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Full Length Article

Comparative Prevalence of Streak Canal and Intramammary Microorganisms and their Contemporaneous Association in a Dairy Cow and Buffalo Herd Lacking Mastitis Control Program

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Abstract

The present study was planned to investigate the prevalence of streak canal and intramammary infections in buffaloes and cows lacking any mastitis control program and to determine the contemporaneous association between streak canal and intramammary infections in these two dairy species. Streak canal swab and quarter foremilk samples were collected aseptically from 20 lactating mastitis free cows and buffaloes each in their first 2 months of second to third lactation and subjected to microbiological examination as per standard procedures. Isolates were speciated using commercially available kits. Contemporaneous association was defined as co-occurrence of a specific microorganism in streak canal swab and quarter foremilk sample of a particular teat simultaneously. The prevalence of streak canal infections as well as intramammary infections was higher in cows (91.25% and 55%, respectively) than in dairy buffaloes (73.75%, and 22.5%, respectively). Of the 78 isolates recovered from streak canal swab samples of buffaloes, *Staphylococcus aureus* (n=18; 23.07%) was the most predominant organism followed by *S. choromogenes* (n=14), *E. coli* (n= 6), unidentified esculin positive *Streptococcus spp*. (n=5). *S. aureus* (n=34) was the most frequent isolate recovered from the streak canals of cows followed by *S. choromogenes* (n=11), *S. hyicus* (n=8), *Escherichia coli* (n=8). In terms of frequency of isolation, *S. aureus* was the most predominant organism (n=6) recovered quarter foremilk samples of buffaloes followed by unidentified coagulase negative *Staphylococcus spp.* (n=3), *Trueperella pyogenes* (*Corynebacterium pyogenes*; n=3), unidentified *Trueperella spp.* and mixed species (n=3). In the case of dairy cows, predominant organism recovered from quarter foremilk samples included *S. aureus* (n=13), *Streptococcus agalactiae* (n=4), yeast (n=4) and mixed species (n=4). Recovery of 44 and 27 isolates, respectively in cows and buffaloes was common to both streak canal swab and quarter foremilk samples (contemporaneous association). © 2015 Friends Science Publishers

Keywords: Streak canal infections; Intramammary infections; Contemporaneous association; Cows and buffaloes; Mastitis

Introduction

Several field investigations of economically significant livestock diseases in Pakistan have indicated that mastitis is the most important health problem of dairy cows and buffaloes (Cady *et al.,* 1983; Ajmal, 1990; Hussain *et al.,* 2005; Ali, 2008). It is reportedly one of the most important reasons for premature termination of lactation and involuntary culling of dairy buffaloes (McDowell *et al.,* 1995) and cows (Samiullah *et al*., 2000) in Pakistan.

Microorganisms associated with mastitis almost invariably enter the udder through the streak canal (ductus papillaris), which is the orifice of mammary gland between internal milk secretary system and the external environment (Milne, 1977; Frandson *et al*., 2013). Streak canal is the first and the most important line of defense against the infections of the udder (Nickerson, 1990; Lacy-Hulbert, 1998).

Owing to its proximity to the external environment, streak canal a priori is very prone to develop infections. It is usually the initial site of infection in mastitis. To date, only a few reports documenting the occurrence of streak canal infections in cow have been published. These infections occur in lactating (Du Preez, 1986; Watts *et al*., 1991; Quirk *et al*., 2012) and non-lactating cows (Woolford *et al*., 2001) as well as heifers (Trinidad *et al*., 1990) and may persist for months. Du Preez (1986) suggested that bacteria colonizing the teat canal produce toxins that diffuse to the interior of the udder, thus producing an inflammatory response and damage to mammary parenchyma. Localized antibiotic infusions applied to the teat canal of cows at drying off have been shown to reduce new intramammary infections (Woolford *et al*., 2001). Nickerson *et al.* (1990) reported that iodophor (1.8%) teat dip was 90% effective in preventing new *S. aureus* teat canal infections and 95.6%

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effective in reducing the progression of *S. aureus* teat canal infections to intramammary infections.

Only a solitary report on the prevalence rate of streak canal infections and their contemporaneous association with the intramammary infections in dairy buffalo (*Bubalus bubalis*) is available in the literature (Tuteja *et al*., 2001). To our knowledge, there is no report comparing the prevalence rate of streak canal and intramammary infections in buffalo with that in cow in dairy herds lacking a mastitis control program. The present study was, therefore, designed to investigate the prevalence of streak canal and intramammary infections in buffaloes and cows under local Pakistani conditions wherein generally no mastitis control program is in place. A subsidiary objective was to determine the contemporaneous association between streak canal and intramammary infections in cows and buffalos.

Materials and Methods

Prevalence of Streak Canal and Intramammary Infections in Buffaloes and Cows

Experimental animals and management: Twenty lactating mastitis free cows and buffaloes each in their first 2 months of 2nd to 3rd lactation and maintained at Livestock Production Research Institute, Bahadurnagar, Okara were selected. All experimental animals were managed in a 'tie-stall' *cum* loose housing system and were fed on concentrate mixture and sorghum + maize fodder. The experimental buffaloes were hosed once or twice daily depending upon the rigor of the weather. All experimental animals were hand milked twice daily between 3-5 a.m. and 3-5 p.m. No mastitis control measures (e.g., post-milking antiseptic teat dipping, dry period antibiotic therapy, mastitis vaccination, segregation of mastitic animals etc.) were in practice.

Collection of samples: Streak canal swab (using Calgiswab type 4, Spectrum Lab., Los Angles, USA) and quarter foremilk samples were taken from 20 lactating buffaloes and 20 lactating cows as per the techniques previously described (Hogan *et al.,* 1990; National Mastitis Council, 1990; Muhammad, 1992; Quirk *et al*., 2012). Briefly, the preparatory steps for streak canal swab samples included: (1) teat ends were prepared for sampling by spraying them with 0.5% iodophor, (2) teat ends were wiped dry with single service individual paper towels, and (3) each teat end was scrubbed with 3 cotton swabs soaked in 70% ethanol. A nasopharyngeal swab (Calgiswab type 4) moistened in sterile phosphate buffered saline (PBS; pH 7.00) containing 0.1% sodium thiosulfate was carefully inserted approximately 2 mm into the teat meatus and rotated 360°. Using a scissors with ends sanitized with 70% ethanol, the swab head was clipped off into a 13x100 mm test tube containing 250 µL sterile PBS with 0.1% sodium thiosulfate. For quarter foremilk samples, each teat end was again sanitized with swabs soaked in 70% ethanol and quarter foremilk samples collected aseptically as previously described by National Mastitis Council (1990). Tubes containing streak canal swabs and milk samples were placed on crushed ice for transportation to the laboratory where plating of all samples was initiated within 1 h of collection.

Laboratory procedures: Tubes containing swab head in PBS rinse solution were vortexed for 10 sec and then 50 µL of the solution was inoculated onto one half of an esculin blood agar plate (5% sheep blood and 0.1% esculin). Quarter foremilk samples (0.01 mL) were streaked onto one quadrant of esculin blood agar plate as described by National Mastitis Council (1990). Plates were examined after 24 and 48 h of incubation at 37°C. Catalase-positive, Gram-positive cocci were presumptively identified as staphylococci and subjected to a tube coagulase test. Isolates of streptococci, coliforms and non-coliforms, *Trueperella* (Corynebacteria), yeast etc., were presumptively identified as per National Mastitis Council (1990) and then maintained at -70°C in trypticase soya broth with 20% glycerol in cryogenic vials until speciated.

Species determination of isolates: Stock isolates were serially sub-cultured three times on Columbia blood agar and identified by utilizing commercial identification systems (Biomerieux, France) for *Staphylococci*, Streptococci, *Trueperella* (*Corynebacterium*), Gram negative rods and yeast.

Determination of Comparative Contemporaneous Association Between Streak and Intramammary Infections in Cows and Buffaloes

Contemporaneous association as used in the context of the present study referred to co-occurrence of a specific microorganism in streak canal swab and quarter foremilk sample of a particular teat simultaneously (Muhammad, 1992; Quirk *et al*., 2012).

Results

Comparative Prevalence of Streak Canal and Intramammary Infections in Cow and Buffalo

Of the 80 streak canal swab samples collected from 20 cows, 73 (91.25%) yielded growth of 106 isolates belonging to 9 different microorganism categories. Similarly, of the 80 streak canal swab samples collected from 20 buffaloes, 59 (73.75%) yielded growth of 78 isolates belonging to 8 different micro-organisms categories (Table 1). *Staphylococcus aureus* (n=34) was the most frequent isolate recovered from the streak canals of cows followed by *S. choromogenes* (n=11), *S. hyicus* (n=8), *Escherichia coli* (n=8), *Streptococcus agalactiae* (n=6), unidentified esculin positive *Streptococcus spp.* (n =5) and yeast species (n=5). Less frequently encountered

Table 1: Comparative prevalence of streak canal isolates in cows and dairy buffaloes

Microorganisms	No. of isolates recovered from the streak canal swab samples of cows (n=20)	No. of isolates recovered from the streak canal swab samples of buffaloes (n = 20)
Staphylococcus aureus	34	18
Staphylococcus choromogenes	11	14
Staphylococcus simulans	3	1
Staphylococcus epidermidis	1	-
Staphylococcus hyicus	8	4
Staphylococcus xylosus	2	1
Staphylococcus haemolyticus	-	1
Staphylococcus warneri	1	1
Staphylococcus saprophyticus	-	1
Staphylococcus hominis	1	2
Unidentifiedcoagulase negative *Staphylococcus spp.*	2	3
Streptococcus agalactiae	6	3
Streptococcus dysgalactiae	1	2
Unidentified Esculin positive *Streptococcus spp.*	5	5
Escherichia coli	8	6
Trueperellapyogenes (Corynebacteriumpyogenes)	3	1
Unidentified *Corynebacterium spp.*	4	3
Bacillus spp.	3	3
Yeast *spp.*	5	4
Nocardia	1	-
Mixed *spp.*	4	3
Unidentified *spp.*	3	2
Total	106	78

microorganisms included unidentified *Corynebacterium spp*. (n=4), mixed *spp*. (n= 4), *S. simulans* (n=3), *T. pyogenes* (*Corynebacterium pyogenes*; n= 3), *Bacillus spp.* (n= 3) and unidentified *spp.* (n= 3). In a descending order, distribution of 78 isolates recovered from streak canal swab samples of buffaloes was as follows: *S. aureus* (n=18), *S. choromogenes* (n=14), *E. coli* (n= 6), unidentified esculin positive *Streptococcus spp*. (n=5), *S. hyicus* (n=4), yeast *spp*. (n=4), unidentified coagulase negative *Staphylococcus spp.* (n=3), *Str. agalactiae* (n=3), unidentified *Corynebacterium spp.* (n=3), *Bacillus spp.* (n=3), and mixed *spp.* (n=3).

Of the 80 quarter foremilk samples of 20 cows subjected to microbiological examination, 44 (55%) yielded growth of one or more than one microbial species (Table 2). *S. aureus* was the most frequent isolate recovered from quarter foremilk samples of cows accounting for 25.49% (n=13) of the total isolates (n=51) recovered from 80 quarter foremilk samples. The other organisms isolated from quarter foremilk samples of cows in their descending order included: *Str. agalactiae* (n=4), yeast (n=4), mixed species (n=4), *S. choromogenes* (n=3), unidentified coagulase negative *Staphylococcus spp.* (n=3), *T. pyogenes* (n=3), unidentified *Corynebacterium spp.* (n=3), unidentified *spp.* (n=3), *S. hyicus* (n=2), *E. coli* (n=2), *S. simulans* (n=1), *S. epidermidis* (n=1), *S. hominis* (n=1), *S. warneri* (n=1), *Str. dysgalactiae* (n=1), unidentified esculin positive

Table 2: Comparative prevalence of microorganisms recovered from quarter foremilk samples of cows and dairy buffaloes

Microorganisms	No. of isolates recovered from quarter foremilk samples of cows (n=20)	No. of isolates recovered from quarter foremilk samples of buffaloes (n=20)
Staphylococcus aureus	13	6
Staphylococcus simulans	1	-
Staphylococcus choromogenes	3	2
Staphylococcus epidermidis	1	-
Staphylococcus hominis	1	1
Staphylococcus xylosus	-	1
Staphylococcus hyicus	2	1
Staphylococcus warneri	1	-
Unidentified coagulase negative *Staphylococcus species*	3	3
Streptococcus agalactiae	4	2
Streptococcus dysgalactiae	1	1
Unidentified Esculin positive *Streptococcus species*	1	1
Escherichia coli	2	1
Trueperellapyogenes (*Corynebacteriumpyogenes)*	3	3
Unidentified *Corynebacterium spp.*	3	3
Bacillus *spp.*	1	1
Yeast	4	2
Mixed species	4	3
Unidentified *spp.*	3	2
Total	51	33

Streptococcus spp (n=1), and *Bacillus spp.* (n=1). Eighteen (22.5%) buffalo quarters yielded growth of one or more than one microbial species (Table 2). Like in cows, *S. aureus* was the most predominant isolate (n=6; 18.18%) followed by unidentified coagulase negative *Staphylococcus spp*. (n=3), *T. pyogenes* (n=3), unidentified *Corynebacterium* spp. (n=3), mixed spp. (n=3), *S. chromogenese* (n=2), *Str. agalactiae* (n=2), Yeast *spp.*, unidentified *spp.* (n=2), *S. hominis* (n=1), *S. xylosus* (n=1), *S. hyicus* (n=1), *Str. dysgalactiae* (n=1), unidentified esculin positive *Streptococcus spp.* (n=1), *E. coli* (n=1), and *Bacillus spp.* (n=1).

Scrutiny of comparative contemporaneous association (defined in the context of the present study as co-occurrence of a microorganism in streak canal swab and quarter foremilk sample) of streak canal organisms with the intramammary organisms in cows and buffaloes revealed that recovery of 44 and 27 isolates, respectively in cows and buffaloes was common to both streak canal swab and quarter foremilk samples (Table 3).

Comparative Contemporaneous Association Between Streak and Intramammary Infections in Cows and Buffalos

Table 3 depicts comparative contemporaneous association (i.e., co-occurrence of streak canal and intramammary microorganisms; identity) of streak canal organisms with

Table 3: Comparative contemporaneous association of streak canal organisms with the intramammary organisms in cows and buffaloes

Microorganisms	No. of isolates recovered simultaneously from streak canal swab and foremilk quarter samples of cows (n= 20)	No. of isolates recovered simultaneously from streak canal swab and foremilk quarter samples of buffaloes (n= 20)
Staphylococcus aureus	12	6
Staphylococcus choromogenes	3	2
Staphylococcus simulans	1	-
Staphylococcus epidermidis	1	-
Staphylococcus hyicus	2	1
Staphylococcus xylosus	-	1
Staphylococcus warneri	1	-
Staphylococcus hominis	1	1
Unidentified coagulase negative *Staphylococcus spp.*	1	3
Streptococcus agalactiae	4	2
Streptococcus dysgalactiae	1	1
Unidentified Esculin positive *Streptococcus spp.*	1	1
Escherichia coli	2	1
Trueperella pyogenes (Corynebacterium pyogenes)	3	1
Unidentified *Corynebacterium spp.*	3	2
Bacillus spp.	1	1
Yeast *spp.*	3	2
Mixed *spp.*	2	2
Unidentified *spp.*	2	-
Total	44	27

the intramammary organisms in cows and buffaloes. In cows (n=20), a total of 44 isolates belonging to 18 microbial categories were recovered simultaneously from streak canal swab and foremilk quarter samples. The highest number of these isolates (n=12) belonged to *S. aureus* followed by *Str. agalactiae* (n=4), *S. choromogenes* (n =3), *T. pyogenes* (n = 3), unidentified *Corynebacterium spp.* (n=3) and yeast *spp.* (n=3). Two isolates each of *S. hyicus, E. coli,* mixed *spp.* and unidentified *spp.* were recovered simultaneously from streak canal and quarter foremilk samples of 20 cows. One isolates each of *S. simulans, S. epidermidis, S. warneri, S. hominis,* unidentified coagulase negative *Staphylococcus spp., Str. dysgalactiae,* unidentified esculin positive *Streptococcus spp.* and *Bacillus spp.* were recovered simultaneously from streak canal and quarter foremilk samples of 20 cows.

In a comparable number (n = 20) of buffaloes sampled in the present study, 27 isolates of 15 microbial categories recovered from streak canal and quarter foremilk samples showed a contemporaneous association (identity). As in the cow, *S. aureus* was the species recovered most frequently (n = 6) in a simultaneous manner from streak canal and quarter foremilk samples followed by unidentifiedcoagulase negative *Staphylococcus spp.* (n = 3), *S. choromogenes* (n = 2), *Str. agalactiae* (n =2), unidentified *Corynebacterium* spp. (n=2), Yeast *spp.* (n=2), Mixed *spp.* (n=2). One isolate each of *S. hyicus* (n=1), *S. xylosus* (n=1), *S. hominis* (n=1), *Str. Dysgalactiae* (n=1), unidentified esculin positive *Streptococcus spp.* (n=1), *E. coli* (n=1), *T. pyogenes* (n=1), and *Bacillus spp.* (n=1) were recovered simultaneously from streak canal and quarter foremilk samples of 20 buffaloes.

Discussion

Of the 80 streak canal swab samples each of cow and buffalo collected in the first two months of the lactation, 73 (91.25%) and 59 (73.75%) respectively were found infected with different microorganisms. Indian workers (Tuteja *et al*., 2001) documented a streak canal infection rate of 78.09% in dairy buffaloes at the time of drying off. A higher streak canal infection prevalence rate observed in the present study than that reported by these workers may relate in part at least to the difference in the sampling time with respect to lactation. A bacteriological survey of post pubertal, non-lactating dairy heifers indicated that 70% of the teat duct and 80% of the secretion samples obtained were infected with staphylococci that persisted for at least one year and well into the first lactation (Boddie *et al.,* 1987), while about 44 and 46 percent of the Ohio State University (USA) dairy herd cows and heifers streak canal, respectively harbored staphylococci at calving (Muhammad, 1992). The higher prevalence of streak canal infections both in cows and buffaloes in the present study than that reported by this worker may partly be explained on the following grounds:

a) Muhammad (1992) reported only staphylococcal infections; infections by other microorganisms which might have infected streak canal were disregarded. Had organisms other than staphylococci been taken into consideration by this investigator, it is entirely possible that the prevalence rates of streak canal infections might have approached close to those observed in the present study.

b) Post-milking antiseptic teat dipping and dry period therapy were not practiced on the experimental animals of the present study. These mastitis control measures are effective in reducing the prevalence of streak canal and intramammary infections (Quirk *et al*., 2012). In the present study, of the 78 isolates recovered from streak canal swab samples of buffaloes, *S. aureus* (n=18; 23.07%) was the most predominant organism recovered followed by *S. choromogenes* (n=14), *E. coli* (n= 6), unidentified esculin positive *Streptococcus spp*. (n=5), *S. hyicus* (n=4), yeast spp. (n=4), unidentified coagulase negative *Staphylococcus spp.* (n=3), *Str. agalactiae* (n=3), unidentified *Trueperella (Corynebacterium*) *spp*. (n=3), *Bacillus spp.*(n=3), and mixed *spp.* (n=3). Of the 80 quarter foremilk samples of 20 cows subjected to microbiological examination, 44 (55%) yielded growth of one or more than one microbial species. *S. aureus* was the most frequent isolate recovered from quarter foremilk samples of cows accounting for 25.49% (n=13) of the total isolates (n=51) recovered from 80 quarter foremilk samples. In the case of buffaloes, microbiological examination of 80 quarter foremilk samples, 18 (22.5%) quarters yielded growth of one or more than one microbial

species. Like in cows, *S. aureus* was the most predominant isolate (n=6; 18.18%). Tuteja *et al*. (2001) reported that *S. epidermidis* was the most frequent isolate (43.18%) recovered from streak canal samples of dairy buffaloes at drying off. This organism was followed by *Corynebacterium spp*. (27.27%), unclassified streptococci (10.23%), *Str. dysgalactiae* (9.09%), *Str. agalactiae* (7.96%) and *S. aureus* (2.27%). Prevalence of streak canal infections was 78.09% whereas only 38.09% of the quarters were found to be infected. Among the microorganisms recovered from quarter milk samples, *S. epidermidis* (37.50%) was the most frequent isolate followed by *Corynebacterium spp*. (27.5%), *S. aureus*(10%), *Str. dysgalactiae* (7.5%), *Str. agalactiae* (5%) and yeast (2.5%). Nickerson *et al*. (1990) conducted a study to determine the prevalence of mastitis in breeding age and pregnant dairy heifers. The results indicated that IMIs were present in 97% of heifers and 75% of quarters. The predominant isolates were *S. aureus*, *S. hyicus*, and *S. chromogenes*. Iranian workers (Chavoshi and Husaini, 2012) reported CNS, streptococcus and bacillus species as the three most important pathogen groups isolated from CMT positive quarters of 400 dairy buffaloes. Only two quarters yielded the growth of *S. aureus*. The results of this study are at a variance with the findings of the present study as *S. aureus* was the most frequent isolate recovered from 80 foremilk quarters samples of each of cows and buffaloes. Coagulase negative staphylococci were the second most frequently isolated pathogens group in the present study. The notion that the prevalence of streak canal infection (diagnosed by means of streak canal swab samples) is higher than the intramammary infections diagnosed by culture of quarter foremilk samples (Du Preez, 1986; Tuteja *et al*., 2001) was borne out by the findings of the present study.

Several authors have reported that there appears to be a relationship between colonization of streak canal and infection of mammary gland (Forbes and Herbert, 1968; Appleman, 1970; Du Preez, 1986; Muhammad, 1992; Quirk *et al*., 2012). This conclusion was based on the observation that *S. hyicus, S. aureus*, *S. xylosus,* and *S. chromogenes* recovered from streak canal were also the predominant microorganisms recovered from secretion (milk) samples. Similarly, Forbes and Hebert (1968) observed that in lactating cows, the majority of *S. aureus* and *S. epidermidis* IMIs were preceded by streak canal colonization by these microorganisms, and they concluded that chronic IMIs may be maintained by the presence of streak canal colonization. In the present study, determination of comparative contemporaneous association (defined in the context of the present study as co-occurrence of a microorganism in streak canal swab and quarter foremilk samples of the same teat) of streak canal organisms with the intramammary organisms in cows and buffaloes revealed that recovery of 44 and 27 isolates, respectively in cows and buffaloes was common to both streak canal swab and quarter foremilk samples. Tuteja *et al*. (2001) reported that of the 40 infected quarters milk samples, 27 (67.50%) were found to be infected with the same organisms as those infecting the teat canals. We know of no other report documenting the commonality (identity) of streak canal and intramammary isolates in dairy buffalo.

The commonality of a very high proportion of microorganisms to streak canal and milk samples (contemporaneous association) is probably reflective of the notion that streak canal infections serve as a source of microorganisms for the secretory tissue of the udders of cow and dairy buffalo. The present study is the preliminary investigation on the streak canal infections of buffaloes as well as on the contemporaneous association of microorganisms of streak canal and quarter milk samples of Pakistani dairy buffaloes.

Streak canal infections which do not progress upward to cause inflammation of the mammary gland are not usually diagnosed by mastitis diagnostic tests based on the detection of inflammation. Based on the International Dairy Federation (2005) criteria of somatic cell count (SCC) for mastitic quarters ($> 200 \times 10^3$ cells per ml of milk), Guha *et al*. (2012), reported that 32.20% of buffaloes and 20.89% of their quarters were categorized as suffering from mastitis. SCC had a lower sensitivity of mastitis diagnosis as compared to microbiological examination of milk samples (Guha *et al*., 2012). The explanation proffered by these investigators for lower sensitivity of SCC as compared to microbiological examination relates to recent latent infections which might be due to colonization of streak canals by mastitis pathogens. In consonance with the findings reported by Guha *et al*. (2012), *Staphylococcus spp.* were the most frequently isolated microorganisms from the milk in the present study.

A single infusion of dry period antibiotic into infected quarter's ≥45 days (d) prepartum led to reduced incidence of IMIs by 59% at calving compared with pretreatment level (Nickerson *et al*., 1990). Even for the difficult to treat organisms like *S. aureus,* the cure rate was higher than 90%. Prophylactic treatment of uninfected quarters ≥45 d prepartum resulted in a 93% reduction in new Streptococcal IMIs. The mean SCC was 50% lower at calving in treated heifers, and milk yield over the first 2 months of lactation was 10% greater than that of untreated controls. Heifers from herds using fly control had a lower prevalence of IMIs than herds not practicing this mastitis control measure.

Conclusion

The prevalence of streak canal infections as well as IMIs was higher in cow (91.25 and 55%, respectively) than in dairy buffaloes (73.75 and 22.5%, respectively). Recovery of 44 and 27 isolates, respectively in cows and buffaloes was common to both streak canal swab and quarter foremilk samples.

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