Screening some major communicable diseases of AI bulls in Bangladesh

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Abstract

Stud bulls must be free from certain diseases owing to their importance in the AI industry and public health. One hundred and thirty eight bulls of Central AI Laboratory, Savar, Dhaka were screened out for the presence of bovine tuberculosis, brucellosis, trichomoniasis and campylobacteriosis. Single intradermal comparative tuberculin test was used for screening tuberculosis, and rose bengal plate test (RBPT) was performed for detecting brucellosis. Preputial washing was cultured and examined under microscope for the presence of the organisms for trichomoniasis and campylobacteriosis.

Thirty eight of 138 bulls (27.5%) were positive reactors to tuberculin test and 1 (0.7%) bull was positive to RBPT. None of the bulls were positive to trichomoniasis or campylobacteriosis. The teaser bull (n=1) was positive to tuberculin test. The prevalence of tuberculin positive reactors was significantly higher ($p< 0.01$) in collecting bulls (36.25%) than that of pre-collecting bulls (14.04%). Collecting bulls were 4 times more likely to be infected by the bovine tuberculosis (OR 4.01; 95% CI 1.48-10.86) than the pre-collection ones.

The high percentage (27.01%) of tuberculin positive bulls and presence of brucellosis in the bull stud emphasizes the need for adopting a standard health protocol in the Central AI Laboratory to minimize the risk of spreading these diseases through artificial insemination in Bangladesh.

Key words: breeding bull, brucellosis, campylobacteriosis, trichomoniasis, tuberculosis

Introduction

International organizations like OIE (Office Internationale des Epizooties) and CSS (Certified Semen Service) provided Standard Health Requirements for sires in AI

industries. FAO/IAEA also proposed the standard health requirements for sires of Asian countries which primarily targeted in supplying fertile semen without apparent spread of disease (FAO/IAEA 2005). This could be possible through diligent attention to technical details and a comprehensive and effective sire health control program (Herman et al 1994). Specific diseases that should be tested for bull health program are: tuberculosis, brucellosis, leptospirosis, bovine viral diarrhea, campylobacteriosis and trichomoniasis (CSS 1993).

Although the prevalence data of the diseases in developing countries are scarce, the information on the occurrence and their control measures does exist (Corbel 1997; Cosivi et al 1998). Of the 36 Asian nations, in 29 countries bovine TB is partly controlled or not controlled at all and only 7 countries apply test and slaughter as a part of control measure. Only 6% and <1%, cattle and buffalo, respectively, are found in the countries where bovine TB is notifiable and test and slaughter policy is in effect. Thus, 94% of the population of this region remains in risk of zoonotic bovine TB. Brucellosis, so far reported, remained as a potential threat in 24 Asian nations. Only 8 islands nations have declared eradication of bovine brucellosis. No reports are available for the rest of the countries.

In Bangladesh, Central AI Laboratory, Savar, Dhaka is the main concerned organization from where semen straws are produced, and distributed throughout the country for nationwide use in AI. This demands that all bulls in Central AI Laboratory should be regularly screened out following a standard bull health protocol.

The present study was conducted to test all bulls resident at Central AI Laboratory to determine the presence of tuberculosis, brucellosis, trichomoniasis and campylobacteriosis with the goal to develop a health check protocol for AI bulls in Bangladesh.

Materials and methods

This study was conducted at the Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh, Central AI Laboratory, Savar, Dhaka and Central Disease Investigation Laboratory (CDIL), Dhaka during the period from February to April 2004. Samples were collected from the bulls and further steps of the investigations viz. sample processing, microscopic examination, culture and serological examination were carried out using the facilities of the CDIL.

Animals

One hundred and thirty eight bulls kept for the national artificial insemination program were tested. Twenty-eight bulls were of zebu (local, Shahiwal, local X Shahiwal) and one hundred and nine bulls were of crossbreed (local X Friesian, local X Holstein, Shahiwal X Friesian, Sindhi X Friesian). One bull used as dummy was of local X Friesian. Date of

birth, breed and body weight of the bulls were recorded from the register book of the Central AI Laboratory.

Bulls were grouped into collecting bulls (the bulls which were engaged in semen collection operation) and pre-collecting bulls (the bulls which were not yet used in semen collection). Pre-collecting bulls aged 12 months or more are regularly brought to the collection premises for training and trials. Collecting bulls and dummy were housed in individual pens and the trial bulls and bull calves were housed in groups of 3 to 4 and 5 to 7, respectively.

Media, chemicals, reagents and antigens

Unless otherwise stated, all the chemicals were procured from Sigma-Aldrich Inc., St. Louis, USA. Sheep blood was collected from the sheep of Livestock Research Institute (LRI), Mohakhali, Dhaka. Rose Bengal Plate Test (RBPT) was performed by using brucella antigen (Brucella Antigen®, # 010599, SA Scientific, San Antonio, USA). Comparative intradermal tuberculin test (Delyed Hypersensitivity Test) was performed by using both bovine type (Bovine Tuberculin PPD®, PL 3326/4006, Veterinary Laboratories Agency, Surrey, UK) and avian type tuberculins (Avian Tuberculin PPD®, PL 3326/4007, Veterinary Laboratories Agency, Surrey, UK). Phosphate buffered saline (PBS tablet, Medicago AB, S-755 98, Uppsala Sweden) was used for the collection of preputial washings for the diagnosis of *Trichomonas foetus* and *Campylobacter foetus*. A modified Stuart's transport medium was prepared for transportation of preputial washing from the bull station to the laboratory for *Trichomonas foetus* culture (Amies 1967). Glucose-tryptose broth supplemented with horse serum and sheep blood agar medium was prepared and used to culture *Trichomonas foetus* and *Campylobacter foetus*, respectively.

Detection of tuberculosis in bulls

The comparative intradermal tuberculin test (Delyed Hypersensitivity Test) was used to identify the bulls infected with *Mycobacterium bovis* (OIE 2004). The injection sites were clipped, shaved and cleansed with spirit soaked cotton. A fold of skin within each clipped area was measured with calipers. A short needled syringe (McLintock® Preset Syringe, # 620840, Germany) was loaded with either avian type or bovine type tuberculin and 0.1ml of the content was injected. The bovine type tuberculin was injected into the lower clipped area while the avian type was injected into the upper clipped area on the same side of the mid-neck of a bull leaving a distance of 10 to 15 cm between the two injection sites. The skin fold thickness of each injection site was again measured 72 hours after the injections. A case was considered positive when the skin reaction with bovine type tuberculin was 4 mm or greater than that with the avian type tuberculin. An inconclusive result was recorded when the bovine reaction was between 1 and 4 mm greater than the avian reaction. A negative result was recorded if bovine reaction was negative or if the positive or inconclusive bovine reaction was equal to or less than the avian reaction site.

Rose Bengal plate test for brucellosis

Serum was prepared from blood samples collected from the jugular vein of individual bulls. Sera samples and the brucella antigen were brought to room temperature. Thirty µL of serum was mixed with the equal volume of antigen on a clear white tile and circled approximately 2 cm in diameter with a manicure. The mixture was rocked gently for 4 min at room temperature and then examined. Any sign of agglutination was considered positive (Morgan et al 1969). The average sensitivity and specificity of the RBPT tests were 77.2% and 99.3%, receptivity.

Detection of *Trichomonas foetus and Campylobacter foetus*

Twenty ml of PBS was introduced into the preputial cavity by using a rubber tube (45 cm long, 10 mm bore) fitted with a 50 ml plastic syringe (Steripack Disposable Syringe®, Opso Saline Ltd, Dhaka, Bangladesh). The preputial orifice was closed by hand pressure and the prepuce was vigorously massaged for 1 minute. The fluid was then withdrawn and divided into two splits. One split was transferred into a vial containing Stuart's transport media for *Trichomonas foetus* and the other portion was transferred into a different vial to culture *Campylobacter foetus*. The vials were marked with bull ID and placed in a thermo flask containing refrigerant to maintain a temperature of 4°C to 8°C and transferred to the laboratory within 2 hours after collection. To identify *Trichomonas foetus, o*ne drop of fresh sample was placed on a glass slide, covered with a cover slip and examined under a microscope (10x objective). Another drop of the sample was inoculated into the glucose-tryptose broth and incubated at 37°C for 4 days. The culture was reexamined under a microscope for the growth of *Trichomonas foetus*.

To detect *Campylobacter foetus*, the samples were centrifuged at 300 g for 10 minutes and precipitates were placed in blood agar plates with the help of a sterilized hook. The plates were placed in a Gas Generating Kit (GGK Oxoid®, Unipath Ltd., Hampshire, England) being the gas generating pack (Gas Pak®, BBL, Hampshire, UK) opened and then placed in the GGK. The GGK was placed in an incubator at 37°C for 3 days and the plates were examined for bacterial growth. The plate was examined for characteristics colonies and any suspected colony was stained with Gram's stain and the slides were examined under microscope (100x objective) for the detection of *Campylobacter foetus.*

Statistical analysis

The prevalence was defined as the number of positive reactors per 100 bulls tested. Chi-square test was used to determine whether any significant difference existed in prevalence between collection status, breed and age groups (Mostafa 1989). The confounder status of a covariate (eg age) was ascertained by comparing between the estimated coefficients for the risk factor variables (eg semen collecting status) for models containing and not containing the covariate. Any "biologically important" change in the estimated coefficient for the risk factor that would dictate the covariate as a confounder was included in the model, regardless of the statistical significance of the estimated coefficient for the covariate (Hosmer and Lemeshow 1989). A standard logistic

regression model was fitted for estimating the effects of semen collection statuses, breeds and age groups on the development of the tuberculosis (Hosmer and Lemeshow 1989). In this model, the dummy variables were created for the categorical variables collection status, Friesian cross and age (> 15 months) with pre-collection, zebu and age (≤ 15 months) as the reference categories. The statistical analyses were performed by using Statistical Package for Social Science 11.5 (Chicago, Illinois)

Results

Thirty eight (27.5%) of 138 bulls tested were positive to tuberculosis and 1 (0.7%) bull was positive to brucellosis. None of the bulls were positive to bovine trichomoniasis or campylobacteriosis.

The bull used as dummy for semen collection was a tuberculosis positive reactor. Because there was only one dummy, it was excluded from the statistical analysis.

The details of the skin reaction changes of the negative, suspicious and positive reactor bulls of different groups are shown in the Table 1.

Table 1. Skin thickness before and after injection of avian and bovine type tuberculin purified protein derivatives in different groups of bulls

Groups	Test status	Number of bulls	Initial skin thickness (mm) (mean)		Skin thickness at 72 hours (mm) (mean)		Difference in reaction at 72 hours (mm) (mean ± SE)
			Avian	bovine	Avian	bovine	
Pre-collecting bull	Negative	43	6.5	7.0	7.0	7.7	0.3 ± 0.1
	Suspicious	6	6.2	6.7	8.2	11.7	3.0 ± 0.0
	Positive	8	6.5	6.6	8.6	16.2	8.9 ± 0.8
Collecting bulls	Negative	44	8.0	9.3	8.3	9.9	0.3 ± 0.1
	Suspicious	7	9	10	9.4	13.4	3.0 ± 0.0
	Positive	29	8.4	9.8	9.8	20.6	9.4 ± 0.8
Zebu	Negative	18	7.8	9.2	7.9	9.5	0.3 ± 0.1
	Suspicious	3	9.0	10.7	9.7	14.3	3.0 ± 0.0
	Positive	7	8.1	9.1	9.0	18.0	8.0 ± 1.0
Crossbred	Negative	69	7.2	7.9	7.6	8.6	0.3 ± 0.1
	Suspicious	10	7.3	7.8	8.6	12.1	3.0 ± 0.0
	Positive	30	8.0	9.1	9.7	20.1	9.6 ± 0.8
Bulls of ≤ 15 months old	Negative	10	5.4	5.9	5.9	6.7	0.3 ± 0.3
	Suspicious	1	4.0	4.0	6.0	9.0	3.0 ± 0.0
	Positive	2	3.0	3.0	7.0	16.0	9.0 ± 1.0
Bulls of > 15 months old	Negative	77	7.5	8.4	7.9	9.1	0.3 ± 0.1
	Suspicious	12	8.0	8.8	9.1	12.9	3.0 ± 0.0
	Positive	35	8.3	9.4	9.7	19.9	9.3 ± 0.7

In the negative and suspicious cases, the mean differences in skin reaction after 72 hours of tuberculin injection were 0.3 and 3 mm, respectively. In positive cases, the mean skin

thickness changes in pre-collecting and collecting, zebu and crossbred, and bulls of ≤15 months and >15 months old were 8.9 mm and 9.4 mm, 8.0 mm and 9.6 mm, and 9.0 mm and 9.3 mm, respectively.

The Table 2 shows a significantly higher percentage of tuberculin positive reactors in the collecting bulls (36.2%) than that in the pre-collecting ones (14.04%) ($p<0.01$). However, the percentages of positive reactors to tuberculin did not differ between breeds and age groups of bulls.

Table 2. Prevalence of tuberculosis with regard to the collection status, breed and age of bulls

Variable	Category	Total no. tested	Reactors		Reactor prevalence, %	χ^2 Value
			Negative or suspicious	Positive		
Collection status	Pre-collection	57	49	8	14.04	8.33**
	Collection	80	51	29	36.25	
Breed	Zebu	28	21	7	25.00	0.07
	Crossbred	109	79	30	27.52	
Age	≤ 15 months	13	11	2	15.38	0.98
	> 15 months	124	89	35	28.23	
Overall		137	100	37	27.01	

***indicates significant (p< 0.01)*

The estimated logistic regression coefficient for collecting status changed from 1.25 to 1.35 when breed was added as a variable (Table 3). When age was added, the coefficient of collection status changed from 1.35 to 1.39. The changes in coefficients made by the breed and age as the effect of collection status on the occurrence of tuberculosis were not significant. Therefore, confounding effect due to breed and age was overruled (Table 3).

Table 3. Estimated logistic regression co-efficient for identifying the confounding effects due to breed or age

Model	Constant	Collection status	Breed	Age
1	-1.81	1.25		
2	-2.27	1.35	0.50	
3	-2.14	1.39	0.72	-1.8

The Table 4 demonstrated that the odds ratio of collecting status as a risk factor was 4.01 with 95% confidence interval between 1.5 and 10.9.

Table 4. Result of fitting logistic regression model to the tuberculosis data on bulls of Central AI Laboratory

Variable	Coefficient (β)	SE (β)	Odds Ratio logistic	95% confident interval
Constant	-2.14	0.89	-	-
Collection status	1.39**	0.51	4.01	1.48-10.86
Breed	0.72	0.51	1.65	0.61-4.45
Age	-0.18	0.89	0.84	0.15-4.76

***indicates significant (p< 0.01)*

This implied that the bulls of collection stage were 4 times more likely to be infected by the bovine tuberculosis than the pre-collection ones.

Discussion

A high percentage of bulls at the Central AI Laboratory, Savar, Dhaka was positive reactor of tuberculosis. Bulls in semen collection suffer at a higher percentage than those which are not yet subjected to collection operation. Although only one bull was positive to brucellosis, it indicates that brucellosis might exist in the herd. None of the bulls were positive to trichomoniasis and campylobacteriosis.

Tuberculosis and brucellosis are not only detrimental to dairy production but also a threat for public health. Tuberculosis is endemic in most livestock farms in South Asian countries and many other developing countries where positive tuberculin reactor animals keep recurring (Samad and Rahman 1986; Cosivi et al 1998; Ahmed et al 1999).

The bull of Central AI Laboratory used to be tested randomly for tuberculosis screening at inconsistent intervals. This might have a role in missing positive cases, if any. O'Reilly and Daborn (1995) regarded tuberculin positive cattle in a herd as a potential source of transmitting infection to other animals and humans. In the Central AI Laboratory, a common dummy was used to tease the bulls for semen collection. The dummy in this study was positive to tuberculin test. The infected dummy might have acted as a constant source of infection for other bulls (Fraser 1986). On the other hand, pre-collecting bulls were comparatively younger and did not come into contact with the dummy. Therefore, infection might have not been established in pre-collecting bulls because of not being exposed. This finding is in agreement with that of the previous reports (Cook et al 1996; Ameni et al 2001). These authors reported younger animals less susceptible than the older ones because of their insufficient exposure time. Moreover, semen collecting bulls frequently (twice per week) visited the collection shed than did the pre-collecting bulls. The Central AI Laboratory was advised to remove the tuberculin positive reactor bulls immediately. Similar recommendations are made by DEFRA (2006) and OIE Terrestrial Animal Health Code (2003).

The tuberculin skin test has been a useful diagnostic and epidemiological tool for monitoring tuberculosis in cattle for many years (Monaghan et al 1994). However, some practical problems exist with the skin test. Lack of absolute diagnostic accuracy ie sensitivity and specificity can cause false-positive reactions and indefinite responses. The ideal screening test would have a sensitivity and specificity of 100%, but biological tests hardly achieve this level of accuracy (Francis et al 1978). Purified protein derivatives it-self is a poorly defined cocktail of antigens, meaning that the present test does not discriminate clearly between individuals infected with tuberculosis and those sensitized by vaccination (if any) or exposure to environmental mycobacteria (Anderson et at 2000). Nevertheless, the bulls at Central AI Laboratory were never vaccinated against tuberculosis.

One bull was found brucellosis positive to rose Bengal plate test in this study. A brucella infected bull may or may not express clinical signs. The infected bull can be fertile and functionally active but it also cause shedding of brucella organisms with semen (McCaughey and Purcell 1973). Semen from infected bulls can cause severe economic losses in the country (Nicoletti 1980). The economic loss arises from the abortion of calves and resulting decreased milk yield, birth of weak calves that die soon after birth, retention of the placenta and consequent poor fertility. In addition to transmission of infection through semen, infected bulls can also serve as a source of infection to other bulls and men

The rose bengal plate test is the most effective screening test for detecting brucellosis and is used worldwide (OIE 2004; FAO 1993). The test is easy to perform and does not require any special laboratory equipment. However, the test sometimes yielded negative results in cattle that were found positive by using compliment fixation test (Rose and Roepke 1957). Although the low pH (+3.6) of the antigen enhances the specificity of the test, the temperature of the antigen and the ambient temperature at which the reaction takes place may influence the sensitivity and specificity of the rose Bengal test. In this study, the test was conducted at room temperature with the antigen and antibodies warmed up to the room temperature before initiating the test.

Bovine genital trichomoniasis and campylobacteriosis are very similar diseases in many aspects and cause economic losses of cow-calf operations and other cattle enterprises from decreased reproductive efficiency (Corbeil et al 1989; Goodger and Skirrow 1986). In beef operations, and even sometimes in dairies, the calf crop can be reduced up to 50% depending on the percentage of bulls infected and the susceptibility of the cows in the herd (Rae 1989).

Many researchers use preputial scraping as samples (Rae et al 1999; Irons et al 2002), but this technique was not practicable in this study because the bulls were engaged with regular semen collection. Parker et al (2003) stated that sampling technique had no effect on the sensitivity of the diagnostic test.

Conclusions

We developed a standard health protocol for bulls of the AI industry in Bangladesh and its regular application will minimize the risk of transmission of semen born diseases in cattle. .

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Go to top