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Isolation and identification of associated bacteria and maggots from myiasis affected wounds of cattle and goats in Bangladesh

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ABSTRACT

The study was carried out to investigate the associated maggots and bacteria in myiasis affected wound in animals. Total 37 myiasis affected cattle (n=25) and goats (n=12) were examined in this study, of which 21 samples were collected from the Veterinary clinics of Bangladesh Agricultural University (BAU), Mymensingh, and the remaining 16 samples were collected from Upazila Veterinary Hospital, Gouripur, Mymensingh. The maggots were collected from the wounds, and were identified under microscope. For microbial identification, samples (n=10) were collected aseptically by using sterile cotton swab. The samples were allowed to grow in media. Colony and staining characteristics were studied. Traumatic, creeping or migratory myiasis wounds were identified in the examined animals. In this study, subcutaneous wound was mostly recorded, of which 43% (n=9/21) was found at the Veterinary Clinics, BAU, and 44% (n=7/16) was found at the Upazila Veterinary Hospital, Gouripur. The maggots were identified as *Chrysomya bezziana*, commonly known as Old World screwworm. Occurrence of myiasis due to *C. bezziana* larvae was recorded as 100%. The associated bacteria were identified as *Staphylococcus* spp. In conclusion, the myiasis wounds are associated with both *Staphylococcus* spp. and larvae of *C. bezziana*. Preventive and control measures against the *C. bezziana* and *Staphylococcus* spp. can be undertaken to save the livestock from myiasis in Bangladesh.

Keywords

Bacteria, Control, Maggot, Myiasis, Wound type

ARTICLE HISTORY

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INTRODUCTION

Myiasis is an invasive infestation in the body of vertebrates caused by maggots (larvae) of flies. There are over 100 species of dipteran flies causing myiasis (John et al., 2006). The clinical and pathological manifestation of myiasis in animals depend on the genus and species of fly involved, mode of invasion by larva, degree and type of migration after invasion, and stage in the life cycle of fly. Myiasis is a well-known condition to veterinarians as this condition in animals are frequently found especially in underdeveloped regions. It causes severe economic losses worldwide (Otranto, 2001).

Although infestation by fly larvae is much more prevalent in animals, it is also frequently found in humans especially in rural, tropical and subtropical regions. Variations in myiasis depends on the fly species and where the larvae are located. Some flies lay eggs in open wounds, some larvae may invade unbroken skin or enter the body through the nose or ears, and some others may be swallowed if the eggs are deposited on the lips or on food (John et al., 2006). Cases of myiasis are found most commonly in tropical and subtropical zones of Africa and the Americas

(Millikan, 1999). The flies responsible for the condition prefer a warm and humid environment. Therefore, myiasis is limited mostly within summer months in temperate zones, but may occur all the year round in the tropics. Patients suffering from infestations by *Dermatobia hominis* are recorded in tropical parts of South America (Marty and Whiteside, 2005; Maier and Honigsmann, 2004; Millikan, 1999; Desruelles et al., 1999) and by *Cordilobia anthropophagi* among those returning from Africa (Adisa and Mbanaso, 2004).

Apart from various infectious diseases, wounds and myiasis are important in livestock health managements. A wound is an injury that typically causes a break the integrity in the skin. Wounds have been defined as sores, abscesses, warts and inflamed skin lesions (Luseba et al., 2007). When there is a break in skin, there is an increased risk of infection. Certain wounds have a higher potential for infections. The goal in wound management is to restore function, repair skin integrity and minimize infection (Robin, 2011). Clinically, myiasis can affect the skin (Poindexter, 1989), eye, auricular or nasal canals or oral cavity and anus (Mendivil and Shamman, 1979).

The secondary invaders infect human and animal through wounds or ulcerations in the skin or subcutaneous tissue; and rarely, through the orifices of the urogenital tract. But wounds, of whatever cause, are frequently contaminated with bacteria, many of which can spread in the bloodstream causing septicemia or toxemia. Wound contamination with *Clostridium welchii* is a frequent cause of death in the battlefield casualties (Erdmann, 2006) and infection (Caballero et al., 1996). There are reports on wound myiasis of animals, but few such work on integrated assessment of severity of wound caused by maggots are available. Therefore, the present research work was undertaken to investigate the wound types found in myiasis, identification and characterization of maggots causing myiasis in cattle and goat, and identification of bacteria infecting the myiasis wound.

MATERIALS AND METHODS

Experimental animals: The research was conducted with the patients that were represented to two Veterinary Hospitals namely Veterinary Clinics of Bangladesh Agricultural University (BAU) and Upazila Veterinary Hospital, Gouripur, Mymensingh. The research was performed covery a period of five months starting from January 2012 to May 2012. Among the affected animals, 8 goats and 13 cattle were recorded in the Veterinary Clinics, BAU, Mymensingh (**Table 1**). Similarly, patients admitted to the Upazila Veterinary Hospital, Gouripur, were taken into consideration for the research purpose. There were 10 cattle and 6 goats admitted to the Upazila Veterinary Hospital. The patients were investigated for wound type, maggot characteristics and categories of the secondary microbial invasions.

Identification of maggots from wounds:

The patient was restrained to examine the wound area. Gauze dipped in oil of turpentine or tincture of iodine was allowed to remain in the wound for 2 min. As a result, the maggots came out of the wound to the surface, and the maggots were removed with sterile forceps. The maggots were kept in plastic vials containing 70% alcohols. Then the vials were brought to the Department of Parasitology, BAU. The maggots were transferred from vials to petridish, and cleaned in 70% alcohol. The maggots were identified under microscope by observing body without fleshy processes and segments with belts of strongly developed spines, as described by Scholl et al. (2009). The length and width of the maggots were also measured.

Identification of microorganisms from wound:

Sources of samples: A total of 37 myiasis samples were collected, of which 21 were collected from Veterinary Clinics of BAU, and the remaining 16 were collected from Upazila Veterinary Hospital, Gouripur, Mymensingh, Bangladesh.

Media used: A number of commercially available synthetic media were used for this study. All the media were of research grade.

Chemicals and reagents: The chemicals and reagents used for the study were: reagents for Gram's staining (Crystal violet, Gram's iodine, acetone alcohol and Safranin), 3% hydrogen peroxide, normal saline solution, alcohol and other common laboratory chemicals and reagents of research grade.

Collection and transportation of samples: The wound area was cleaned by using normal saline, and the samples were collected aseptically by using sterile cotton swab. Then the samples were brought to the laboratory with necessary precautions for bacteriological examination. Samples were disinfected with a swab soaked in methylated sprits. The samples were allowed to inoculate into Nutrient

agar (NA) media and mannitol salt agar (MSA) media for the isolation and identification of bacteria. Nutrient borth (NB), NA, and MSA were prepared according to the manufacturer's instructions.

Isolation and identification of bacteria: Isolation of bacteria from the collected samples was done by inoculating the samples onto NA and MSA. The inoculated media were then incubated aerobically at 37°C for 24 h for growth. The isolates were identified based on their morphology and staining, cultural characteristics and biochemical properties. The representative bacterial colonies were characterized microscopically using Gram's method (Islam et al., 2007; Begum et al., 2007; Nasrin et al., 2007; Roy et al., 2012; Asaduzzaman et al., 2013). Catalase test was done for differentiation of *Staphylococcus* sp. from other bacteria like *Streptococcus* sp.

RESULTS AND DISCUSSION

In the present study, various types of wounds were found such as incised, subcutaneous, migratory, traumatic, and burrowing. In Veterinary Clinics of BAU, out of 21 myiasis wounds, subcutaneous wound was found as the highest (43%; n=9/21). In contrast, traumatic and burrowing types of wounds had the lowest occurrence (9.5%; n=2/21). In Upazila Veterinary Hospital, Gouripur, out of 16 myiasis wounds, subcutaneous wound had the highest occurrence (44%; n=7/16) and traumatic types of wound had the lowest occurrence (12.5%; n=2/16), as shown in **Table 1**. The findings of the present study of myiasis wound were closely agreed with the findings of Mcgraw and Turiansky (2008) who reported that cutaneous involvement was the most common type of myiasis.

A total of 37 animals (cattle and goats) were examined to find out the occurrence of myiasis.All the 37 samples were infested with the larvae of *Chrysomya bezziana.* Among these 37, 21 were identified at the Veterinary Clinics of BAU, Mymensingh, and the remaining 16 were identified at the Upazila Veterinary Hospital, Gouripur, as indicated in **Table 2**.

In the present study, out of 37 myiasis affected cases, 12 animals aged up to 6 months, 10 animals were between 6 months and 2-year, and 15 animals were of >2-year. Among the affected 37 animals, 26 were female, and 11 were male. In Veterinary Clinics of BAU, the adult animals of >2-year had the highest occurrence (38%; n=8/21). The occurrence of myiasis in the animals aging up to 6 months was 33% (n=7/21). The animals aged between 6 months and 2-year had the lowest occurrence (29%; n=6/21). Among the 21 affected animals, 14 (67%) were female and 7 (33%) were male. In Upazila Veterinary Hospital, Gouripur, out of 16 animals, the adult animals of >2-year had the highest occurrence (44%). Among the 16 affected animals, 12 (75%) were female and 4 (25%) were male. The results are shown in the **Table 3** and **Table 4**.

In the present study, at Veterinary Clinics of BAU, average 15 larvae were found in the patients. At best 22 larvae were found in a calf sufferings from umbilical myiasis. In contrast, 6 larvae could be extracted from cattle as minimum. From Upazila Veterinary Hospital, Gouripur, average 13 larvae were found in patients, and highest 31 maggots were recovered from a cow suffering from genital (vulvar) myiasis.

A total of 37 wounds infested with maggots were collected. Ten samples were collected for bacteriological examination. All 10 samples were found positive for *Staphylococcus* spp.; among these 10 samples, 7 were collected from Veterinary Clinics, BAU, and the remaining 3 were of Upazila Veterinary Hospital, Gouripur, as shown in **Table 5**.

Staphylococci organisms were found as Gram-positive cocci, and were arranged in grapes like cluster. Catalase test was performed to differentiate *Staphylococci* (catalase producer) from *Streptococci* (non-catalase producer). Slide calatase test was performed as *Staphyloccoci* could breakdown hydrogen peroxide into oxygen and water, indicated by bubble formation.

The occurrence of such type of problems in cattle and goats is a concern for veterinarians. This might be caused due to poor management practices, and unawareness of farmers. Wounds due to tick bites, parturition, castration, and barbed wire injury could be good conditions to harbor parasitic infestation and microbial infection. The most damaging myiasis causing flies (*Chrysomya* spp.) deposit their eggs in and around the wounds. The fly larvae crawl into the wound, and burrow deep into the underlying tissues to feed. They are developed through three larval stages, causing extensive local tissue damage. *Lucilia sericata* is found in wounds and natural orifices of animal body particularly the mouth, eyes, and sinuses. It causes itching, pain, erythema, bleeding, eosinophilia, and sometimes secondary bacterial infections (Daniel et al., 1994; Kaufman et al., 1989). Pays and Haas (1996) reported a case of myiasis due to *L. sericata*, and Cho et al. (1999) found the first myiasis in human wound in Korea.

Table 1: Different types of wounds in goats and cattle.

Type of wound	Veterinary Clinics, BAU (n=21)		Total (%)	Upazila Vet. Hospital, Gouripur (n=16)		Total (%)
	Cattle (n=15)	Goat (n=6)		Cattle (n=10)	Goat (n=6)	
Incised	4	0	19	2	0	12.5
Subcutaneous	7	2	43	3	4	44
Migratory	3	1	19	4	1	31.25
Taumatic	1	1	9.52	1	1	12.5
Burrowing	0	2	9.52	0	0	0

Table 2: Occurrence of myiasis in cattle and goats.

Source of Maggot sample	No. of samples examined	Myiasis affected (%)
Vet. Clinics, BAU, Mymensingh	21	100 (n=21/21)
Upazila Vet. Hospital, Gouripur	16	100 (n=16/16)

Table 3: Effects of age on the occurrence of myiasis in cattle and goat.

Source of myiasis sample	Age group					
	< 6 months		>6 months to <2 years		> 2 years	
	Cattle	Goat	Cattle	Goat	Cattle	Goat
Veterinary Clinics, BAU	6	1	4	2	5	3
Total (%)	7 (33)		6 (29)		8 (38)	
Upazila Vet. Hospital, Gouripur	4	1	0	4	6	1
Total (%)	5 (31)		4 (25)		7 (44)	

Table 4: Effects of sex on the occurrence of myiasis in cattle and goat.

Source of Maggots sample	Male		Female	
	Cattle	Goat	Cattle	Goat
Veterinary clinics, BAU	4	3	9	5
Total (%)	7 (33)		14(67)	
Upazila Veterinary Hospital, Gouripur	2	2	8	4
Total (%)	4 (25)		12(75)	

Table 5: Invasion of myiasis wounds by the microorganisms.

Source of Maggots sample	No. of sample examined	Wound infected with bacteria	Wound infected (%)
Vet. Clinics, BAU, Mymensingh	7	7	100
Upazila Vet. Hospital, Gouripur	3	3	100
Total	10	10	100

Table 6: Cultural characteristics of *Staphylococci* isolated from myiasis affected wound.

Sources of isolates	Colony characteristics		Interpretation
	Nutrient agar	Mannitol salt agar	
Myiasis affected wound samples	Circular, small, smooth raised a white or yellowish colonies appearance	White or yellowish colonies with fermentation	*Staphylococcus* spp.

In the present study, a total of 37 samples were examined to find out the occurrence of myiasis. All 37 samples were infested with larvae of *C. bezziana.* Among the 37 *C. bezziana*, 21 were identified at the Veterinary Clinics of BAU, Mymensingh, and 16 were identified at the Upazila Veterinary Hospital, Gouripur. At the Veterinary Clinics of BAU, average 15 larvae were found in the patients. Highest 22 maggots could be isolated from a calf sufferings from umbilical myiasis. In the Upazila Veterinary Hospital, Gouripur, average 13 larvae were found in the patients, and highest 31 maggots were found in a cow suffering from

genital (vulvar) myiasis. Daniel et al. (1994) had reported a case of traumatic myiasis of wound and could separate 50 larvae from nose, paranasal sinuses and mouth of the patient. Leach bite may facilitate infection. It carries danger of bacterial and myiasis super infection. Slesak et al. (2011) extracted 22 *C. bezziana* larvae from a patient's heel, which was sufferings from myiasis. Kilic et al. (2011) reported that *L. sericata* often caused myiasis in open wound. A total of 97 larvae were found in a patient with cancer wound after operation. *C. chloropyga* had been reported to be a primary and secondary causes of sheep myiasis in South Africa, and *L. cuprina* was responsible for the condition known as blowfly strike of sheep in a number of countries including South Africa (Hall, 1991).

In Veterinary Clinics of BAU, the highest occurrence (38%) of myiasis wound was found in the adult animals aging >2-year, and the lowest occurence (29%) was found in the animals aging between 6 months and 2-year. Female animals were mostly affected as compared to males. In the present study, 67% of the myiasis affected animals were female and 33% were male. In Upazila Veterinary Hospital, Gouripur, the adult animals of >2-year had the highest occurrence (44%). The animals aged between 6 months and 2-year had the lowest occurrence (25%). Among the myiasis affected animals at Upazial Veterinary Hospital, Gouripur, 75% were female and 25% were male. The occurrence of myiasis wound was more frequent in the adult female cattle. These findings could not be compared due to paucity of similar information in the available literature. Adult animals, however, may sustain wounds more frequently than the young animals. Some of these wounds may afterwards be converted to myiasis. Affections in female animal is more; this might be due to moist condition or discharge on the vaginal orifice.

The skin is the major obstacle for the establishment of infections by bacterial pathogens in internal tissues. When bacteria could breach this barrier, infection can be occurred easily (Janda et al., 1997; Bisno and Stevens, 1996). The most common underlying event for all wounds is trauma. Trauma could be accidental or intentionally induced. In the present study, 10 samples were collected and examined to find out microbial invasion revealing that all the samples were positive for *Staphylococcus* spp. Among the samples, 7 were identified from Veterinary Clinics of BAU, and 3 were identified from Upazila Veterinary Hospital, Gouripur. All the wounds were infected with *Staphylococcus* spp. Caballero et al. (1996) isolated bacteria associated with the screwworm fly, *Cochliomyia hominivorax* causing myiasis. They took swabs from wound, before, during, and after the myiasis infestation.

CONCLUSION

The myiasis are associated with the larvae of *Chrysomya bezziana*. The microorganisms associated with myiasis is *Staphyloccocus* spp. The occurrence of myiasis wound is more frequent in the adult female cattle. Identification of the flies which are mostly responsible for myiasis in cattle and goats in Bangladesh are suggested.

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