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Abortions and infertility in domestic livestock due to brucellosis in Himachal Pradesh, India

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ABSTRACT

A total of 431 morbid materials were collected from 43 buffaloes (*Bubalus bubalis*), 110 cows, 115 does and 163 ewes, suffering from various reproductive disorders. These samples comprised 51 abortions, 80 repeaters, 277 endometritis, 8 cervicitis, and 15 vaginitis cases. Additionally, 98 female genitalia showing endometritis from 50 ewes and 48 does were also processed for isolation of brucellae. *Brucella abortus* biotype III was isolated from 2 of 7 aborted cows, while one isolate each of *B. melitensis* biotype I was isolated from 15 abortions in does and from the stomach contents of an aborted kid. One isolate of *B. melitensis* biotype I was identified from 93 cases of endometritis among does. Four isolates of *B. melitensis* biotype I were encountered in 28 abortions in ewes. The isolates were identified on the basis of cultural, morphological and physiological behaviour. Serotyping was carried out at the reference laboratory of the Division of Public Health, Indian Veterinary Research Institute, Izatnagar. The antibiogram of 9 isolates was performed against 16 chemotherapeutics. One of the does suffering from endometritis demonstrated negative titres for *B. melitensis*, although it yielded this organism in its pure form. This again indicates that isolation and identification of brucellae from clinical and morbid materials is possibly the most reliable method for diagnosis.

Key words: buffalo, *Bubalus bubalis*, cow, doe, ewe, *Brucella melitensis* biotype I, *Brucella abortus* biotype III, India

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Introduction

Reproductive proficiency is one of the core profiles of economic consideration in any livestock production enterprise. Loss of a calf, lamb or kid due to abortion and its sequelae frequently leads to infertility. It hardly needs to be emphasized that known causes of female infertility are many and involve a wide range of aetiologic agents, both specific and non-specific. Non-specific infectious agents are influenced by some perpetuating causes, whereas specific agents contribute directly to manifestation of infertility. The role of specific aetiological agents such as *Brucella* spp., *Campylobacter* spp., *Leptospira* spp., *Listeria monocytogenes*, *Chlamydia psittaci*, *Coxiella burnetii*, *Mycoplasma bovigenitalium* and *Salmonella* spp. has been well established, as documented by RADOSTITS et al. (1995).

Strictly speaking, the different species of *Brucella* are not highly host specific. However, their evolutionary adaptability and inter-host transmission continues to change. Nevertheless, brucellae are potentially highly pathogenic and an insidious human health hazard. Isolation and identification fail in a surprisingly high proportion of cases, due to the fact that many of the fastidious brucellae are elusive and their presence in the body is fleeting. Some are extremely delicate and survive poorly in rapidly decaying carcasses. Brucellosis among livestock is one of the important zoonotic maladies implicated primarily for inducing abortions. From a scrutiny of the available literature it emerges that abortion and infertility due to *Brucella* spp. is by far the most important of the identifiable infectious causes. The present study was contemplated to reveal the occurrence of various brucellae from aborted and infertile animals in Himachal Pradesh, India.

Materials and methods

In the present investigation a total of 431 morbid materials/specimens from various parts of Himachal Pradesh were collected. These accrued from 43 buffaloes (*Bubalus bubalis*), 110 cows, 115 does and 163 ewes. These 431 morbid samples included 51 abortions, 80 normally cyclic repeaters, 277 endometritis, 8 cervicitis and 15 vaginitis, and included 98 female genitalia from abattoirs having endometritis (50 sheep and 48 goats) as a sequelae of abortions. In order to attempt isolation of *Brucella* organisms, primary inoculation was done on sheep blood agar plates in duplicate by directly streaking the swabs/discharge to cultivate and to have discrete colonies of *Brucella* spp. The plates were separately incubated at 37 °C aerobically and micro-aerophilically in the presence of about 3%

CO_2 in candle jars for 72-120 hours. The isolates were initially recognised on the basis of their cultural and morphological features. They were also biochemically characterised as described by BUCHANON and GIBBONS (1974) and CARTER (1995). Serological identification of the isolates was done at the Division of Public Health, Indian Veterinary Research Institute, Izatnagar. Antimicrobial susceptibility test was performed employing Disc diffusion test.

Results

This study revealed that the percentage of different *Brucella* organisms was 2.1 as nine (9) isolations were made from different gynaecological disorders in cows, does and ewes. *B. abortus* biotype III were isolated from two of 110 samples emanating from cows, while *B. melitensis* biotype I was encountered in three of 115 does and four of 163 samples collected from ewes, whereas no isolation of this organism was achieved from the samples processed from buffaloes (Table 1.)

Table 1. *Brucellae* isolates encountered from different livestock species in Himachal Pradesh, India

Species of livestock	No. of samples cultured	Positive for *Brucella* spp.	Per cent isolation	Indetification of isolates*
Buffaloes	43	-	-	-
Cows	110	2	1.81	*B. abortus* biotype-III
Does	115	3	2.60	*B. melitensis* biotype-I
Ewes	163	4	2.45	*B. melitensis* biotype-I

*Based on cultural, morphological and physiological features

In cows, two of the seven samples of abortions yielded *B. abortus* biotype III and no isolate emanated from 33 samples of endometritis. *B. melitensis* biotype I was encountered in 2 of 15 and 4 of 28 samples of abortions from does and ewes, respectively. Only one isolate of *B. melitensis* biotype I accrued from 93 samples of endometritis in does (Table 2.)

All brucellae isolates encountered in the study were characterised on the basis of cultural, morphological and biochemical characteristics. They manifested positive reaction for catalase, oxidase, nitrate reduction, as well as growth both in the presence of 1:5000 w/v thionin and 1: 5000 w/v basic fuchsin. These isolates grew in the absence of CO_2 and yielded

negative results for H_2S production, motility, methyl red, Voges Proskauer, and indole production. Biochemical features were typical of the *Brucella* species. Subsequently, serological identification of these isolates was carried out at the Indian Veterinary Research Institute, Izatnagar (Distt. Braeilly).

Table 2. Reproductive disorder prevalence of *Brucella* in different species of animals in Himachal Pradesh, India

Livestock species	No. of samples	Yelded *Brucella*	*Brucella* spp. isolated
Abortion (Cows)	7	2	*B. abortus* biotype III
Abortion (Does)	15	2	*B. melitensis* biotype I*
Endometritis (Does)	93	1	*B. melitensis* biotype I
Abortion (Ewes)	28	4	*B. melitensis* biotype I

*One isolate each recovered from stomach contents and also from vaginal discharge of the same aborting doe

Table 3. Antibiogram of different isolates of *Brucella* from different species of animals in Himachal Pradesh, India

Species	No. of isolates tested	Pb	S	C	Ct	A	Nf	Ak	Na	T	Cx	Am	Cf	K	Fr	G	Cm
B. melitensis biotype I	7	-	6	5	4	4	3	5	1	6	-	3	7	7	1	6	-
B. abortus biotype III	2	-	1	2	1	2	2	2	-	2	-	1	2	2	-	2	1
Total	9	-	7	7	5	6	5	7	1	8	-	4	9	9	1	8	1

Concentration of different antimicrobials used: A-Ampicillin-25 μg; Ak-Amikacin-30 μg; Am-Amoxicillin-30 μg; C-Chloramphenicol-30 μg; Cf- Ciprofloxacin-10 μg; Cm-Co-trimazine-25 μg; Ct-Chlortetracycline-30 μg; Cx-Cloxacillin-1 μg; Fr-Furozolidone-50 μg; G-Gentamicin-10 μg; Na-Nalidixic acid-30 μg; Nf-Nitrofurantoin-300 μg; Pb-PolymyxinB-300 μg; S-Streptomycin-10 μg, and T-Tetracycline-30 μg

All seven *B. melitensis* biotype I and two *B. abortus* biotype III were tested for their sensitivity on Müller Hinton Agar and brain heart infusion agar supplemented with 6% sterile sheep blood. The sensitivity pattern was tested against 16 different chemotherapeutics (Table 3.) All seven *B. melitensis* biotype I were resistant to polymyxin B, cloxacillin and co-trimazine. Both isolates of *B. abortus* biotype III were resistant to polymyxin B, nalidixic acid, cloxacillin and furazolidone. All seven *B. melitensis* biotype I were sensitive to ciprofloxacin and kanamycin, while both isolates of *B. abortus* biotype III were sensitive to chloramphenicol, ampicillin, nitrofurantoin, amikacin, tetracycline, ciprofloxacin, kanamycin,

and gentamicin. Only one isolate of *B. abortus* biotype III was sensitive to streptomycin, chlortetracycline, amoxicillin, and co-trimazine. The sensitivity profile, in descending order, was manifested as 6 each to streptomycin, tetracycline and gentamicin, 5 were sensitive to chloramphenicol and amikacin, while four each were sensitive to chlortetracycline and ampicillin, each to nitrofurantoin and amoxicillin. Only one of each isolate was sensitive to nalidixic acid and furazolidone.

Discussion

Achievement of an infallible diagnosis of brucellosis is a tedious process, since isolation is influenced by a number of factors, such as highly fastidious growth requirements, a lesser number of viable organisms in the sample, delay in transportation (leading to putrefaction), earlier treatment with chemotherapeutics. Also, a prolonged incubation period for isolation may lead to failure in its isolation. Brucellosis is a disease of economic importance to any livestock enterprise as it induces abortion in infected animals. The disease very often spreads from animal to animal in a herd by several modes of transfer, chief among these being contact with infected discharges from an aborted dam and its foetus.

The aetiologic role of *B. melitensis* biovar I among cows, ewes and does in infertility has been extensively quoted by several researchers (MUSTAFA and CORBEL, 1988; BANAI et al., 1990; RIBEIRO et al., 1990; ERDOGAN et al., 1993) citing varying percentages of its involvement. The present study did not encounter the involvement of *B. melitensis* biovar I among infertility cases either in buffaloes or cows. Two aborted cows yielded *B. abortus* biotype III in this study. No isolation of *B. abortus* biotype III could be made from buffaloes, ewes and does, while REFAI et al. (1991) isolated *B. abortus* biotype III from buffaloes. ERDOGAN et al. (1993) documented nine isolates of *B. abortus* biotype III from bovine abortions. Thus, their observations are at variance with observations in this study, recording its involvement to the extent of 1.81%, consequently in conformity with ZOWGHI and EBADI (1988) who recorded *B. abortus* biotype III as causing abortions in cows. A single document evidencing its occurrence in aborted cows has been cited by PAT and PANIGRAHI (1966). They isolated five strains of *B. abortus* from six samples of milk and vaginal discharges in Orrisa, and they classified five strains as *B. abortus* biotype III (1), *B. abortus* biotype I (3) and *Brucella* A/M (intermediate-1).

All the isolates of brucellae encountered in this investigation displayed typical physiological behaviour, as described by CARTER (1995)

and BUCHANON and GIBBONS (1974). The present study recorded the association of *B. melitensis* biotype I in ewes as 2.45%. This finding is almost in accord with MOORTHY and SINGH (1982), who isolated *B. melitensis* biovar I to the extent of 3.2%. SAVALGI et al. (1987) reported the prevalence of *B. melitensis* biotype I in buffaloes and cows as 12 and 20% respectively. The pharmacokinetics of chemotherapeutics against brucellosis in different species of livestock does not appear to have been undertaken. The biological half-life of such chemotherapeutics in uterus needs to be assayed both in clinical and carrier animals. Only scant references are available with regard to treatment of brucellosis, both in humans and animals. SAMARINA et al. (1991) tested 25 *B. melitensis* strains from humans and animals for drug resistance, and were resistant to cephalexin, phosphomycin, chinoxydine, dioxydine and oxolinic acid. The combination of sisomicin and trimethoprim at 1:10 and sisomicin and nitroxoline at 1:2 had synergistic effects. NAGAL et al. (1994) reported that *B. melitensis* biotype III was sensitive to tetracycline and gentamicin but was resistant to penicillin G, streptomycin, cotrimoxazole and furazolidone. In the present study the seven *B. melitensis* biotype I were sensitive to ciprofloxacin and kanamycin. The sensitivity profile in descending order by six such isolates to gentamicin, tetracycline and streptomycin, while five isolates were sensitive to chloramphenicol, amikacin and four to chlortetracycline, and ampicillin; three to nitrofurantoin and amoxicillin. Only one isolate was sensitive to nalidixic acid and furazolidone. They were all resistant to polymyxin B, cloxacillin and co-trimazine. In view of antibiotic resistant isolates of brucellae in nature, their public health hazard and occupational risk, a carefully thought-out decision must be made before recommending therapy. Here, it is again emphasized that for better management of brucellosis in livestock, owing to economic reasons the treatment is not recommended unless a particular animal is highly valued. However, this study found ciprofloxacin and kanamycin to be drugs of choice, followed by gentamicin and tetracycline. One of the does suffering from endometritis demonstrated negative titres for *B. melitensis*, although it yielded this organism in its pure form. This again indicates that isolation and identification of brucellae from clinical and morbid materials is possibly the most reliable method for diagnosis.

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SAŽETAK

Prikupljen je 431 uzorak od 43 bivola (*Bubalus bubalis*), 110 krava, 115 koza i 163 ovce, s različitim poremetnjama u razmnožavanju. Ti su uzorci potijecali od 51 pobačaj, 80 životinja koje ni nakon ponovljenog osjemenjivanja nisu bile oplođene, 277 endometritisa, 8 cervicitisa i 15 vaginitisa. Osim toga je i 98 ženskih spolnih organa koji su imali znakove endometritis u 50 ovaca i 48 koza također pretraženo radi izolacija brucela. *B. abortus* biotip III je izolirana iz 2 od 7 krava s pobačajem, dok je po jedan izolat *B. melitensis* biotip I izoliran iz 15 pobačaja koza i iz sadržaja želuca jednog pobačenog jareta. Jedan izolat *B. melitensis* biotip I je pronađen među 93 slučaja endometritisa koza. Četiri izolata *B. melitensis* biotip I su pronađena među 28 pobačaja u ovaca. Izolati su identificirani na osnovi kulturoloških, morfoloških i fizioloških osobitosti. Serološko tipiziranje je izvršeno u

referentnom laboratoriju Division of Public Health, Indian Veterinary Research Institute, u Izatnagaru. Za 9 izolata su načinjeni antibiogrami s po 16 kemoterapeutika.

Ključne riječi: bivol, krava, koza, ovca, *B. melitensis* biotip I, *B. abortus* biotip III, India