

Exploring bacterial pathogens and risk factors associated with the occurrence of navel ill in calves

Research Article

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ABSTRACT

The study mentioned here was designed to investigate both bacterial pathogens and risk factors associated with the occurrence of navel ill in calves. A total of 350 calves diagnosed to have navel ill formed the population in our study. Incidence density reports representing the period between 2009 and 2018 were obtained from Veterinary Teaching Hospital (VTH), Bangladesh Agricultural University where information about age, sex, breed, seasonal effect was also included. Umbilical swab from each individual suffering from confirmed navel ill was collected for microbiological study. The occurrence of navel ill in the age of <30 days (n = 244, 69.71%) was noted higher compared to the age of ≥30 days (n = 106, 30.29%). Male calves were highly susceptible (n = 192, 54.86%) compared to females (n=158, 45.14%). In cross breed individuals, the occurrence was higher (n = 330, 94.29%) in regard of indigenous calves (n=20, 5.71%). The occurrence of the illness in summer (March-June) was more common (n = 159, 45.43%) in comparison to both rainy (n = 111, 31.71%) and winter (n = 80, 22.86%) seasons. In term of microbiological study, *Staphylococcus aureus*, *E. coli* and *Proteus* spp. were identified and isolated from the infected areas of the calves with the disease. In conclusion, navel ill occurred more commonly in male calves with the age of less than one month. The appearance of navel ill was noted to be more frequently in summer season (March-June) in cross breed calves. The results also demonstrated that the condition is mediated by mixed bacterial infection formed by gram-positive and gram-negative agents which induce the initial inflammation.

Keywords: calf, navel ill, *Staphylococcus aureus*, *E. coli*, *Proteus* spp.

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Introduction

Navel ill is a disease of young calves, usually less than a week of age. The infection enters via umbilical cord or soon after birth with a prevalence of 5-15% (Mee, 2008a). In Bangladesh, 6.14 million cattle of a total sum of 24.09 million, is dairy cattle (DLS, 2019). Mortality in calves less than one year of age is about 9% (Debnath et al., 1990). Most of the animals die at young age due to different

infectious diseases and surgical disorders like navel ill (Samad et al., 2002). The facultative myiasis producing flies can be a responsible agent for navel infection. There is usually a mixed bacterial flora including *E. coli*, *Proteus* spp., *Staphylococcus* spp., *Actinomyces pyogenes* etc. (Sherif et al., 2017). This infection can result in a wide range of signs depending on where the

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bacteria spread to. In some calves, infection spreads from the navel to the liver causing a liver abscess (Mee, 1990; Biss et al., 1994). Though individual antibody levels alter the course of the infection, the health situation remains also highly dependent to postnatal management (Waldner and Rosengren, 2009). The occurrence of this condition is strongly associated with poor hygienic maintenance of both calf and shed as well as with the intake of low-quality colostrum (Waltner-Toews et al., 1986).

In Bangladesh, several studies have been conducted in calves with neonatal diseases and their recurrence such as *Schistosomus reflexus* infection and umbilical hernia while on the other hand only few works about the healing of navel ill have been arranged and very few of these studies were mostly confined to revealing the prevalence of the disease (Hoda et. al., 2018; Jaman et al., 2018; Mishra et al., 2020). Therefore, the present research work has been implemented to harvest an incidence data of calves diagnosed with navel ill-treated at Veterinary Teaching Hospital (VTH), Bangladesh Agricultural University (BAU), Mymensingh and belonging to a decade between 2009-2018 in order to explore the bacterial pathogens and risk factors responsible for the occurrence of the disease.

Material and methods

Collection of occurrence data: Incidence data of navel ill was collected from clinical data records of Veterinary Teaching Hospital (VTH), Bangladesh Agricultural University (BAU), Mymensingh. The temporal period contained approximately a decade between 2009 and 2018 while details such as age, sex, breed, and occurrence season were also taken into consideration.

Experimental animals: Calves with confirmed navel ill at Veterinary Teaching Hospital (VTH), Bangladesh Agricultural University (BAU), Mymensingh were adopted as the individuals of the research study mentioned here and umbilical swab for microbiological study was collected from each one, between January 2019 and May 2019.

Collection of bacteriological samples: All of the samples were collected aseptically from the deep navel region with the use of sterile cotton buds. Before the procedure, the area was soaked with saline solution and swab samples were collected by circling the cotton bud into the infected region before quickly dipping it into screwed capped test tubes containing nutrient broth.

Culture of navel ill causing bacteria: The collected broth samples were incubated at 37 °C for 2 hours for enrichment. The samples were inoculated into different selective medias such as Mannitol Salt agar (MSA) and MacConkey agar and incubated at 37 °C overnight. Next day bacterial growth in various medias were observed and pure culture of each bacteria were obtained by repeated culture preparations of a single colony.

Identification of isolated bacteria by Gram's staining: The pure cultures of isolated bacteria were subjected to Gram's staining for an observation of bacterial morphology, arrangement and staining characteristics under light microscope at 10x magnification.

Statistical analysis: Descriptive analysis was performed. Data were collected and calculated to determine the occurrence of navel ill in the above-mentioned species. The connecting link between the disease's occurrence and variables such as age, sex, breed and seasonal differences was evaluated and presented in percentage for each group.

Results

Effects of different variables on the occurrence of navel ill: A total amount of 350 calves diagnosed with navel ill were recorded from 2009 to 2018 at VTH, BAU, Mymensingh. Different attributes on which the occurrence of the condition was based on are shown below.

Occurrence of navel ill based on age:

The effect of age on navel ill in calves is presented in Figure 1. Occurrence was higher (n = 244, 69.71%) at the age of less than 30 days in comparison with the occurrence at the age higher than or equal to 30 days (n = 106, 30.29%).

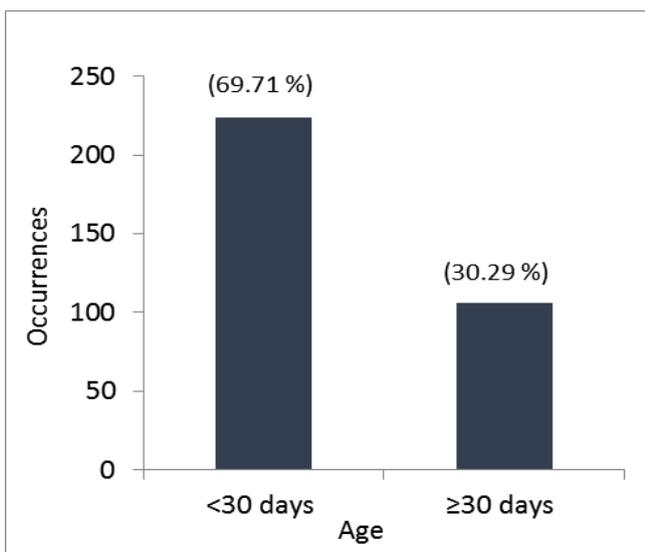


Figure 1. Occurrence of navel ill in calves based on age.

Occurrence of navel ill based on sex: The effect of sex on navel ill occurrence in calves is presented in Figure 2. Occurrence was higher (n = 192, 54.86%) in males than in females (n = 158, 45.14%).

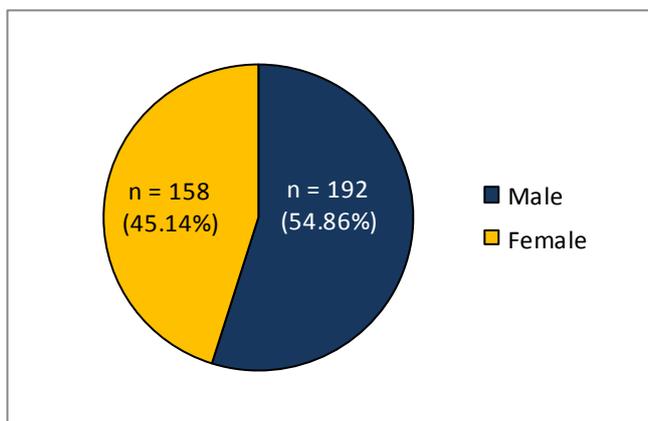


Figure 2. Graphical presentation of occurrence of navel ill based on sex in calves.

Occurrence of navel ill based on breed: The effect of breed on navel ill appearance in calves is shown in Figure 3. Occurrence was higher (n = 330, 94.29%) in cross bred calves than in indigenous calves (n = 20, 5.71%).

Occurrence of navel ill based on season: The effect of season on navel ill appearance in calves is presented in Figure 4. Highest occurrence of the disease was noted (n = 159, 45.43%) in summer season (March-June) while the lowest level was detected (n = 80, 22.86%) in winter season (July-October).

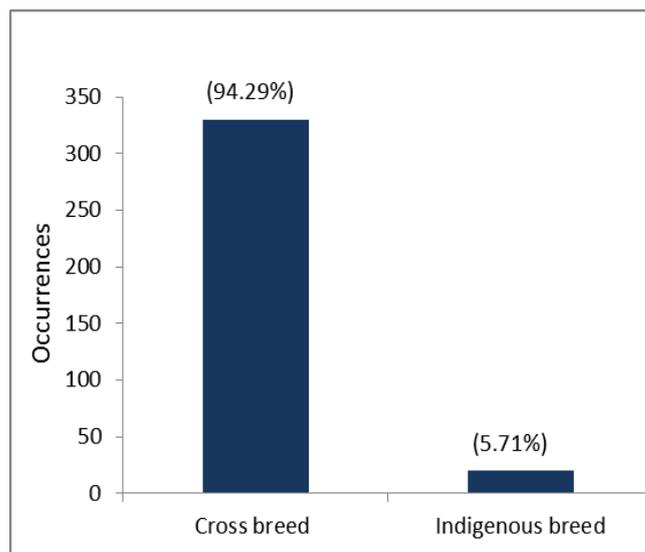


Figure 3. Occurrence of navel ill based on breed in calves.

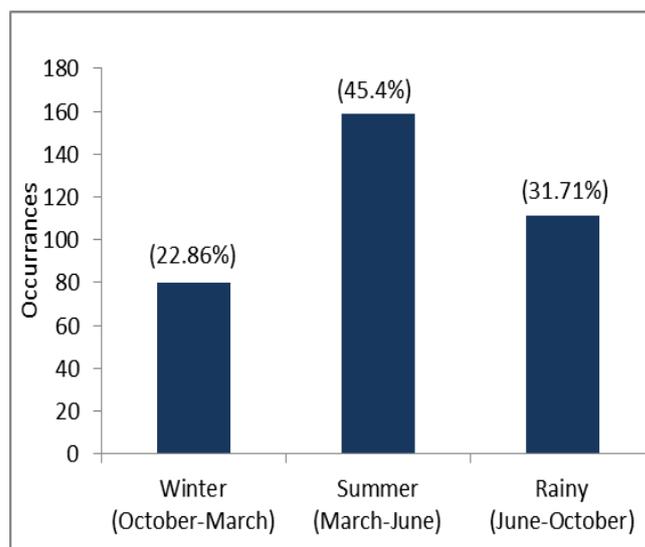


Figure 4. Graphical representation of occurrence of navel ill in calves based on season

Bacteriological findings

Spreading of swab on agar plate: On the basis of cultural characteristics and staining properties, *Staphylococcus* spp., *E. coli* and *Proteus* spp. were identified from the samples of all calves suffering from navel ill. On mannitol salt agar (MSA), *Staphylococcus* spp. produced a golden yellow colony due to the fermentation of Mannitol indicating that the isolates were *Staphylococcus aureus* (Figure 5A). On MacConkey Agar, *E. coli* caused only pink apparition and *Proteus* spp. produced colorless colonies (Figure 5B).

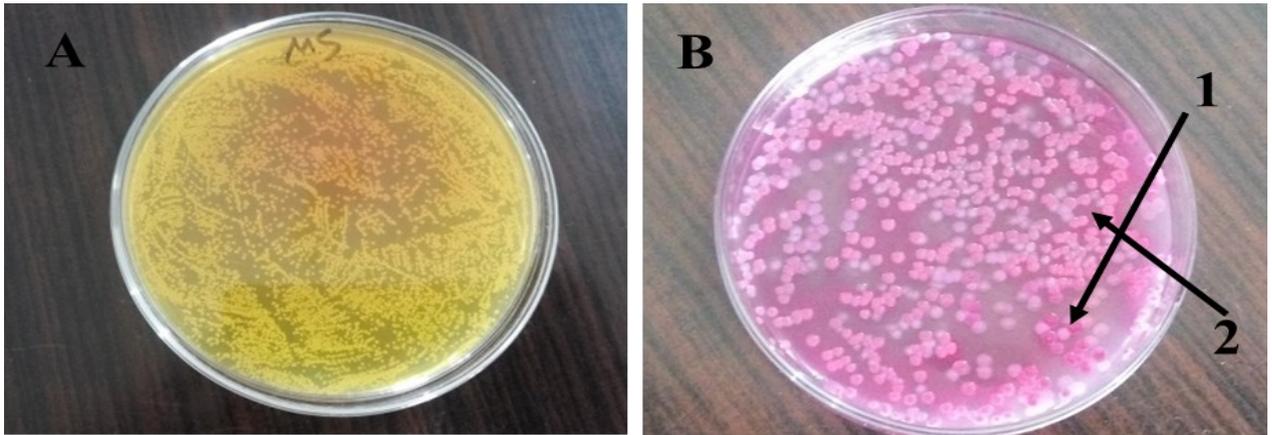


Figure 5. Isolated bacteria from navel ill patient. (A) Growth of *Staphylococcus aureus* in MSA (B) Growth of suspected (1) *E. coli* and (2) *Proteus* spp. on MacConkey Agar.

Pure culture finding: A single colony from each plate were spread on the same agar plate respectively. On MSA, a golden yellow colony were found; which was suspected to be *Staphylococcus aureus* (Figure 6A) and on

MacConkey Agar, a pinkish colony was found with metallic sheen, which was thought to be *E. coli* (Figure 6B1) and a colony with lose color were found, which was suspected to be *Proteus* spp. (Figure 6B2).

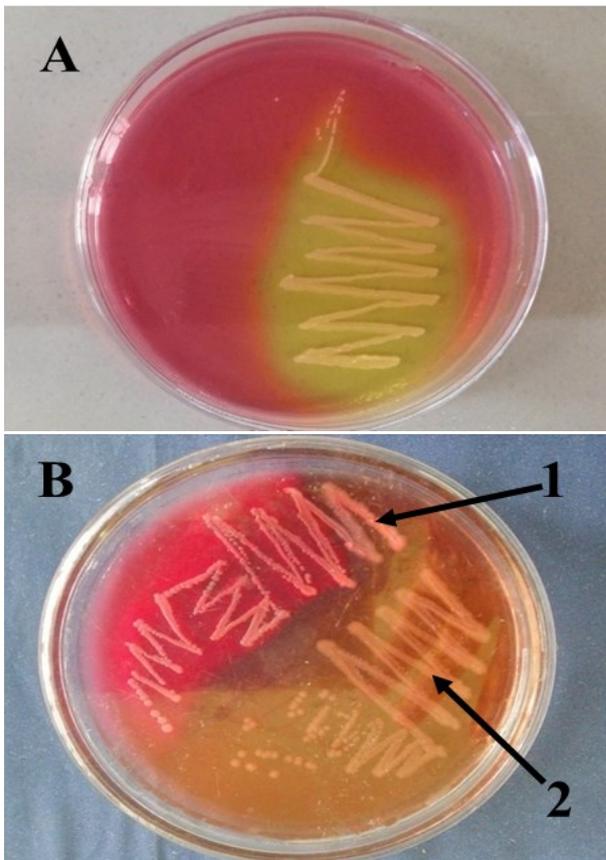


Figure 6. Colony characteristics of bacteria. (A) A Golden yellow color colony was found which was the characteristics of *Staphylococcus aureus*, (B1) A characteristics pinkish colony with metallic sheen was detected and the colony was evaluated as *E. coli* and (B2) a pale color colony was found which are usually recognized as the colony characteristics of *Proteus* spp.

Characteristics of isolated bacteria: On Gram's staining, The *Staphylococcus aureus* showed Gram positive, spherical shaped in cluster form (Figure 7A), the *E. coli* showed Gram negative rod-shaped organisms arranged in single or short chain like (Figure 7B) and the *Proteus* spp. showed Gram negative short rod arranged with periodic cycles of migration producing concentric zones or spread in a uniform film (Figure 7C).

Discussion

During the study period it was found that among 350 of infected calves, 244 were brought to VTH earlier than thirty days and 106 calves were of ≥ 30 days of age. Samad et al. (2001) reported that the infection rate was higher (73.03%) in those aged between 0-30 days compared to individuals aged between 31-90 days (24.72%) while the same rate was measured very low (2.25%) for those aged >90 days. Blowey and Weaver (2011) also reported that navel ill usually occurs in calves younger than one weeks of age, as a result of inflammation, due to umbilical tissue infection following parturition in insanitary environment; which was compatible with our study.

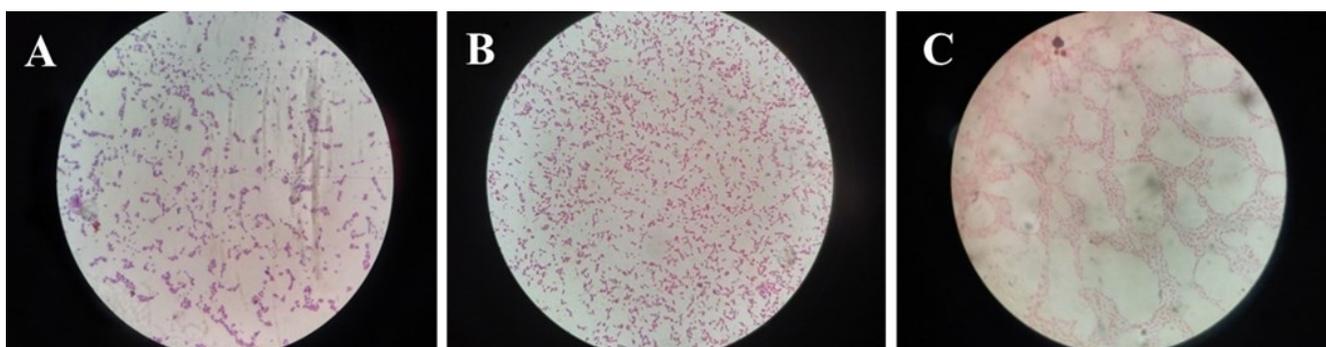


Figure 7. Staining characteristics of bacteria. (A) Smooth, convex and grape like clusters were found which is the characteristics of *Staphylococcus aureus*, (B) Rod shaped, irregular arrangement and pink in color was found which is the characteristics of *E. coli* and (C) Rod shaped with ranging in length from coccoid rods to long wire forms was detected which is the characteristics of *Proteus* spp.

In the present study, we found that the infection rate was higher in males (54.86%) than in females (45.14%); which was also in concordance with Radostits et al. (2007) having also reported that, male calves were more susceptible to the infection than the females. Since urethral opening was placed nearer to the navel area compared to the location of same opening in females, the navel area of male calves exhibit a higher possibility of being soiled by urine and getting infected.

In cross breed calves, the rate of infection was higher than in indigenous breed. However, Rweyemamu et al. (2008) reported that in local breeds, the infection rate was higher than in crossbred calves. This breed-related variation is estimated to be correlated to genetic predisposition.

In our study we observed that the rate of infection (45.43%) augmented in summer season. Watson (2004) also reported that the rate of incidence for navel ill and conjunctivitis were significantly ($p < 0.05$) higher in summer season compared to other seasons. As the condition is commonly seen during summer months, it is suspected that the odds are in favor of ultraviolet light, dust and flies acting as predisposing factors.

In bacteriological study we found *Staphylococcus aureus*, *E. coli* and *Proteus* spp. in samples obtained from navel region of the calves infected with naval ill. Sherif et al. (2017) reported that there is usually a mixed bacterial flora including

E. coli, *Proteus* spp., *Staphylococcus* spp., *Actinomyces pyogenes* etc. According to Blood and Radostits (1989), bacteria commonly involved include *E. coli*, *Streptococcus* spp., *Staphylococcus* spp., *Actinomyces (Corynebacterium) pyogenes* and other soil and fecal contaminants. A wide range of opportunistic bacteria were found in samples taken from the umbilical lesions (Jaman et al., 2018).

In conclusion, based on the findings of our foregoing study, navel ill occurred mostly in calves of less than one month of age and more common in male calves than that in female calves. The higher occurrence of navel ill encountered in the cross bred calves in contrast to indigenous calves. This disorder is more prevalent in summer season (March-June). The results also demonstrated that the condition is mediated by mixed bacterial infection (gram-positive and gram-negative bacterial infection) which induce the initial inflammation. Therefore, after birth the navel area should manage properly, calves should keep in dry and hygienic environment where light and air can easily enter.

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