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A LONGITUDINAL STUDY OF BRUCELLA ABORTUS INFECTION IN A CATTLE BREEDING FARM IN BHUTAN

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ABSTRACT: Brucellosis is a zoonotic disease that affects multiple species of animal and humans. Following importation of cattle in late 2014, the National Jersey Breeding Center (NJBC), Samtse a nucleus breeding experienced unusual rate of abortion (54.17%; 13/24) within a short period of time. A detailed investigation by the National Centre for Animal Health, Serbithang confirmed Brucellosis as the cause of abortion in the nucleus breeding farm. Subsequently, a study was conducted to investigate the progression of disease in the farm. Between February 2016 to November 2016, serum and milk samples were collected from the entire farm animal at five different time points and subjected to Rose Bengal Test (RBT), ELISA (enzyme linked immunosorbent assay), CFT (Complement Fixation Test), bacterial culture and PCR (polymerase chain reaction). The mean farm prevalence of brucellosis was 32.80 (SE ± 2.32, range: 25.00-38.18) by RBT, 40.11 (SE ± 2.42, range:35.94-38.18) by ELISA and 37.17 (SE ±1.01, range:36.15-38.18) by CFT. The mean percentage of milking cows found shedding *Brucella abortus* in the milk was found to be 37.17 (SE± 1.01, range:13.33-30.30). Overall, the prevalence of the disease did not show significant variation during the study period. However, this study has shown that there is active infection and shedding of organism occurring in the farm animals. Therefore, there is an urgent need to control and prevent the spread of infection among the animals, to the farm workers and outside the farm.

Keywords: Brucella; cattle; food safety; health safety; infection; zoonoses

1. INTRODUCTION

Brucellosis is an important bacterial zoonosis that affects varieties of livestock like cattle, sheep, goats, camels and also wild life and humans (Olsen and Palmer 2014). Office International des Epizooties (OIE) declared Brucellosis as multiple species disease, infection and infestation (OIE 2018). The disease is caused by an intracellular gram-negative coccobacillus of the genus *Brucella* and the main *Brucella* spp that affect livestock species include *B. abortus* (cattle), *B. melitensis* (sheep, goats), *B. suis* (pigs), and *B. ovis* (sheep). The disease is known to cause economic losses to farmers by causing abortions and subsequently decreased milk yield in the livestock (Abernethy et al. 2011; Xavier et al. 2009). Brucellosis accounts for about 20-30% decrease in milk production as estimated in Brucellosis-affected farms (Herrera et al. 2008; Havelaar et al. 2019). Animal Health workers including veterinarians, laboratory workers, farmers, butchers, abattoir workers, and meat inspectors are people at high risk of *Brucella* infections. The disease could be transmitted to humans via consumption of contaminated raw milk, cheese and butter (Dadar et al. 2020). Dairy processing techniques from raw milk poses high risk of exposure to the agent. Further, it was found that approximately 20% of the person who assisted calving or abortion had exposure to *Brucella* against 9.7% seropositivity in people having just direct contact with the large ruminants (Holt et al. 2021).

Vaccination of cattle above four months of age is the most economic measure for Brucellosis control (Nicoletti 1984). However, for effective control or eradication of Brucellosis, the most important strategy should include prevention, test and removal programs, sanitation and vaccination as

well as whole herd depopulation (Olsen and Stoffregen 2005). Currently in different ASEAN countries (Indonesia, Malaysia, Philippines, Singapore, Thailand, Brunei, Laos, Myanmar, Cambodia and Vietnam), 'Test-and-slaughter' strategy is being practiced for the control of Brucellosis. Considering its zoonotic potential, implementation of control program through 'one health' principles with national vaccination, testing and prevention education, improving public health capacities and international collaborations across endemic regions can significantly reduce the burden of Brucellosis in dairy farms (Dadar et al. 2021). Bhutan does not have officially approved vaccination programme against Brucella. Animal health authority in Bhutan also does not have officially endorsed national Brucellosis control plan.

Bhutan is essentially an agrarian country with about 56.7% of the population engaged in agriculture and the livestock farming form an integral part of the agriculture system with about 62% of the household rearing livestock. Approximately 90% of the rural farmers rear livestock (RGoB 2009) and among different livestock, cattle are the preferred species in the country (Tshering and Thinley 2017). In Bhutan, dairy crossbreeding has contributed to higher gross margins and the farmers have high preference over Jersey crossbreeds (Samdup et al. 2010). National Jersey Breeding Centre (NJBC) in Samtse was established during the first FYP (1961-1965) as a nucleus farm in the country for maintaining purebred jersey breed germplasm. The main objective of the farm is to produce and supply superior quality breeding bulls to various districts for cross breeding program. As an economic stimulus plan of the government the Department of Livestock imported around 30 adult Karan Fries cows towards the end of June 2014 for adaptability trial and the animals were reared at NJBC, Samtse.

The farm management at NJBC, Samtse experienced unusual rate of abortion (54.17%; 13/24) among pregnant animals within a short period of time (one month). The abortion was observed after the importation of animals in late 2014. In the early spring of 2015 National Centre for Animal Health (NCAH), Serbithang initiated detailed investigation of the incident. All the animals in farm were screened for Brucellosis using IDEXX serum antibody enzyme linked-immunosorbent assay (ELISA) and found 24.56% (28/114) of animals were reactive. Additionally, abortion materials such as placenta, fetal stomach content and vaginal swabs were cultured and colonies confirmed as Brucella abortus by the conventional polymerase chain reaction test (PCR). The cause of abortion was confirmed due to bovine Brucellosis. This study describes a detailed investigation where a longitudinal study was conducted to understand the progression of Brucella infection in animals at NJBC, Samtse.

2. MATERIALS AND METHODS

2.1 Sampling and storage

2.1.1 Sampling design

Series of sampling and laboratory testing at five different time points were conducted over a period of nine months from February 2016 to November 2016 to isolate and identify the agent and serologically monitor the status of disease in the farm (Table 1). Initially sampling for the first three time points (1st, 2nd and 3rd) were done on monthly basis (February, March and April). The 4th time-point sampling was done in June two months after the 3rd sampling. The last (5th) sampling was done in November five months after the 4th sampling. Since the animals were managed in a farm, the farm was considered one epidemiological unit. Therefore, the farm prevalence of Brucellosis during each time-point of sample collection was calculated as the total number of animals that tested positive by the number of animals present on the farm during the sampling. However, in this study, we did not follow the status of Brucellosis of individual animal for different collection time. Neither was the test result by different tests for each animal's sample was compared. Therefore, we do not report the status of Brucellosis in individual animal during different collection time-point and if the positive animal tested positive to all the tests deployed for diagnosis. Serum samples from 1st and 2nd time -points were tested by both RBT and ELISA. However, samples only from 4th and 5th time-points were tested by CFT. Molecular analysis by PCR was performed on all milk samples from 1st to 4th time points (Table 1).

Table 1: Laboratory findings of the samples tested

Time point	Positive percentage			
	RBT (+/n)	ELISA (+/n)	CFT (+/n)	Culture PCR (+/n)
Feb 2016 (1st)	31.67 (38/120)	39.17 (47/120)	NA	30.30 (10/33)
Mar 2016 (2nd)	32.23 (39/121)	49.59 (60/120)	NA	16.67 (8/48)
Apr 2016 (3rd)	25.00 (32/128)	35.94 (46/128)	NA	29.27 (12/41)
Jun 2016 (4th)	36.92 (48/130)	37.69 (49/130)	36.15 (47/130)	13.33 (6/45)
Nov 2016 (5th)	38.18 (42/110)	38.18 (42/110)	38.18 (42/110)	NA
Mean ± SE	32.80 ± 2.32	40.11 ± 2.42	37.17 ± 1.01	22.39 ± 4.32

NA: not applicable; +/n; No of positive/No of sample

2.1.2 Blood sample

Around 8 ml of blood samples were collected from entire farm animals, both young and adult for each time point. Blood samples were used to separate serum.

2.1.3 Milk sample

Around 40 ml of milk sample from all the milking cows were collected in a sterile 45 ml Falcon tube for each time point. Udder and teats were cleaned and milk samples collected directly into the tube.

2.1.4 Storage

All samples collected from NJBC, Samtse were transported to the NCAH, Serbithang and stored frozen until used for laboratory testing. Separated serum and milk samples were labeled and stored at -20ºC.

2.2 Laboratory analysis

2.2.1 Rose Bengal test

Rose Bengal Antigen (ID Vet, France) was used to perform Rose Bengal Test (RBT) and screen sample for antibody against *Brucella* species (Blasco 1994). The test serum (0.03 ml) was mixed with 0.03 ml of RBT antigen on a glass slide to produce a zone of approximately 2 cm in diameter. The mixture was agitated gently for 4 min at ambient temperature and then observed for agglutination. Tests were considered positive when any visible reaction or agglutination were observed.

2.2.2 Enzyme linked-immunosorbent assay

Sera samples were screened against *Brucella* antibodies using, IDEXX Brucellosis Serum X2 Ab test (IDEXX US, part number BAT1132T) following manufacturer's instruction. The Enzyme linked-immunosorbent assay **(**ELISA) results were interpreted as the signal of the test sample as a proportion of the positive control. The optical density (OD) values of each sample were processed into sample to positive percentage (SP%) to remove background noise. Sample with SP% ≥ 80 was considered positive and accordingly any sample with SP% < 80 was considered negative. The test was considered valid if the OD values of the controls were within the range prescribed in the manufacturer's instruction.

2.2.3 Complement fixation test

Serum samples were referred to the National Institute of Animal Health, Bangkok, an OIE reference laboratory for Brucellosis diagnosis for complement fixation test (CFT). All serum samples were tested to determine antibody titre using CFT validated as per OIE standards. Sample with antibody titre of IU ≥ 20 was considered positive and accordingly any sample with IU < 20 was considered negative.

2.2.4 Culture

Milk samples for culture were referred to the National Institute of Animal Health, Bangkok, the OIE reference laboratory for Brucellosis diagnosis. All milk samples were cultured in selective media to recover *Brucella abortus* organism using validated test as per the OIE standards. The growth colonies on media were confirmed by molecular test – conventional polymerase chain reaction (PCR) test.

2.2.5 Polymerase chain reaction

The colonies were harvested for extraction of deoxyribonucleic acid (DNA), the genomic materials of *Brucella abortus* for PCR test. The DNA samples were used as source of nucleotides in conventional PCR test. The samples in PCR run that produced a band of 450 bp amplicon of *bp26* gene was considered positive.

3. RESULTS AND DISCUSSION

3.1 RBT result

Samples from all five time-points were subjected to RBT. The mean percentage of RBT positive samples over five time-points was 32.80 ± 2.32 (95% CI: 25.00 - 38.18) with lowest and highest positive cases detected in 3rd and 5th time-points, respectively. Samples from all five time-points were subjected to ELISA. The mean percentage of ELISA positive samples over five time-points was 40.11 ± 2.42 with lowest and highest positive cases detected in 3rd and 2nd time-points, respectively.

The RBT and ELISA test results showed the lowest positive cases detected in the 3rd time-point testing. When compared, ELISA detected more positive cases than by RBT. This could be attributed to higher diagnostic sensitivity of ELISA than that of RBT. Samples from only two time-points (4th and 5th) of the study were tested by CFT. The samples from first three time-points could not be referred to Bangkok due to some logistic challenges. The mean percentage of CFT was 37.17 ± 1.01 with highest positive cases detected in 5th time-point samples. Due to the limited volume of serum samples referred, the CFT could not be performed in the initial three time-point samples. While all the test detected 42 positive samples from the 110 (38.18%) during the fifth sampling time, the ELISA detected the highest (37.69%), followed by RBT (36.92%) and lowest by CFT (36.15%). All milk samples except for 5th time-point were cultured and tested by Brucella species specific PCR. The mean percentage of PCR was 22.39 ± 4.32 with lowest and highest positive cases detected in 4th and 1st timepoints, respectively. Milk samples from 5th time-point were also referred but got contaminated, thus could not be processed to pure culture and subject to PCR. About 22% of the animals were found to be shedding organisms in milk in addition to heavy shedding during abortion. This situation can pose serious public health hazards if required interventions are not put in place to safeguard occupational health and food safety. Overall, through nine months of longitudinal study the prevalence of the disease remained same and did not show significant variation. This could be due to the management practices applied to prevent further spread which was reportedly done through segregation of infected animals and managing separately. Although all the four species of Brucella can affect humans but the majority of cases are caused by *B. abortus* and *B. melitensis* (Alton et al. 2014). Commonly Brucella organisms are concentrated in shed in the urine, placental, or foetal tissue and it can transmit through contact or aerosols; it is also additionally found in undercooked meat products or unpasteurized dairy, creating a food safety risk for the consumers (He 2012).

Brucellosis has been considered as high-risk disease among veterinarians (Pereira et al. 2020; Alzuheir 2021); abattoir workers and herdsmen (Mubanga et al. 2021). For the effective overall control of Brucellosis, routine screening of farms and people, targeted vaccination, intervention on milk safety, farmer awareness would be the best implementation strategies (Phillip et al. 2021).

4. CONCLUSIONS & RECOMMENDATIONS

The investigation confirmed that the abortion was due to Brucella *abortus* infection and the infection persisted for quite a long period of time. Over a period of nine months of monitoring at five different time points, the mean prevalence of 22.39% shedders is a real concern for farm as well as the occupational health safety of the farm workers. There is an urgent need to control and prevent the spread of infection. To prevent the spread of infection to human, personal protections of farm workers were enhanced and installation of pasteurization unit in all the suspected farms. To prevent the spread of infection to other animals, infected animals were isolated and managed separately and distribution of breeding stock put on hold. Public health authority is in the process of screening risk group humans for possible exposure.

Based on the findings of this study and until a concrete policy decision is reached concurrent to international standards on control of this disease, the management at NJBC, Samtse is recommended to take immediate actions in the following areas: i) education of farm workers on *Brucella*; ii) providing protective gears to farm workers; iii) installation of milk pasteurization unit at the farm; iv) segregation of infected animals and managed separately by designated set of attendants and, v) putting complete moratorium on supply of breeding stock to other districts. Additionally, considering one of the main mandates of NJBC, Samtse being the distribution of superior quality breeding stocks in the country for cross-breeding, there is an urgent need to conduct national level sero-surveillance to determine the extent of spread of cattle Brucellosis in Bhutan.

REFERENCES

Tshering and Thinley Pastoralism. (2017). Research, Policy and Practice 7:5; DOI 10.1186/s13570-017-0077-1

Olsen S and Palmer M. (2014). Advancement of knowledge of Brucella over the past 50 years. Vet Pathol. 51(6):1076–89. DOI: 10.1177/0300985814540545

OIE. (2018). OIE-listed diseases, infections and infestations in force in 2018. http://www.oie.int/en/animal-health-inthe-world/oie-listed-diseases-2018/. Accessed 24th March 2022.

Samdup T, Udo HMJ, Eilers CHAM, Ibrahim MNM and Van der Zijpp AJ. (2010). Crossbreeding and intensification of smallholder crop– cattle farming systems in Bhutan. Livestock Science 132:126 –134.

Abernethy DA, Moscard-Costello J, Dickson E, Harwood R, Burns K, McKillop E, McDowell S and Pfeiffer DU. (2011). Epidemiology and management of a bovine brucellosis cluster in Northern Ireland. Preventive Veterinary Medicine. 98: 223–229. https://doi.org/10.1016/j.prevetmed.2010.11.002 PMID: 21144605

Xavier MN, Paixão TA, Poester FP, Lage AP and Santos RL. (2009). Pathological, Immunohistochemical and Bacteriological Study of Tissues and Milk of Cows and Fetuses Experimentally Infected with Brucella abortus. Journal of Comparative Pathology. 140: 149–157. https://doi.org/10.1016/j.jcpa.2008.10.004 PMID: 19111839

Holt HR, Bedi JS, Kaur P, Mangtani P, Sharma NS and Gill JPS et al. (2021). Epidemiology of brucellosis in cattle and dairy farmers of rural Ludhiana, Punjab. PLoS Negl Trop Dis 15(3): e0009102. https://doi.org/10.1371/journal.pntd.0009102

Herrera E, Palomares G and Díaz-Aparicio E. (2008). Milk production increase in a dairy farm under a six-year Brucellosis control program. Ann NY Acad Sci. 1149:296–299. Crossref. PubMed.

Havelaar A, Grace D and Wu F. (2019). Foodborne diseases from dairy products in developing countries: hazards and health implications.

Olsen SC and Stoffregen W. (2005). Essential role of vaccines in brucellosis control and eradication programs for livestock. Expert Rev Vaccines. 4(6):915–928. Crossref. PubMed.

Dadar M, Fakhri Y, Shahali Y and Khaneghah AM. (2020). Contamination of milk and dairy products by Brucella species: a global systematic review and meta-analysis. Food Res Int. 128:108775. Crossref. PubMed

Dadar M, Tiwari R, Sharun K and Dhama K. (2021). Importance of brucellosis control programs of livestock on the improvement of one health. Veterinary quarterly, 41(1):137-151.

Nicoletti P. (1984). The control of brucellosis in tropical and subtropical regions. Preventive Veterinary Medicine. 2(1–4):193–196. Crossref

Alton GG and Forsyth JRL. (2014). Brucella. In: Baron S, editor. Molecular Medical Microbiology, 2nd ed. Texas: Elsevier Ltd. p. 1781–8. doi: 10.1016/ B978-0-12-397169-2.00101-3

He Y. (2012). Analyses of Brucella Pathogenesis, Host Immunity, and Vaccine Targets Using Systems Biology and Bioinformatics. Front Cell Infect Microbiol, 2:2. doi: 10.3389/fcimb.2012.00002

Karlsson PA, Persson C, Akoko J, Bett B, Lundkvist Å and Lindahl JF. (2021). Using a One Health Case Based Investigation for Improved Control of Brucellosis in Isiolo, Kenya. Front. Trop. Dis. 2:711425. doi: 10.3389/fitd.2021.711425

Pereira CR, Cotrim de Almeida JVF, Cardoso de Oliveira IR, Faria de Oliveira L, Pereira LJ and Zangerônimo MG et al. (2020). Occupational exposure to *Brucella* spp.: a systematic review and meta-analysis. PLoS Negl Trop Dis. 14: e0008164. 10.1371/journal.pntd.0008164

Alzuheir I, Zabadi HA and Helal MA. (2021). Occupational Exposure Assessment and Seroprevalence of ***Brucella*** Specific Antibodies Among Veterinarians in the Northern Palestine. Front Veterinary Science; 8: 813900. doi: 10.3389/fvets.2021.813900

Mubanga M, Mfune RL, Kothowa J, Mohamud AS, Chanda C, Mcgiven J, Bumbangi FN, Hang'ombe BM, Godfroid J, Simuunza M and Muma JB. (2021). Brucella Seroprevalence and Associated Risk Factors in Occupationally Exposed Humans in Selected Districts of Southern Province, Zambia. Front. Public Health 9:745244. doi: 10.3389/fpubh.2021.745244