs mainly of testis and epididymis which was easily recognised from a distance signs. Clinical signs were observed in breeding rams at the farm from time to time and *B. melitensis* was isolated from their testis and epididymis (Chand *et al.* 2002). This situation in rams caused culling of infected animals from the farm year after year, as a preventive measure.

Considering the endemic situation, it was decided to adopt an appropriate strategy to contain and control the disease so that it could be eliminated from the farm in shortest possible time. However, before implementing any control strategy it was necessary to identify all infected animals at the farm. Accordingly, all the animals were tested by RBT, mRBT and ELISA. It was noticed that all the RBT or mRBPT positive animals were also positive in ELISA while a large numbers of ELISA positive animals did not yield positive results in either of the RBT (Table 3). These results clearly suggest that RBT and even its modification, mRBT, are not suitable to be used in chronically infected flocks to identify all infected animals. The efficacy of RBT as an individual test is questionable (Blasco *et al.* 1994a, Teshale*et al.* 2006) and its sensitivity is low particularly in chronically infected flocks (Kulshereshta *et al.* 1978, Blasco *et al.* 1994b). While the sensitivity of ELISA is much better than other serological methods in small ruminants (Blasco *et al.* 1994b, Marin *et al.* 1999, Chand *et al.* 2005, Teshale *et al.* 2006). Hence, animals detected positive in ELISA were taken as infected with brucellosis. All the infected animals were segregated and culled and serologically negative animals only were vaccinated with *B. melitensis* Rev. 1 vaccine.

The vaccine prepared from *B. melitensis* Rev.1 strain is presently recognized as the best available vaccine world over and has also been recommended by OIE for sheep and goats (European Commission 2001). Numerous independent field trials in many countries have confirmed its suitability (Alton and Elberg 1967, Elberg 1996, Blasco 1997).

The selection of strategy for the control of brucellosis in sheep and goats could vary from country to country and even ecologically distinct areas within the country, depending on the prevailing epidemiological and socioeconomic conditions. An implementation of test and slaughter policy for eradication is feasible when incidence of the disease is very low (WHO 1986) and adequate financial support is available. If the *B. melitensis* infection is endemic and widespread in the area, control by immunization of animals is strongly recommended as the preliminary step for the final elimination of infectious agent (Minas 2006). In a closely controlled conditions, such as on a farm, where re-entry of infection can be prevented, veterinary diagnostic laboratory for testing is available and vaccination of clean animals is coupled with removal of reactors, the success of controlling brucellosis and achieving rapid elimination of the infectious agent from the area/flock is very high (Alton 1987). Hence, strategy involving vaccination of clean animals and removal of infected animals coupled with other managemental measures was adopted to achieve complete and rapid elimination of *B. melitensis* from the farm.

Vaccination of animals on the farm was completed in September 2007. The effect of vaccination on the farm was assessed by on looking on the incidence of abortion in ewes and development of enlargement of testis in rams. More than 3 years have passed but cases of abortion in ewes and enlargement of testis in rams were not recorded which explains the success of vaccination in controlling the disease at the farm.

Brucellosis is endemic in most part of India and evidence suggests its high prevalence in sheep and goats (Renukaradhya *et al.* 2002). According to 2003 Livestock Census 61.47 million sheep and 124.36 million goats are reared in various management practices and agro-climatic conditions in the country. Under the circumstances and animal husbandry practices being followed by sheep and goat breeders/farmers/organizations and the nature of the disease which is insidious and continue to persist in infected areas and flocks the economic losses due to this disease, which have never been assessed, could be expected to be enormous. Nevertheless *B. melitensis* is highly virulent to human beings and always a threat to individuals who are in-contact with the infected animals. Hence, efforts are urgently needed by all the concerned including State and Central Government bodies to have an appropriate policy in place to combat this disease. The present study on an organized sheep farm could work as a model at least for other private or Government farms where the disease is prevalent.

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REFERENCES

Alton G G. 1987. Control of *Brucella melitensis* infection in sheep and goats-a review. *Tropical Animal Health Production* **19**: 65–74.

Alton G G and Elberg S S. 1967. Rev. 1 *Brucella melitensis* vaccine. A review of ten years of study.*Veterinary Bulletin* **37**: 793–800.

Alton G G, Jones L M, Angus R D and Verger J M. 1988. *Techniques for the Brucellosis Laboratory*. INRA, Paris.

Blasco J M. 1997. A review of the use of *B. melitensis* Rev. 1 vaccine in adult sheep and goats. *Preventive Veterinary Medicine* **31**: 275–83.

Blasco J M, Garin-Bastuji B, Marin C M, Gerbier G, Fanlo J, Jimenez De Bagues M and Cau C.1994a. Efficacy ofdifferent rose bengal and complement fixation antigens for the diagnosis of *Brucella melitensis* infection in sheep and goats. *Veterinary Record* **134**: 415–20.

Blasco J M, Marin C M, Jimenez De Bagues M, Barberan M, Hernandez A, Molina L,Velasco J, Diaz R and Moriyon I. 1994b.

Evaluation of allergic and serological tests for diagnosis *Brucella melitensis* infection in sheep. *Journal of Clinical Microbiology* **32**: 1835–40.

Chand P, Sadana J R and Malhotra A K. 2002. Epididymo-orchitis caused by *Brucella melitensis* in breeding rams in India. *Veterinary Record* **150**: 84–85.

Chand P, Rajpurohit B S, Malhotra A K and Poonia J S. 2005.Comparison of milk-ELISA and serum-ELISA for the diagnosis of *Brucella melitensis* infection in sheep. *Veterinary Mircrobiology* **108**: 305–11.

Elberg S S. 1996. Rev. 1 *Brucella melitensis* vaccine. Part III: 1981–1995. *Veterinary Bulletin* **66**: 1193– 1200.

European Commission. 2001. Scientific Committee on Animal Health and Animal welfare. *Brucella melitensis* in sheep and goats.

Farrell I D. 1974. The development of a new selective medium for the isolation of *Brucella abortus* from contaminated sources. *Research in Veterinary Science* **16**: 280–86.

Ferreira A C, Cardoso R, Travaassos D I, Mariano I, Belo A, Rolao P I, Manteigas A, Pina F A and Correa De Sa M I. 2003. Evaluation of a modified Rose Bengal test and an indirect enzyme linked immunosorbent assay for the diagnosis of *Brucella melitensis* infection in sheep. *Veterinary Research* **34**: 297–305.

Jimenez de Bagues M P, Marin C M, Blasco J M, Moriyon I and Gamazo C. 1992. An ELISA with lipopolysaccharide antigen for the diagnosis of *B. melitensis* infection in sheep and for the evaluation of serological response following subcutaneous or conjunctival *B. melitensis* strain Rev.1 vaccination. *Veterinary Microbiology* **30**: 233–41.

Kulshreshtha R C, Kalara D S and Vasudevan B. 1978. A study on the evaluation of a few serodiagnostic tests in sheep brucellosis. *Indian Veterinary Journal* **55**:181–83.

Marin C M, Moreno, E Moriyon I, Diaz R and Blasco J M. 1999. Performance of competitive and indirect enzyme linked immunosorbent assays, gel immunoprecipitation with native hapten polysacchride, and standard serological tests in diagnosis of sheep brucellosis. *Clinical and Diagnostic Laboratory Immunology* **6**: 269–72.

Mathur T N. 1966. Investigation of brucellosis among cattle with regard to human brucellosis. Part II. Brucellosis among goats and sheep. *Indian Journal of Medical Research* **54**: 615–22.

Minas A. 2006. Control and eradication of brucellosis in small ruminants. *Small Ruminant Research* **62**:101–07.

OIE. 2004. Caprine and Ovine Brucellosis (excluding *Brucella ovis* infection) Chapter 2.4.2. *Manual of Standards Diagnostic Tests and Vaccines*. Paris.

Radostits O M, Gay C C, Blood D C and Hinchliff K W. 2000.*Veterinary Medicine*. 9th edn, Pp 888. London, W. B. Saunders.

Renukaradhya G J, Isloor S and Rajasekhar M. 2002. Epidemiology, zoonotic aspects, vaccination and control/eradication of brucellosis in India. *Veterinary Microbiology* **90**: 183–95.

Teshale S, Muhie Y, Dagne A and Kidanemariam A. 2006. Seroprevalence of small ruminant brucellosis in selected district of Afar and Somali coastoral areas of Estern Ethiopia: the impact of husbandry practice. *Revue Medicine Veterinary* **157**: 557–63.

WHO.1986. Joint FAO/WHO Expert Committee on Brucellosis. *Technical Report.* **740**. WHO.