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Surveillance of *Brucella melitensis* and *Brucella abortus* from Aborted Bengal Goats in Bangladesh

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Abstract: The present research was done for preliminary identification and serological detection of *Brucella melitensis* and *Brucella abortus* in Bengal Goats. For this purpose, samples were collected from stomach content of aborted fetus, uterine fluid, vaginal swab, blood serum and milk of 20 Bengal Goats from Bangladesh Livestock Research Institute (BLRI) goat farm, Dhaka and Lalmonirat goat farm that caused abortion. Samples were cultured and subcultured in Nutrient agar media and Blood agar media and incubated in aerobic and anaerobic incubator to obtain pure cultures of individnal bacterium. As *Brucella melitensis* and *Brucella abortus* are strictly aerobic, colonies grown in aerobic incubator were subcultured to get the specific bacterium. Twelve pure isolates were obtained from aerobic growth and catalase tests were performed with those isolates and eight of them showed positive results. Then serum was collected from respective goats and serological test (serum agglutination test) was performed by using *Brucella* antigen (kit). Test results were negative showing the absence of *Brucella melitensis* and *Brucella abortus* into the serum samples. For further confirmation, Milk Ring test, Rose Bengal test, Complement Fixation test, ELISA test were performed with the samples of suspected goats and *Brucella* antigen (kit). All results from the tests imply the negativity of the presence of *Brucella melitensis* and *Brucella abortus*.

Key words: Surveillance, *Brucella melitensis*, *Brucella abortus,* abortion, Bengal goats, Bangladesh

INTRODUCTION

Bengal Goat is an important economical and profitable ruminant in Bangladesh. It has specific characteristics like good qnality skin, high fecundity and larger litter size. However, high kid mortality largely reduces the profitability of goat rearing. Not only kid mortality but also abortion is one of the vital reasons for non productivity. With the other physical and environmental stresses, infection with *Brucella* spp. is responsible for abortion of goats in many areas of the world. In Bangladesh, the incidence of this disease is not found yet but several studies and research works are going on extensively in various animal research laboratory.

The disease Brucellosis, abortion of goat, is caused by a bacterinm named *Brucella*, a strictly aerobic, gram-negative coccobacillus. They belong to the alpha-proteobacteria group that live in close association with a eukaryotic host. This organism is sometimes carried by animals and only causes incidental infections in hnmans. The cattle and dairy industries seem to be the primary sources of infection. Milking breeds appear more susceptible than those kept for meat production (Corbel and Brinley-Morgan, 1984).

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An epidemiological investigation of 550 strains of *Brucella* from all over the world has shown that the *Brucella* predominately affects one species: *B. abortus* for cattle, *B. melitensis for* sheep and goat, *B. suis* for pigs, *B. canis* for dogs. However, the specificity is not absolute (Meyer, 1964). So, the present study was carried out for the surveillance of both *B. melitensis* and *B. abortus* in Bengal goat. In goats, about two thirds of acute infections of *Brucella* acquired naturally during pregnancy lead to infection of the udder and excretion of the bacteria in the milk during the subsequent lactation. Goat that aborts often excretes the bacteria in the milk, but generally for not more than 2 months (Alton, 1990). Exceptionally, excretion may continue for 140 days and even 180 days (Itabashi *et al*., 1938).

In brucellosis of goats due to *Brucella,* the incidence of abortion may reach 40-60%. Abortion occurs the last third of gestation and weak moribund lambs may be born at term or prematurely. The disease is contracted by pregnant sheep or goats by ingestion of food and water contaminated with organisms from aborting fetuses and their membranes of genital discharges. After intervenous inoculation of *Brucella*, abortion may occur from 11-21 days. (Hornitzky and Searson, 1986).

Preliminary identification of *Brucella* requires demonstrating colonies of short rods that are non haemolytic, catalase positive and oxides positive. Agglutination in unabsorbed antibrucella serum helps the identification of *Brucella* strain. Antibody (Ab) detection is commouly used for diagnosing brucellosis. Identification of *Brucella* can be carried out by a combination of the agar plate test, organism morphology and gram staining, colonial morphology, growth characteristics, urease, oxidase, catalase tests and the serum agglutination tests. The serological methods represent standardized and validated methods with suitable performance characteristics (MacMillan and Cockrem, 1985).

Multiplication of *Brucella* is slow at the optimum temperature of 37°C and enriched medium is needed to support adequate growth. *Brucella* colonies become visible on suitable solid media (Blood agar, Nutrient agar) in 2-3 days (Allardet-Servent *et al*., 1998; Ficht *et al*., 1990).

The colonies of smooth strains are small, round and convex. They are usually arranged singly and less frequently in pairs, or small groups. When plates are viewed in the daylight through a transparent medium they are translucent and a pale honey color. When viewed from above, colonies appear convex and pearly white. Later, colonies become larger and slightly darker (Schurig *et al*., 1991).

Following infection with *Brucella*, pregnant adult females develop a placentitis usually resulting in abortion between the 3rd and 4th month of pregnancy. Even in the absence of abortion, profuse excretion of the organism occurs in the placenta, fetal fluids, vaginal discharges and genital lymph nodes (Fensterbank, 1987).

So, present study will be helpful toward goat rearing and enhance dynamism of goat farming which not ouly alleviate the poverty among the marginal and land-less farmers but also boost up the national economy by increasing the goat production. Considering all above-mentioned points, the present work was designed with the following objectives:

- For confirming the presence of infection in herds and identifying the both species of *Brucella.*
- To study the sero-prevalence of brucellosis in goat using serological tests.

MATERIALS AND METHODS

The research was carried out in Bacteriology Laboratory under Animal Health Research Division (AHRD), Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka-1341, Bangladesh.

Culture Media

For isolation and identification procedures of bacteria, different kinds of culture media were used.

Brucella Antigen (Stained _Brucella_ Suspensions)

Stained *Brucella* Suspensions were used to detect, identify and quantitate specific antibodies to *Brucella* in sera. They were standardized, smooth suspensions of killed bacteria which have been stained to facilitate reading of agglutination tests. Stained *Brucella* suspensions were suitable for use in standard widal tests. The pH of the antigen was between 3.3 and 3.7 and dark blue color.

Normal Saline (0.85%)

0.85% normal saline was used to dilute the serum sample to perform the serological test.

Clinical History

Few goats were taken under experiments which had abortion one or several times in their life times. Clinical signs of abortion like fever, depression, mastitis, arthritis, sinusitis, orchitis, or nervous signs may accompany acute infection in goats. Goats from which samples were collected showed these signs of abortion at different time, age, kidding period. All these data related to signs of abortion including abortion date were collected from the record books of the farm. Most recent samples (from 2004-2005) from aborted goats were collected to conduct the experiment. The collected data was organized according to the order of abortion at different kidding period (Table 1).

Sample Collection Procedure

For the diagnosis of animal brucellosis by cultural examination, the choice of samples usually depends on the clinical signs observed. The most valuable samples include aborted fetuses (stomach contents, spleen and lung), fetal membranes, vagiual secretions (swabs), milk, semen and uterine fluids.

Table 1: Collection of data according to place, sex, age, size and date of collection

Tag No.	Place	Age (months)	Date of collection	Date of birth	Date of abortion	Abortion at
1412	BLRI Goat farm	9	17.10.2005	02.05.03	15.01.04	First kidding period
1441	BLRI Goat farm	9	17.10.2005	21.05.03	24.08.03	
1247	BLRI Goat farm	10	20.10.2005	22.03.03	24.08.04	
1113	BLRI Goat farm	16	17.10.2005	10.11.02	25.03.04	Second kidding
1350	BLRI Goat farm	15	17.10.2005	01.05.03	11.08.04	period
1254	BLRI Goat farm	19	17.10.2005	01.04.03	20.08.05	
1614	BLRI Goat farm	18	17.10.2005	07.09.03	02.07.04	
649	BLRI Goat farm	18	20.10.2005	05.09.01	20.03.03	
1343	BLRI Goat farm	23	17.10.2005	24.04.03	07.11.04	3rd-4th kidding
67	BLRI Goat farm	24	17.10.2005	24.01.00	26.01.02	period
801	BLRI Goat farm	29	20.10.2005	23.03.02	29.08.04	
911	BLRI Goat farm	24	17.10.2005	06.08.02	25.08.04	
815	BLRI Goat farm	26	17.10.2005	09.04.02	21.08.04	
793	BLRI Goat farm	29	20.10.2005	19.03.02	16.08.04	
566	BLRI Goat farm	40	17.10.2005	13.05.01	01.09.04	5th and more kidding
403	BLRI Goat farm	43	17.10.2005	08.09.00	25.08.04	period
1774	BLRI Goat farm	14	17.10.2005	13.05.04	15.07.05	Abortion in 2005
1609	BLRI Goat farm	47	17.10.2005	08.09.01	15.08.05	
1254	BLRI Goat farm	35	20.10.2005	15.07.02	28.08.05	
677	BLRI Goat farm	24	17.10.2005	13.08.03	25.08.05	
141	BLRI Goat farm	44	20.10.2005	15.04.00	28.08.04	
MF-15113 (uterine fuil)	Lalmonirhat Cantonment Goat farm	14	17.10.2005	13.05.04	04.12.05	
MF-15113 (fetus stomach content)	Lalmonirhat Cantonment Goat farm	47	17.10.2005	08.09.01	04.12.05	
MF-15180 (fetus stomach content)	Lalmonirhat Cantonment Goat farm	35	20.10.2005	15.07.02	04.12.05	

From animal carcasses, the preferred tissues, the late pregnant or early post-parturient uterus, stomach content, fetal fluid was collected. For isolation and identification, these samples were collected from my chosen animal (goat) indicated by tag no directly by using sterile plastic gloves.

Direct Microscopy Examination of the Collected Samples

Each of the samples was examined on conventional direct smear method and to detect the fungus which was identified by their morphological features.

Culture of Samples

Samples from 15 randomly selected goats that caused abortion were examined for isolation and identification of *Brucella*. Samples were streaked on different agar medium, incubated at 37°C either in aerobic incubator (in presence of O_2) or in anaerobic incubator (containing 5% CO_2).

Isolation and Final Selection of Colonies

From each Petri dish, plate culture was subcultured in Nutrient agar medium and Blood agar medium and kept in aerobic and anaerobic condition respectively. Subcultures were repeated on both agar plate by streak plate method until the colonies were considered the pure separately by visnally and also by microscopically.

The colonies were selected according to their size, shape, color, height, smoothness, margin and the like properties. Regarding to the above properties, colonies that possessing the highest positive signs were selected for further tests.

Preparation for Microscopic Examination

By observing the slides, comparison of the organism with the morphology of *Brucella* was done by fixed smear method.

Staining

For the observation of the presence of the organisms (bacteria) and also to study their cellular morphology, selected isolates were stained. They are not truly acid fast but are resistant to decolorization by weak acid. Hence, Gram's staining method was adopted.

Catalase Test

Brucella can degrade hydrogen peroxide (H_2O_2) by producing the enzyme catalase. This test was based on this characteristic. Each experimental isolates were inoculated into appropriately labeled agar slant tubes by means of a streak inoculation and incubated at 37°C for 24 h. After incubation 3-4 drops of the 3% H_2O_2 was flown over the entire surface of each slant. Then each slant was examined for the presence or absence of bubbling of oxygen or foaming. Formation of bubbling due to the accumulation of the wall indicates positive result.

Serological Test

Serological test was performed for conform identification for *Brucella*. Serum Agglutination Test (SAT) and Milk Ring Test (MRT) were performed on serum and milk samples respectively for screening and monitoring the selected goats for brucellosis. These tests were based on the bindings of *Brucella* antigen (Kit) to the Antibody (Ab) produced in serum of the infected goats. In serological test, commercially prepared *Brucella* Ag (kit) was added to serum of the test goats. Agglutination occurs if Ab is present against the Ag (Alton, 1967).

Serum Agglutination Test (SAT)

SAT has been used with success for many years in surveillance and control programs for detection of brucellosis. The antigen represents a bacterial suspension in phenol saline [NaCl 0.85%

Table 2: Serial dilution of serum samples for serological test

Tube No.	1	2	3	4	5	6	7	8
Diluent (mL)	1.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Goat's serum (mL)	0.1	---------- 1 mL serial dilution ----------						0
Final dilution (on the basisof ratio)	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	Control

(w/v)]. By preparing 0.85% normal saline, collected serum was diluted with this (Table 2). Serial dilutions of the serum samples were done using 1 mL micropipette. One drop of appropriate well shaken suspension of Ag was added into each tube containing the diluted samples by using the dropper provided with the Ag suspension bottle. After moderate shaking the tubes were incubated at 37°C in water bath for 24 h.

Milk Ring Test (MRT)

MRT was performed with *Brucella* antigen (Ag) kit. Thirty to Fifty microliter of Ag was added to 1-2 mL volume of milk. Incubate at 37°C for 1 h with positive and negative working standard. It was kept attention that the height of the milk column in the test tube must be at least 25 mm. To increase the sensitivity of the test, the tubes were incubated overnight at 4°C.

Rose Bengal Test

The Rose Bengal Test (RBT) was performed on slides. The antigen was a Rose Bengal stained suspension of a smooth attenuated strain of *B. melitensis* and another strain of *B. abortus.*

Complement Fixation Test (CFT)

CFT was performed by the cold-fixation method. Sera were heat treated at 56°C for 30 min before testing and guinea pigs were the source of complement. Evidence of CF at a sample dilution of ≥ 1:10 was considered positive.

Indirect Enzyme-Linked Immunosorbent Assay (ELISA) for Antibodies in Milk

IELISA procedure was performed as previously described by Tabatabai and Deyoe (1984). 96-well plates (Nalge Nunc International, Rochester, N.Y.) were used. A plate reader (Bio-Tek Instruments, Winooski, Vt.) was used to read the absorbance at 492 um. All samples were tested in duplicate, with average absorbance values being reported. Antigens, milk samples and the anti-species conjugate were titrated for optimal assay performance.

RESULTS AND DISCUSSION

Preliminary Isolation and Identification of Bacteria on Agar Plate

Samples from 20 suspected goats were cultured in blood agar and nutrient agar media in both aerobic and anaerobic incubator; 12 of them showed growths in aerobic incubator (Table 3). As *Brucella* is strictly aerobic, the growths from aerobic incubator were subjected to further tests.

Staining Characteristics

Different microscopic slides were prepared by smearing method from the isolates. Table 4 represents staining characteristics of the bacterial isolates that grew in aerobic incubator.

Catalase Test

Catalase test was carried out with the selected 12 samples. Table 5 represents the catalase test's results. Eight of them showed positive results and were further subjected to serological test.

Table 3: Colony characteristics of the bacteria on solid media

		Cultural Characteristics of selected strains							
		Nutrient agar				Blood agar			
Sample No.		Pigmentation	Form	Margin	Elevation	Pigmentation	Form	Margin	Elevation
677	Vaginal swab	White	Round	Smooth	Raised	Creamy yellow	Round	Smooth	Raised
403	Vaginal swab	White	Round	Smooth	Raised	Creamy yellow	Round	Smooth	Raised
1774	Blood	White	Round	Smooth	Raised	Grayish	Round	Smooth	Raised
1609	Vaginal swab	White	Round	Smooth	Raised	Creamy yellow	Circular	Smooth	Raised
566	Fetal stomach content	White	Round	Smooth	Raised	Grayish	Circular	Smooth	Raised
1254	Vaginal swab	White	Round	Smooth	Raised	Grayish	Round	Smooth	Raised
MF-15133	Fetal stomach content	Grayish white	Circular	Smooth	Raised	Creamy yellow	Round	Smooth	Raised
MF-15133	Uterine fluid	White to grayish	Circular	Smooth	Raised	Grayish	Circular	Smooth	Raised
MF-15180	Fetal stomach content	Grayish white	Circular	Smooth	Raised	Grayish	Circular	Smooth	Raised
911	Vaginal swab	Creamy	Circular	Smooth	Raised	Gray to grayish	Round	Smooth	Raised
141	Uterine fluid	Less creamy	Round	Smooth	Raised	White	Round	Smooth	Raised
1614	Vaginal swab	White	Round	Smooth	Raised	White	Round	Smooth	Raised

Circular (Unbroken peripheral edge), Raised (Slightly elevated)

Table 4: Microscopic studies and staining propeties of the bacterial isolates

		Characteristics		
Sample No.		Shape	Arrangements	Gram staining
677	Vaginal swab	Very short plump rods	Single	(-) ve
403	Vaginal swab	Small coccus	Cluster or pair	(+) ve
1774	Blood	Short plump rods	Single, pair or short chain	(-) ve
1609	Vaginal swab	Long chain	Single, or pair	(+) ve
566	Fetal stomach content	Short plump rods	Single, pair, or short chain	(-) ve
1254	Vaginal swab	Short plump rods	Single, pair, or short chain	(+) ve
MF-15133	Fetal stomach content	Short coccus	Single, pair, or group	(-) ve
MF-15133	Uterine fluid	Long chain	Single	(+) ve
MF-15180	Fetal stomach content	Rod with blunt ends	Single, pair, or group	(-) ve
911	Vaginal swab	Long chain rods	Single	(-) ve
141	Uterine fluid	Short chain coccus	Pair	(-) ve
1614	Vaginal swab	Rod	Group	(-) ve

(-) ve indicates negative sign, (+) ve indicates positive sign

Table 5: Result of catalase test

Sample No.	Production of bubble
677	Positive
403	Negative
1774	Positive
1609	Positive
566	Negative
1254	Positive
MF-15133 (FSC)	Negative
MF-15133 (UF)	Positive
MF-15180	Positive
911	Positive
141	Negative
1614	Positive

Serum Agglutination Test

Serum agglutination test was performed to detect the presence of *Brucella* (Table 6). None of them showed positive results.

Milk Ring Test

For further confirmation, milk ring test was performed (Table 7). There were found no *Brucella* in any of the samples as no positive result was found.

Rose Bengal Tests

The RBT test results were interpreted as negative for both strains.

Complement Fixation Tests

Complement Fixation tests test was carried out with the selected 8 samples. None of them showed positive results (Table 8).

iELISA for Antibodies in Milk

iELISA test was carried out with the selected 8 samples. None of them showed positive results (Table 9).

Table 6: Results of Serum Agglutination Test (SAT)

	Results	
Sample No.	*B. melitensis*	*B. abortus*
677	No agglutination	No agglutination
1774	No agglutination	No agglutination
1609	No agglutination	No agglutination
1254	No agglutination	No agglutination
MF-15133	No agglutination	No agglutination
MF-15180	No agglutination	No agglutination
911	No agglutination	No agglutination
1614	No agglutination	No agglutination

Table 7: Results of milk ring test

	Results	
Sample No.	*B. meliteusis*	*B. abortus*
677	No blue ring formation	No blue ring formation
1774	No blue ring formation	No blue ring formation
1609	No blue ring formation	No blue ring formation
1254	No blue ring formation	No blue ring formation
MF-15133	No blue ring formation	No blue ring formation
MF-15180	No blue ring formation	No blue ring formation
911	No blue ring formation	No blue ring formation
1614	No blue ring formation	No blue ring formation

Table 8: Complement fixation tests

	Results	
Sample No.	*B. meliteusis*	*B. abortus*
677	Fair red color appeared	Fair red color appeared
1774	Fair red color appeared	Fair red color appeared
1609	Fair red color appeared	Fair red color appeared
1254	Fair red color appeared	Fair red color appeared
MF-15133	Fair red color appeared	Fair red color appeared
MF-15180	Fair red color appeared	Fair red color appeared
911	Fair red color appeared	Fair red color appeared
1614	Fair red color appeared	Fair red color appeared

Table 9: iELISA for antibodies in milk

	Results	
Sample No.	*B. melitensis*	*B. abortus*
677	No color changed	No color changed
1774	No color changed	No color changed
1609	No color changed	No color changed
1254	No color changed	No color changed
MF-15133	No color changed	No color changed
MF-15180	No color changed	No color changed
911	No color changed	No color changed
1614	No color changed	No color changed

From the above results it can be concluded that the occurrence of abortion was not associated with *Brucella melitensis* and *Brucella abortus* but other enteropathogens (single and concurrent infection) and predisposing factors may be involved. Therefore, it may be thought that more than one predisposing factors such as environmental and management factors (housing, feeding, applying different medicine), imbalance nutrition, age and immune status of the animal might involve in the occurrence of abortion in goats.

CONCLUSIONS

Because brucellosis is a disease of major economic and zoonotic importance, strategies for its control in small ruminants is essential in endemic areas. The initial aim of the strategy selected will be the reduction of infection in the animal population to such a level that the impact of the disease on human health as well as on animal health and production will be minimized. There is an occupational risk to health veterinarians and livestock related professionals who handle infected animals and aborted materials. As Brucellosis is one of the most easily acquired laboratory infections and strict safety precautions are recommended to maintain while handling *Brucella* suspected materials (WHO Laboratory Biosafety Mannal, 1993).

Further research is needed on the identification, isolation, characterization and cloning of both inner and outer membrane proteins which could be used as diagnostic antigens for detection and elimination of brucellosis.

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