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Prevalence of Brucellosis and Awareness of its Spread

Nimita Kant, Parul Kulshreshtha*, Rashmi Singh, Anuradha Mal, Shashikant Kumar, Mohit Tehlan, Amita Dwivedi, Rinkle Mehra, Riya Ahuja, Shilpa Kausik and Paritosh Ahmed
Shivaji College, University of Delhi, Raja Garden, Delhi. 110 027
*parulkuls26@gmail.com

ABSTRACT

Brucellosis is a disease in cattle and can transmit to humans on consumption of infected beef and dairy products. Most of the suburban and rural households depend on dairy farming for their living. In NOIDA, Delhi NCR, buffalo population is very high. Most people living in the high rise apartments there, also consume unpasteurized dairy products from the local dairies. In this scenario, we ventured to investigate the knowledge, attitude and practices of cattle farmers of Noida to assess their habits which may lead to spread of Brucellosis. In the wake of it, 50 cattle farms were visited which housed 150 cattle in total. At the same time, the prevalence of *Brucella*-specific antibodies was assessed by WHO- prescribed 'on site'/ 'on farm' tests and, each farmer was informed about the disease status of their cattle. Since none of the cattle was vaccinated previously with the *Brucella* vaccine, serological detection was therefore appropriate for this population. Results showed that the prevalence of *Brucella*-specific antibodies was very high (50%) in the buffalo population of Noida. It was concluded that the habits of the cattle keepers were the prime reasons behind the high sero-prevalence observed. Hence, we took upon the task of spreading awareness and distributed *Pashudhan se sambandhit karya*, a document in Hindi prescribed for cattle care by Indian Council of Agricultural Research. We also created awareness about the Brucellosis vaccination schedule as per Indian Veterinary Research Institute.

Keywords: *Brucella abortus*, Brucellosis, buffalo, Noida, Seroprevalence, survey.

INTRODUCTION

NOIDA has a high population of buffalo. Most rural people have dairy farming as their profession. With the declaration of NOIDA as the Special Economic Zone (SEZ), there has been a boom of housing societies in Noida. Thus the dairy farming business of natives of Noida has also seen a rise. This is because most people staying in high end societies still prefer to consume buffalo milk. In this scenario, it was pertinent to find out the knowledge, attitudes and practices of cattle keepers that may lead to spread of Brucellosis in particular and other infectious diseases in general. Brucellosis is a reproductive disease of livestock which induces infertility, delayed heat, interrupted lactation, abortion and loss of calves, loss

of milk and meat production. Brucellosis is endemic in countries belonging to the Mediterranean region, Asia, Middle East, Latin America and Africa (1). The disease causes economic loss and is endemic to all states of India (2). The causative agent of this disease is a *proteobacteria* belonging to genus *Brucella*. *Brucella* is gram negative, aerobic, facultative intracellular coccobacilli. There are several infective species of this genus; *B.abortus* infects cattle, *B.melitensis* infects sheep *and goats, B.suis* infects pigs, *B.canis* infects dogs. All the species of *Brucella* can infect humans, but *B.melitensis* is the most common human pathogen (3). In humans it causes a severely debilitating disease that requires prolonged antibiotic treatment with a propensity of serial recurrence (4). *Brucellae* transmits from one animal to other through cuts or abrasions in the skin or by inhalation. The factors that facilitate transmission include poor farm hygiene, use of semen from infected bulls for artificial insemination, unrestricted trade and movement of animals from one district to another, use of local cattle yards and fairs for trading where mixing of cattle with sheep/goats occurs and chance of transmission rises. Human transmissions are also common. In rural areas, human beings come in regular contact with cattle and consumption of unpasteurized/unboiled milk (rich in *Brucella*) is common. In urban areas *Brucella* transmits through consumption of infected meat and of dairy products prepared from unpasteurized milk like yoghurts, ice creams and soft cheese (5). Control and preventive measures include improved farm hygiene, mass vaccination of cattle and diagnosis of Brucellosis followed by removal of infected animal. Vaccination is crucial for eradication, but due to lack of awareness amongst farmers with large number of buffalos, vaccination of each buffalo in the country is an unfathomable task. The diagnosis of brucellosis is based on detection of anti-*Brucella* antibodies in the blood of host because in 15-35% of cases (active disease) blood stays negative for *Brucella* bacterium by blood culture study(6). Isolation and identification of *Brucella* from blood of infected organism is a method of diagnosing *Brucella* infection. But this method is time consuming (45 days) and not safe for laboratory personnel and impractical for large number of animals. WHO prescribes Rose Bengal Plate Test (RBT) wherein the serum from the animal is added to the test reagent and agglutination on slide is observed(7). This test is qualitative and indicates whether the animal is infected with *Brucella* or not. We conducted this test on the farm and informed cattle keepers about possible disease status of their cattle. We conducted the Serum Agglutination Test (8) in the college laboratory, which diagnosed the degree of infection of each animal found positive with RBT.

METHODOLOGY

KAP [Knowledge, Attitudes and Practices] Survey:

A cross-sectional study was carried out to estimate the awareness of cattle keepers about Brucellosis and other infectious diseases. A standardized structured questionnaire including both open end and close end questions scripted in Hindi was distributed in Noida. Fifty farms which housed about thousand cattle were covered. The questionnaire was prepared to know the animal maintenance practices of the cattle keepers. The survey in these farms was conducted by ten students (enrolled in the project) and the concerned faculty members of Shivaji College. The format of the questionnaire has been provided as Appendix.

Ethical Clearance:

Experimental protocol was submitted and approved by the animal ethical committee of Shivaji College, University of Delhi. We followed the international standards of animal handling throughout the study.

Blood Collection:

Animal handling was done by Animal Lab technician trained at National Institute of Immunology (NII) for handling animals. To collect blood, a small area of ear was cleaned with spirit swab, a prick was made using a 16 guage needle to collect blood in a 5ml disposable syringe (Dispovan) from a visible ear vein from buffalos and saved in microfuge tubes. These tubes containing buffalo blood (0.5ml) were kept in vertical orientation in a collection box. The tubes were brought to college within 3-4 hours of collection from village and centrifuged at 500x*g* to obtain blood serum. Sera were then treated at 60 degree C for 30 minutes for inactivating probable infective bacteria and stored at -20^0 C till further investigation. After the blood collection the prick was pressed for some time to stop bleeding with a cotton swab followed by applying Neosporin powder at the prick to avoid any infection.

Rose Bengal Test Assay:

RBT is a reagent approved by WHO for detection of Brucellosis. It has a pink coloured dye called as Rose Bengal mixed with dead *Brucella abortus* in a solution of pH 3.5 to 3.7. This is a qualitative test and was done 'on the cattlesheds'. Rose Bengal test reagent (100 µl) was mixed with a drop of blood on a microslide and reaction was observed for five minutes by tilting the slide.

Agglutination Assay:

This assay was done by using the Brucella Serum Agglutination Test [SAT] antigen. SAT antigen is a suspension of a pure smooth culture of *Brucella abortus* in phenol saline obtained from Indian Veterinary Research Institute, Izatnagar. This was done in the college laboratory. The assay was adapted from the original (8) to reduce the volume of sera to be used and was carried out in 96–well ELISA plates. Phenol saline was prepared by adding 0.5% phenol to normal saline and was dispensed in volumes of 80µl in first well of each row followed by 50 µl in the subsequent wells. Sera were added in a volume of 20µl to first well, mixed well and two fold serially diluted four more times by picking 50µl from first well. The sera and agglutination antigen was mixed well and incubated at 37 deg C for 20 ± 1 hours. All 100 sera samples were tested by SAT and study was done three times. The result was observed after incubating plates for half an hour at the room temperature.

Social Cause served by the Innovation Project:

After the questionnaires were answered by the respondents they were given the right answers and were apprised of the practices which can prevent their cattle from contracting Brucellosis. The Animal Maintenance Directives by the name of *Pashudhan se sambandhit karya* in the local language − Hindi is available on the website of the National Dairy Research Institute. We printed these directives and distributed them to the cattle keepers.

RESULTS & DISCUSSION

Brucellosis is endemic to all states of India. There are regular reports of brucellosis alerts in Punjab and Southern states of India (9, 10). In these two regions, brucellosis control programs are well established and effective and other parts of the country also need to catch up. Since this disease is cattle-borne and spreads to humans via dairy products and meat, we believe its containment requires utmost priority. Hence we attempted to understand the awareness of cattle keepers regarding the infectious diseases and investigated the local sero prevalence of *Brucella* in Noida. We could not convince all the farmers approached by our team to get the blood test done for their buffaloes since many were not open to our initiative. Hence, while the KAP survey was conducted for 100 cattle sheds (housing 3000 buffalos)

spread across different sectors of Noida, in all, 60 buffalos from 50 cattle farms in Noida were subject to blood test for detection of Brucella infection.

Survey:

As is evident from Table I, the awareness about Brucellosis and its vaccine S19 did not exist amongst the cattle keepers. The diseases they were cognizant of were Anthrax and Foot and Mouth disease. We also realized that there was a general lack of awareness about the safety measures that cattle farmers must follow to prevent spread of zoonotic diseases. Though the number of abortions during third trimester was low among the cattle in Noida suggesting low rate of infection with *Brucella abortus,* majority of cattle keepers appeared to be potentially at high risk of contracting Brucellosis and other infectious diseases due to their negligent and unsafe practices.

As can be observed from Table I, most cattle keepers were of the belief that they cannot acquire infection from their cattle and hence did not follow any safety measures while handling animals. Their risk of acquiring *Brucella* infection could be attributed to consumption and application (on cracked lips) of raw milk, sleeping in cattle sheds and assisting in animal birth and disposing off the aborted fetus & placenta with bare hands. Additionally, the cattle keepers are usually exposed to *Brucella* present as spores in the cattle sheds and in body fluids of cattle. A dangerous practise by majority of the cattle keepers was not to isolate their sick animals from the rest. This ignorance often leads to the spread of disease to other animals with an impending threat of reaching an epidemic scale! Our survey also revealed that none of the cattle keepers get the cattle tested for any infectious diseases including Brucellosis before buying them. Another cause of worry was the observation that most of cattle keepers do not use any disinfectant to clean their cattle shed, hence increasing their chance of contracting the infections.

However, it was a relief to observe that only 1% of cattle keepers rear small ruminants like goat and sheep together with buffalo. This practise saves spread of infectious diseases from small ruminants to the large ones.

Table I: Knowledge, Attitude and Practices (KAP) Survey in NCR of Noida

S.N.	Risk Factors	Yes %	No %	Sometimes %	Don't Know %
1	Drink raw milk	80	15	5	0
2	Milking	75	22	3	0
3	Sleeping in Animal Sheds	70	11	19	0
4	Assisting Animal Birth	100	0	0	0
5	History of abortion in [third trimester] on farm	35	65	0	0
6	Disposed aborted fetus with bare hands	45	55	0	0
8	Disposed placenta with bare hands	35	53	2	10
9	Applying raw milk on cracked lips	44	50	6	0
10	Vaccination of animals	30	44	14	12

11	Isolation of sick animals	25	75	0	0
12	Can you acquire disease from your cattle?	15	85	0	0
14	Use of disinfectant to clean cattle shed	5	85	10	0
15	Blood test before buying the animal	0	100	0	0
16	Do you rear goat and sheep with buffalo and cow?	1	97	2	0
17	Have you heard of Brucellosis?	0	100	0	0
18	Have you heard of S19 vaccine?	0	100	0	0

Analysis of Rose Bengal Test (RBT):

RBT is a qualitative test for checking Brucellosis infection. We found 35/60 (58%) samples to show positive agglutination with RBT. The agglutination was correlated with blood samples that were negative for agglutination (Figure 1).

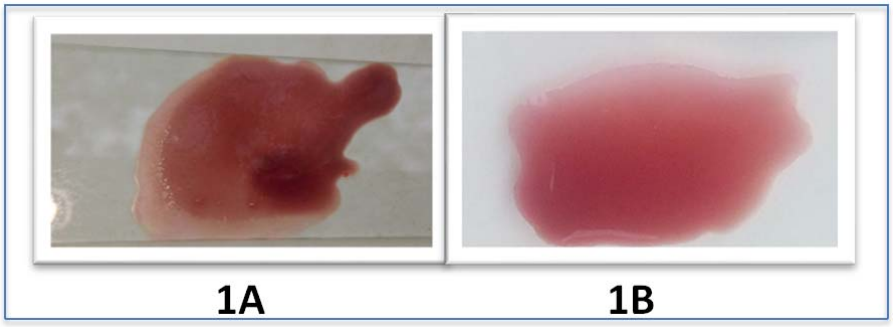

Figure 1: Rose Bengal Test with buffalo blood. 1A − this figure shows clumping of blood with RBT agent. 1B− Control slide: (no agglutination with RBT).

Serum Agglutination Test (SAT):

This test helps to determine the degree of *Brucella* infection. International units of *Brucella* infection was calculated by using the following formula:

> I.U.= Titre of unknown serum x 1000 / the titre of International serum with the antigen to give 50% agglutination

Here, the titer of the international serum with the antigen specified on the reagent bottle was 1:500. This value was used to calculate IU of infection. The titer of unknown was the last dilution showing agglutination with the antigen. An IU equal to or more than 80 IU indicated infection with *Brucella abortus.* It was found that 30 out of 60 samples gave IU of infection of 160. Five samples that were positive by RBT assay had a 40 IU of infection. But in cattle 40 IU represents doubtful case of *brucella* infection. Thus 50% was the seroprevalence which is way higher than the 13.5% of stable endemic equilibrium predicted by IARI (7, 11,12).

Social cause served by the Innovation Project:

The cattle farmers lack access to the internet or computer facilities and therefore, after the survey was done, we also distributed the Animal Maintenance Directive document to them. This directive has been written in Hindi language and gives month-wise instructions on how to take proper care of the cattle. Since, this document also lacks information about Brucellosis. Therefore, students and faculty members involved in the Innovation project made efforts to educate the respondents about harmful effects and the spread of Brucellosis and provided awareness training as intervention to the local population in the region.

CONCLUSIONS

This study showed that the prevalence of *Brucella*-specific antibodies among buffalo population of Noida (50%) was significantly higher than the baseline value (13.5%) predicted by IARI for all states of India. *Brucella* infection is endemic for India but given the size of a country, prevalence among different states may be different. Therefore, reports about seroprevalence of Brucellosis are extremely important to plan the regional public awareness and prevention programs. Results of KAP survey in this study indicate that unhygienic cattle sheds and risky demeanour of cattle keepers contribute to this high seroprevalence or spread of Brucellosis. Therefore, we conclude that if the country aims to curb this rising threat of Brucellosis then Government and the scientific community must carry out active awareness and preventive programs.

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