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IDENTIFICATION OF BACTERIAL AGENTS FROM THE FAECAL SAMPLES OF DIARRHOEIC SHEEP AND THEIR ANTIBIOTIC SENSITIVITY

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ABSTRACT

The current study was carried out to identify the bacterial species in the faecal samples of 20 diarrhoeic sheep and to observe their sensitivity to different antibiotics. This investigation was performed by collecting diarrhoeal samples from the sheep (n=20) under goat and sheep research farm of Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka, Bangladesh. The average age and body weight of the animals were 25 days and 2.5 kg respectively. Of the bacteria responsible for diarrhoea in case of sheep, *Escherichia coli* alone was found in 6 samples (30%), *Escherichia coli* combined with *Proteus mirabilis* was found in 12 samples (60%), and no bacteria could be detected in 2 of the 20 samples tested. Both the bacteria were highly sensitive to ceftriaxone, and moderately sensitive to ciprofloxacin and gentamicin. It could be stated that ceftriaxone is the antibiotic of first choice for the treatment of diarrhoea in sheep, where *Escherichia coli* is suspected to be the principal causal agent of diarrhoea.

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INTRODUCTION

Diarrhoea characterized by an increased frequency, fluidity, or volume of faecal excretion is a major problem in the farm animals. It is caused by a wide range of microbial, parasitic and environmental factors namely–bacteria, virus, helminths, protozoa, toxic substances, sands, lush pasture, overfeeding, overcrowding, poor sanitation, inadequate intake of colostrum, poor quality colostrum, poor quality milk replacers, feeds difficult to digest, etc. On the basis of literature the bacterial agents causing diarrhoea in sheep are *Escherichia coli*, *Salmonella spp*. and *Clostridium perfringens,* where *Proteus spp.*, and some other species of bacteria may be associated in some cases (Rahaman, 1995).

According to Hindson and Winter (2002), a combination of reduced reabsorption from the lumen of the intestine, and increased fluid loss through damaged mucosa into the intestine is found in many forms of both gastroenteritis and inflammatory change in the lower intestine. Such a condition ranges from bacterial infections such as *E. coli* and *Salmonella* to parasitic diseases such as coccidiosis and parasitic gastroenteritis. Diarrhoea or enteritis, to a greater extent, is related with clostridial diseases. These clostridial diseases of sheep have been recognized clinically for over 200 years, but not until the end of the nineteenth century did their bacterial nature start to be unraveled, a process that continued over the next 50 years (Sterne, 1981). Even during the 1990s, new information came to light as the importance of Clostridium sordellii as a cause of abomasitis and enteritis in all ages of sheep was established (Lewis and Naylor, 1998). The haemorrhagic enteritis affecting lambs in the first few days of life is caused by *C. perfringens* types (either beta or beta 2 toxin) or C, and differs from lamb dysentery only in the gross pathology and being marginally less acute, affecting lambs up to 3 weeks of age (Lewis, 2007).

E. coli is a major cause of diarrhoea in calves, piglets and lambs, and the term 'colibacillosis' is commonly used. It causes huge economic loss in this age group of animals (Radostits *et al*., 2000). However, selection of suitable antibiotics is a good strategy for successful treatment of bacterial diarrhoea. But all antibiotics are not always useful for the treatment of diarrhoea because of development of antibiotic resistance. So, the present study was carried out to identify the bacterial species in the faecal samples of diarrhoeic sheep and to observe their sensitivity to different antibiotics.

MATERIALS AND METHODS

This investigation was performed in the bacteriological laboratory of Bangladesh Livestock Research Institute (BLRI) by collecting diarrhoeal samples from the sheep (n=20) in BLRI goat and sheep research farm. The average age and body weight of the animals were 25 days and 2.5 kg, respectively. The duration of the experiment was from August, 2010 to May, 2011.

Sample collection

The faecal samples were collected aseptically into stool containers directly from the rectum with the help of small polybags.

Laboratory tests for identification of bacteria

The collected samples were then allowed for culture in bacteriological media (nutrient agar, EMB agar, SS agar, Blood agar, Mac Conkey agar), Gram's staining and biochemical tests (Methyl Red test, Indole test, Catalase test, etc) for isolation and identification of the bacterial species.

Antibiotic sensitivity test

The antibiotic sensitivity test was done by using disc diffusion method (Cowan and Steel, 1965). The commercially available antibiotic discs such as, oxytetracycline, gentamicin, amoxycillin, penicillin G, cloxacillin, sulphamethoxazole, streptomycin, ciprofloxacin and ceftriaxone were used to know the sensitivity of *Escherichia coli* and *Proteus mirabilis* to these drugs.

RESULTS AND DISCUSSION

The isolates were identified as *Escherichia coli* on the basis of morphology (Gram negative rod), cultural characteristics (Green metallic sheen on EMB agar as in fig.1) and biochemical characteristics and *Proteus mirabilis* on the basis of morphology (Gram negative rod), cultural characteristics (Swarming growth on Mac Conkey agar as in fig.2 and Brilliant Green Agar) and biochemical characteristics. Among the different kinds of bacteria responsible for diarrhoea in sheep, only *Escherichia coli* was found in 6 samples (30%), *Escherichia coli* alone combined with *Proteus mirabilis* was found in 12 samples (60%), and no bacteria were found in 2 of the 20 samples tested (Table 1).

Both the bacteria were highly sensitive to ceftriaxone, and moderately sensitive to ciprofloxacin and gentamicin (Table 2). The inhibition zones, in case of ceftriaxone, were 29 mm for *Escherichia coli* and 27 mm for *Proteus mirabilis* while these zones were 17 mm for *Escherichia coli* and 21 mm for *Proteus mirabilis* in case of ciprofloxacin and 17 mm for both *Escherichia coli* and *Proteus mirabilis* in case of gentamicin (Table 2). These bacteria were found resistant to other antibiotics used in the sensitivity test (Table 2). Wang *et al.* 2014 noticed decreased susceptibility of *P. mirabilis* in Taiwan to some broad spectrum antibiotics, including 3rd-generation cephalosporins and ciprofloxacin, whereas Kwiecińska-Piróg *et al.* 2013 found that in most of the tested concentrations, ciprofloxacin was more efficient than ceftazidime against the *P. mirabilis* biofilm. They became able to prove that the efficiency of antibiotics against *P. mirabilis* biofilm depends on its maturity and strains' origin.

Bacterial resistance to a particular antibiotic might occur due to use of that antibiotic for a longer period of time. The present study revealed that *E. coli* isolates were resistant to several antibiotics like penicillin G, amoxycillin, cloxacillin, streptomycin and oxytetracycline. These findings are in support of Ershaduzzaman *et al.* 2007, Tadesse *et al.* 2012. But Islam *et al.* 2007 found streptomycin as a sensitive antibiotic to *E. coli* which contradicts the present findings. However, this might happen due to strain variation of *E. coli*.

In this investigation the highest sensitivity was recorded for ceftriaxone because it is a new generation of cephalosporin and has not been used by the physician for long time.

Table 1. Prevalance of bacteria found in the diarrhoeal samples of sheep

Bacteria Found	Total Number of Samples Tested	No. of Positive Samples	Percentage
Escherichia coli and *Proteus mirabilis.*	20	12	60 %
Escherichia a coli	20	6	30 %
No bacteria	20	2	10 %

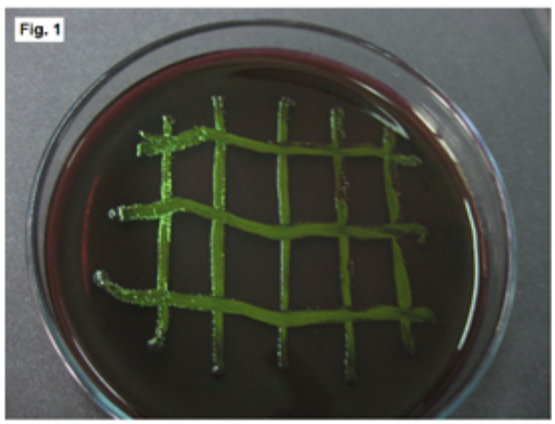

Figure 1. Growth of *E. coli* in EMB agar with Metallic sheen.

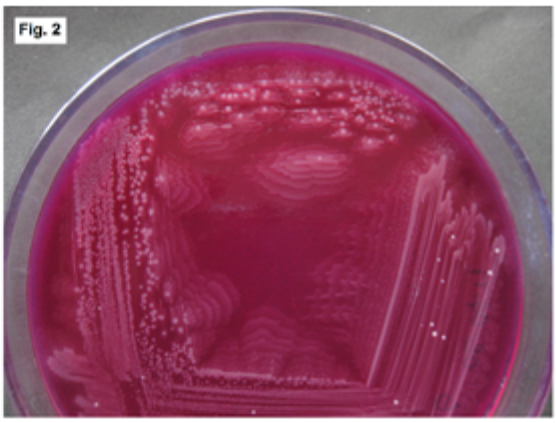

Figure 2. Swarming growth of *Proteus mirabilis* in MacConkey agar.

Table 2. Inhibition zones produced by antibiotics used against *Escherichia coli* and *Proteus mirabilis* in the sensitivity test.

Antibiotics discs used	Bacterial species	Zone of inhibition	Result
Oxytetracycline	*Escherichia coli*	7 mm	Resistant
	Proteus mirabilis	0 mm	Resistant
Gentamicin	*Escherichia coli*	17 mm	Moderately sensitive
	Proteus mirabilis	17 mm	Moderately sensitive
Amoxycillin	*Escherichia coli*	0 mm	Resistant
	Proteus mirabilis	7 mm	Resistant
Penicillin G	*Escherichia coli*	0 mm	Resistant
	Proteus mirabilis	0 mm	Resistant
Cloxacillin	*Escherichia coli*	0 mm	Resistant
	Proteus mirabilis	0 mm	Resistant
Sulphamethoxazole	*Escherichia coli*	0 mm	Resistant
	Proteus mirabilis	0 mm	Resistant
Streptomycin	*Escherichia coli*	0 mm	Resistant
	Proteus mirabilis	0 mm	Resistant
Ciprofloxacin	*Escherichia coli*	17 mm	Moderately sensitive
	Proteus mirabilis	21 mm	Highly sensitive
Ceftriaxone	*Escherichia coli*	29 mm	Highly sensitive
	Proteus mirabilis	27 mm	Highly sensitive

N.B.: The interpretation was done as resistant (≤10 mm), less sensitive (11-14 mm), moderately sensitive (15-18 mm) and highly sensitive (≥19 mm) except penicillin G, where zone of inhibition for resistance range is ≤28 mm according to Kirby-Bauer Method.

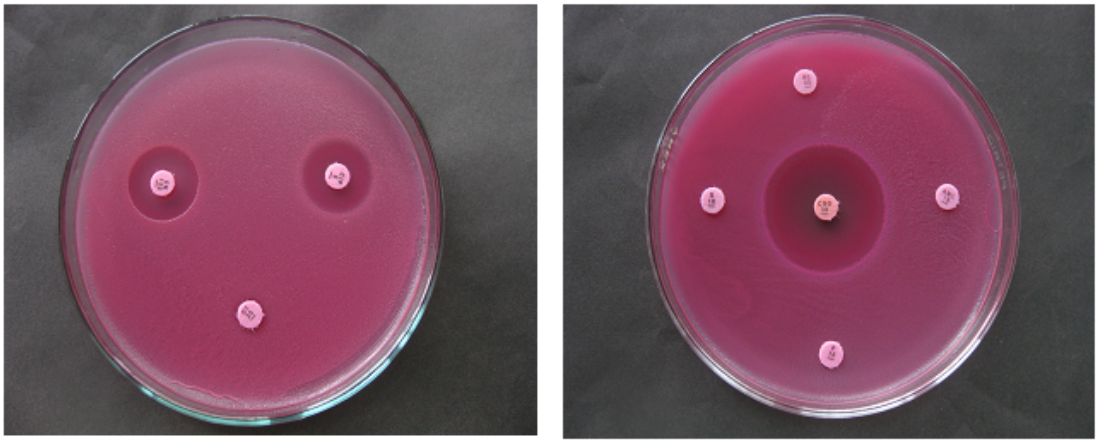

Figure 3. Production of clear zone of inhibition by Ceftriaxone (at the right-central), Ciprofloxacin (at the left-left one), Gentamicin (at the left-right one) of *E. coli in* Mac Conkey agar.

CONCLUSION

In conclusion, it might be stated that ceftriaxone is the antibiotic of first choice, and ciprofloxacin and gentamicin are the antibiotics of second choice to be used for the treatment of diarrhoea in sheep, where *Escherichia coli* is suspected to be principal causal agent of diarrhoea.

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