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Isolation and identification of buccal and intestinal bacteria in goats in Chittagong, Bangladesh

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Abstract

An investigation was carried out to determine the prevalence of common bacterial pathogens in goats admitted at S. A. Quaderi Teaching Veterinary Hospital (SAQTVH) in Chittagong Veterinary and Animal Sciences University (CVASU), Chittagong, Bangladesh. Two hundred swab samples (100 buccal and 100 faecal) were taken to find out the bacterial burden in the study population. All samples were subjected to various cultural and biochemical tests to isolate the bacterial pathogens from goats. From these tests *Staphylococcus* spp, *Escherichia coli* and *Salmonella* spp were identified. Out of 100 buccal swab samples, prevalence of *Staphylococcus* spp found 14%. A total of 20 *E. coli* isolates were screened out from fecal swab samples that reflected the prevalence was 20% in goats. 12% prevalence of *Salmonella* spp was recorded and found to be positive in all cultural and biochemical tests from faecal swabs. Although there was no significant difference ($p<0.05$) between different variables like age, sex and breed with prevalence of bacteria but these variables were proportionately differed. Extensive studies are recommended for the molecular study of these bacterial pathogens and for assessing the effects on goat population.

Keywords: Prevalence, *Staphylococcus* spp, *E. coli*, *Salmonella* spp, goat

Introduction

Bangladesh is an agriculture based country. Goat rearing is considered as superior in the agricultural sector because of an utmost assured return in a relatively short period of time. In many parts of the world, it is kept as a source of meat, milk and fiber. Among the notable diseases of goat, pneumonia, enteritis, different respiratory and gut associated illness are considered as common throughout the world that increase production costs with expensive treatments. The disease is mostly caused by bacteria (Tsolis et al., 2012). However, a very little is known on the associated bacterial agents in goats or a few is published until now in the literature. In Bangladesh, large number of goat population die each year due to bacterial diseases at the early stage of their lives (Asaduzzaman et al., 2013). There are both infectious and non-infectious species of the organisms in goats causing high morbidity and mortality of adult animals

and their young offspring (Momin et al., 2011). Most common bacterial pathogens include *Staphylococcus* spp, *Escherichia coli*, *Salmonella* spp, *Bacillus* spp. etc.

Staphylococcus spp is a ubiquitous commensal bacterium on skins and anterior nares, but frequently causes severe infections. Rapid and direct identification of *Staphylococcus* spp is crucial for proper management of patients with skin infections, abscesses, septicemia or bacteremia, gastroenteritis, endocarditis, toxic shock syndrome and certain food intoxications (Kateete et al., 2010). *Staphylococcal* food poisoning includes symptoms of sudden onset of nausea, vomiting, abdominal cramps and diarrhea (Balaban and Rasooly, 2000).

Escherichia coli is considered as the normal bowel flora of different species of mammals and birds but some strains of *E. coli* possess pathogenic characters due to the acquisition of virulent factors (Dho and Lafont, 1984). *E. coli* is responsible of white scour in goat (Bhat et al., 2008). Animals suffering from white scour have severe colitis characterized by abdominal pain, pasty feces, sever enteritis may culminates into death due to severe dehydration. Systemic infection caused by *E. coli* in kids resulting in septicemia and enteritis, characterized by fever, anorexia, and weakness, followed by coma and death which is similar to colibacillosis in calves (Abdullah et al., 2010).

Amongst the common zoonotic bacterial diseases of adult goats characterized by diarrhea, the most frequent one is salmonellosis (Radostits et al. 2007). *Salmonella* Enteritidis produces enterotoxins which are invasive to cause inflammatory change within the intestine leading to diarrhea. Bacterial enteritis remains the most common clinical problem in the goats (Meshram et al., 2009). Three common conditions caused by *Salmonella* are gastroenteritis, enteric fever, and bacteremia. Cases of clinical salmonellosis in sheep and goats are infrequent. The most common serotype found in gastroenteritis cases is *S.* Typhimurium, but many other serotypes have also been isolated. Serotype *S.* Abortus ovis, which causes abortions in the last two months of pregnancy and gastroenteritis in sheep and goats, seems to be restricted to Europe and the Middle East (PAHO, 2001).

Poor management, transportation stress, overcrowding pens, sudden environmental changes, poor housing conditions, concurrent viral infections (e.g. parainfluenza-3virus), lung parasites and other stressful conditions increase goat's susceptibility to diseases. Keeping on view the importance of goat as a vital source of meat, and a potential zoonotic threat, the present study was designed to conduct with the two objectives: 1) to estimate the prevalence of *Staphylococcus* spp, *Escherichia coli* and *Salmonella* spp in the study population of goats and 2) to determine the phenotypic characterization of bacterial isolates in different bacteriological culture media and biochemical tests.

Materials and Methods

Study population and sample collection

The study was carried out during January to March, 2015. The samples were collected from S. A. Quaderi Teaching Veterinary Hospital (SAQTVH) in Chittagong Veterinary and Animal Sciences University (CVASU). About one hundred buccal and one hundred faecal samples were collected during the study period. The samples were collected by inserting a sterile swab into oral cavity and RAJ (Recto-anal junction) of the animal. The collected swab was placed into a falcon tube (5ml) containing Stuart's transport medium (Oxoid, UK), and sent to the Microbiology Laboratory, CVASU for laboratory analysis.

Experimental design

The entire study was divided into three major steps: The first step included the collection of samples, their transportation to the laboratory and inoculation into different culture media. In second step, isolation and identification of the bacterial isolates was done based on their cultural characteristics including hemolytic activity, Gram's staining character etc. In third step, characterization of the organism was done using various biochemical tests.

Bacteriological investigation

Isolation and identification of *Staphylococcus* spp

Buccal swabs (n=100) from transport media were placed onto sterile Buffered Peptone Water (BPW) (Oxoid, UK) and enriched for 24 hours at 37°C (Thaker et al., 2013). Both Mannitol salt agar (MSA) medium and Blood agar base were prepared according to the instructions of manufacturer (Oxoid, UK). Blood agar was prepared by adding 5% citrated-bovine blood with blood agar base. A loopful of

inoculum from enrichment was streaked on Blood Agar (Oxoid, UK) and incubated at 37°C for 24 hours for detection of hemolysis. Growth of yellow colonies on MSA (Oxoid, UK) surrounded by yellow zones as a result of fermentation of mannitol after 24 hours of incubation at 37°C indicated a positive result (Kateete et al., 2010). Smear was prepared from the isolated colony on clean grease free microscopic glass slide and stained with Gram's Method of staining. All the positive samples were subjected to coagulase and catalase tests for biochemical confirmation of *Staphylococcus* spp as described by Monica, (1991).

Isolation and identification of *Escherichia coli*

Samples from Recto anal junction (n=100) were subjected onto BPW broth (Oxoid, UK) as pre-enrichment for *E. coli* (Thaker et al., 2013). A loopful of culture inoculates streaked on MacConkey (Oxoid, UK) agar. Pink colonies obtained from MacConkey agar were taken and streaked on Eosin methelene blue (EMB) (Oxoid, UK) agar to verify whether the bacterial population was *E. coli*, or not. Dyes Eosin and Methylene Blue react with products released by *E. coli* from lactose or sucrose as carbon and energy source, forming metallic green sheen regarded as positive isolate (Virpari et al., 2013). IMViC test were performed for confirmation of *E. coli* as biochemical tests (Edward and Ewing, 1972).

Isolation and identification of *Salmonella* spp

Swabs were collected from Recto-anal junction (RAJ) and pre-enriched on BPW (Oxoid, UK), incubated at 37˚C for 16 hours. 1 ml of inoculums was transferred into Selenite-cystein broth (Oxoid, UK) after pre-enrichment (Putturu et al., 2013). A loopful of inoculums plated onto Xylose Lysine Deoxycholate (XLD) (Oxoid, UK) medium and incubated at 37˚C for 24 hrs. Black centered colony from XLD was inoculated in Brilliant Green Agar (BGA) (Oxoid, UK) and incubated as well. TSI, MIL, Urease and Citrate tests were performed for the confirmation of *Salmonella sp.*

Microscopic study by staining method

Grams staining method was done to study morphology and staining characters. Suspected colonies from EMB (*E. coli*), BGA (*Salmonella* spp) and Mannitol salt agar (*Staphylococcus* spp) was stained as described by Manual of veterinary investigation laboratory technique (OIE, 2000). The procedure was as follows:

A small colony was picked up with a bacteriological loop, smeared on glass slide and fixed by gentle heating. Crystal violate solution was then applied on smear to stain for two minutes and then washed with running water. Few drops of Gram's iodine were then added to act as mordant for one minute and then again wash with running water. Acetone alcohol was then added for few seconds who act as a decolorizer. After washing with water, safranin was added as counter stain and allowed to stain for 2 minutes. The slides were then washed with water, blotted and dried in air and then examined under microscope with high power objective (100X) using immersion oil.

Data entry and statistical evaluation

All the data like age, sex and breed from the goats were entered into MS excel (Microsoft office excel-2007, USA). Data management and data analysis were done by STATA version-13 (STATA Corporation, College Station, Texas). The prevalence of three bacterial isolates with different variables compared for statistical significance using chi-square ($\chi 2$) test. A p value of <0.05 was considered statistically significant.

Results

An overview of the samples collected and tested

The study was undertaken at SAQTVH, Chittagong Veterinary and Animal Sciences University, Chittagong. A total of 200 goats were sampled and investigated to determine the occurrences of common bacterial isolates and distribution of different factors during the study period.

Prevalence of *Staphylococcus* spp in goat

A total number of 14 samples were positive out of 100 collected buccal swab samples. Prevalence was found 14%. The samples were further characterized by coagulase test. 10 samples out of 14 were found positive to coagulase test.

Table 3.1 reflected the statistics of 100 buccal swab samples based on different variables. There was no significant difference among different variables such as breed, age and sex of animals. Female goat showed higher prevalence (17%) than male goat (11%). Prevalence was higher in adult (16%) than young (11%). 19% prevalence of *Staphylococcus* spp found in Jamuna Pari. Detection of *Staphylococcus* was analyzed with different factors like age, sex and breed by $\chi 2$ test but none of them statistically significant.

TABLE 1: Association of different variables with prevalence of *Staphylococcus* spp (Buccal swab) in goat

Variables	Category	N	Positive	Negative	Prevalence (%)	χ2 value	P value
Age	Young (≤1 year)	36	04	32	11	0.389	0.532
	Adult (>1 year)	64	10	54	16		
Sex	Male	54	06	48	11	0.814	0.367
	Female	46	08	38	17		
Breed	Black Bengal	27	03	24	11	0.088	0.957
	Jamuna Pari	32	06	26	19		
	Cross	41	05	36	12		

NB: N=Number, p= statistically significant (p<0.05)

Prevalence of *Escherichia coli* in goat

The prevalence of *E. coli* from faecal swabs was 20%. Adult (23%), female (24%) and cross breed (24%) showed highest prevalence than other variable. The occurrence of *E. coli* in fecal isolates based on different variables (age, sex and breed) was not significantly associated shown in Table 3.2.

TABLE 2: Association of different variables with prevalence of *E. coli* (faecal isolates) in goats

Variables	**Category**	**N**	**Positive**	**Negative**	**Prevalence (%)**	**χ2 value**	**P value**
Age	Young	36	05	31	14	1.313	0.252
	Adult	64	15	49	23		
Sex	Male	54	09	45	17	0.815	0.367
	Female	46	11	35	24		
Breed	Black Bangle	27	4	23	15	0.113	0.945
	Jamuna Pari	32	6	26	19		
	Cross	41	10	31	24		

NB: N=Number, p= statistically significant (p<0.05)

Prevalence of *Salmonella* spp in goat

Out of 100, 12 were isolated as *Salmonella* spp, prevalence found 12%. Among different variables, prevalence in adult (14%) was higher than young (8%) goats. Female revealed 15% prevalence and male 9% whereas Black bangle showed 15% prevalence. None of the variables were statistically significant (Table 3).

TABLE 3: Association of different variables with prevalence of *Salmonella* spp (faecal isolates) in goats

Variables	Category	N	Positive	Negative	Prevalence (%)	χ2 value	P value
Age	Young	36	03	33	08	1.160	0.281
	Adult	64	09	55	14		
Sex	Male	54	05	49	09	0.088	0.767
	Female	46	07	39	15		
Breed	Black Bangle	27	04	23	15	0.614	0.736
	Jamuna Pari	32	03	29	09		
	Cross	41	05	36	12		

NB: N=Number, p= statistically significant (p<0.05)

Identification of *Staphylococcus* spp, *E. coli and Salmonella* spp

Identification of *Staphylococcus* spp (Fig 1), *E. coli* (Fig 2) *and Salmonella* spp (Fig 3) were based on their staining, cultural, morphological and biochemical properties.

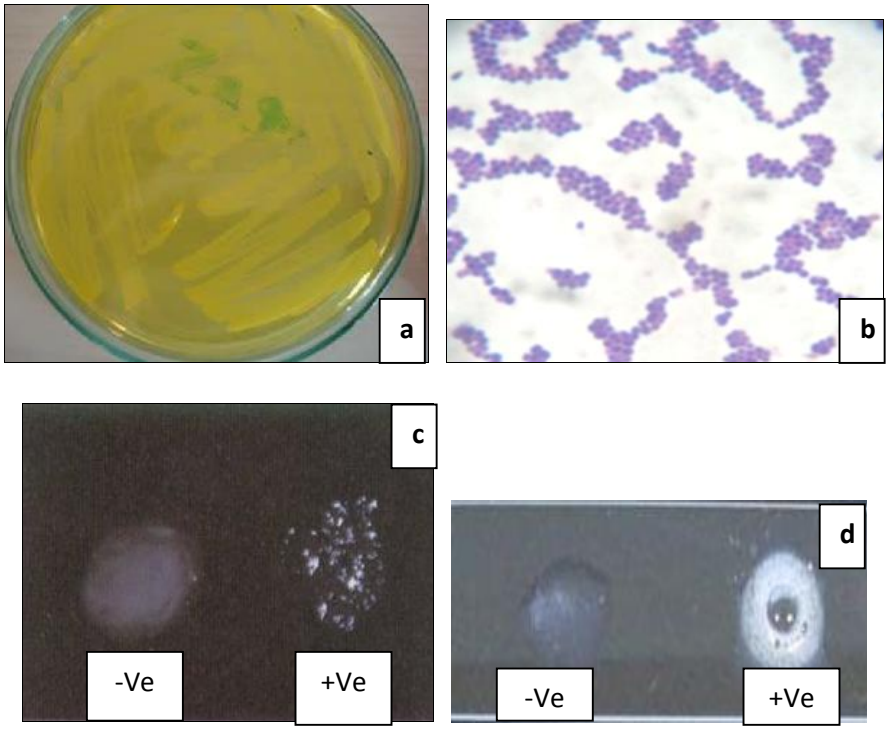
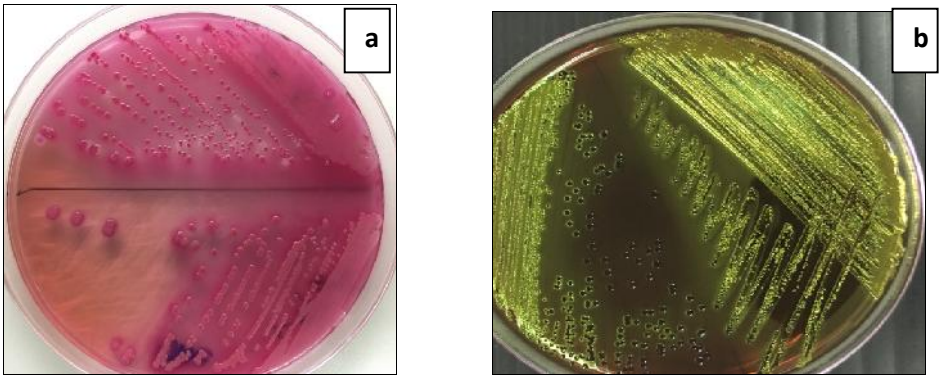


Figure 1: Biochemical test of *Staphylococcus* spp (a) The growth of *Staphylococcus* spp on Mannitol salt agar plates indicating bright yellow colonies (b) Grape like cluster under microscope (c) Slide coagulase test for *Staphylococcus* spp (d) Slide catalase tests for *Staphylococcus* spp.



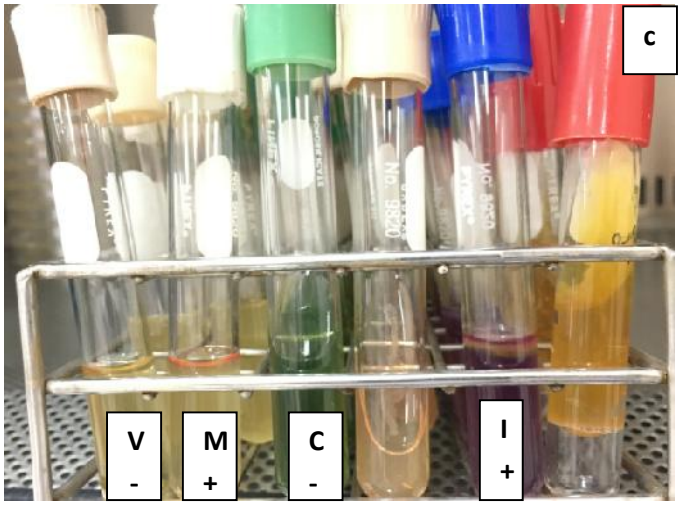


Figure 2: (a) Growth of *E. coli* on MacConkey agar (b) Growth of *E. coli* on EMB agar (c) IMViC test for confirmation of *E. coli*

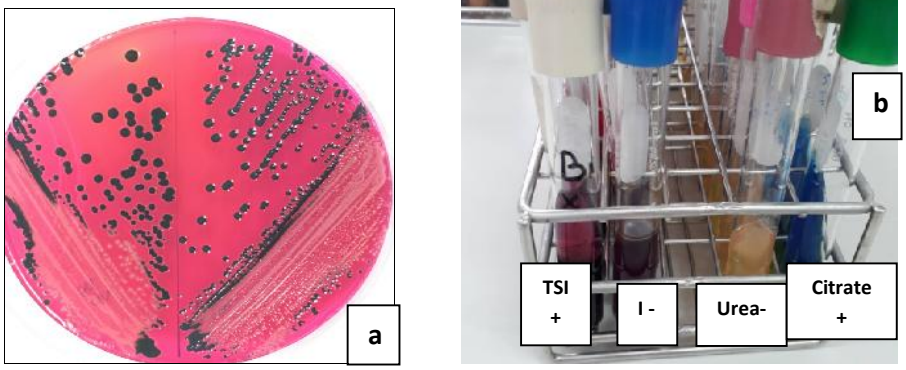


Figure 3: (a) *Salmonella* spp on XLD (b) Biochemical confirmation of *Salmonella* spp

Discussion

In this study we found that out of 100 buccal samples, 14% were positive to *Staphylococcus* spp, 20% of *E coli* and 12% of *Salmonella* spp from 100 rectal swab samples.

Staphylococcus spp is considered as one of the major cause of respiratory infection and frequently isolated from goats (Islam et al., 2006). Prevalence of *Staphylococcus* spp was 14% in our study. This finding is lower than that of the findings of Adamu et al., (2010) that depicted 30% prevalence in goat population. Emikpe et al., (2009) found 26% prevalence of *Staphylococcus* spp in West African dwarf goats. This type of variation in isolation of *Staphylococcus* spp might attributable to geographic variation of the region from where the samples were collected, mixed bacterial population in animals, variation of the techniques adopted by different laboratories for conducting the experiments.

The data obtained from the epidemiological study of the occurrence of *Staphylococcus* spp are presented in Table 3.1. The prevalence of *Staphylococcus* spp was found to be higher in female goats than male goats, but the difference was not statistically significant ($p>0.05$). However, because the small ruminants producers want to keep more females for breeding purposes, their probability to expose to bacterial invasion thus might higher compared with males which is in agreement with Abdulla et al., (2010). 0Jamuna Pari goats were showen to be slightly higher prevalence than other breeds, this might be due to breed differences of animals, disagreement with Loomba et al., (2010).

Apparently the prevalence does not differ significantly ($p>0.05$) between different age groups, but adult animals found to be comparatively higher than young goats might be due to malnutrition, poor immunity and poor management systems.

Fecal swabs were collected from one hundred goats to characterize *Escherichia coli* in the study population. Out of 100 swab samples, 20 (20%) isolates were found to be positive in all cultural tests. The prevalence was lower than the findings of Mugalu et al., (2006) who published 31.2% prevalence of *E. coli* in the clinical goats. The reason behind this variation might be due to sampling variation, climatic and geographical diversity of the animal examined. There was no significant association between different variables like age, sex and breed with the prevalence of *E. coli* in the goats.

Salmonella is an important human food-borne pathogen and is found in the intestinal tract of many animals. In the present study, the prevalence of *Salmonella* spp was 12% which varies with the findings of earlier works reporting 9.01% and 46.3% in goat fecal samples (Duffy et al., 2009; Turkyilmaz et al., 2013). Variation in the prevalence of *Salmonella* spp might be attributable to varying climate and husbandry practices at different places in different countries and to number and type of goats sampled in different studies.

There was no significant difference between prevalence of *Salmonella* spp and different variables like breed, age and sex of goats. However, the prevalence of *Salmonella* spp was significantly higher ($p<0.05$) in female goats than male which is in the agreement with Carattoli, (2003). Higher prevalence in female might be attributable to reduced body defense, impact of breeding, lactation which expose animals to multiple bacterial invasions. Apparently the prevalence does not differ significantly ($p<0.05$) between different age groups, but adult goats showed higher prevalence compared to young goats supported by Carattoli, (2003). This might be due to malnutrition, poor immunity and poor management systems.

Conclusion

The isolation of *Staphylococcus* spp, *E. coli* and *Salmonella* spp from goats is a silent threat to public health. Its presence instigates the steps required to control such a disease of zoonotic potential, which may lead to dire consequences if not addressed. Special attention should be paid to the identification of possible sources and measures are adapted for its prevention in the food animals, to ensure the supply of safe and healthy meat in the market for human consumption. The literacy rate of the people should be improved and short courses on improving hygiene and livestock management should be provided.

References

Abdullah, A.B.M., Khan, M.S.R., Alam, M., Haq, F. and Hassan, J. 2010. Isolation and Molecular Characterization of *Escherichia coli* from Goat of Apparently Healthy and Clinical Cases. Bangladesh Journal of Microbiology. 27: 14-17.

Adamu, J.Y., Raufu, A.I., Chimaroke, F.C. and Ameh, J.A. 2010. Antimicrobial susceptibility testing of *Staphylococcus aureus* isolated from apparently healthy humans and animals in Maiduguri, Nigeria. International Journal of Biomedical and Health Sciences. 6 (4): 191-195.

Asaduzzaman, M., Ahmed, K.S., Khan, M.F.R., Nazir, N.H. and Rahman, M.M. 2013. Isolation and identification of bacteria from upper respiratory tract of Black Bengal goat in Bangladesh and investigation of some epidemiological parameters related to pneumonia. Scientific Journal of Microbiology. 2(11): 207-213.

Balaban, N., and Rasooly, A. 2000. Staphylococcal enterotoxins. Int. J. Food. Microbiol. 61: 1-10.

Bhat, M.A., Nishikaw, Y. and Wani, S.A. 2008. Prevalence and virulence gene profiles of Shiga toxin-producing *Escherichia coli*and enteropathogenic *Escherichia coli*from diarrheic and healthy lambs in India. Small. Ruminant. Res. 75(1): 65-70.

Carattoli, A. 2003. Plasmid-mediated antimicrobial resistance in *Salmonella enterica*. Curr. Issues. Mol. Biol. 5: 113-122.

Dho, M. and Lafont, J.P. 1984. Adhesive properties and iron uptake ability of Escharichia coli lethal and nonlethal forchicks. Avian Dis. 28: 1016-1025.

Duffy, L., Barlow, R., Fegan, N. and Vanderlinde, P. 2009. Prevalence and serotypes of Salmonella associated with goats at two Australian abattoirs. J. Appl. Microbiol. 48: 193–197.

Edward, P.R. and Ewing, W.H. 1986. Identification of Enterobacteriaceae, Fourth Edition, Burgess Company, Minnesota.

Emikpe, B.O., Oyero, O.G. and Akpavie, S.O. 2009. Isolation and Antibiogram of Aerobic Nasal Bacterial Flora of Apparently Healthy West

African Dwarf Goats. Rev Elev Med Vet Pays Trop.. 62 (1): 17-21.

Islam, M.Z., Ahad, A. and Biswas, P.K. 2013. Antimicrobial resistance profile of sorbitol non–fermenting Shiga toxin producing *Escherichia coli* isolated from smallholdings cattle. J. Infec. Mol. Biol. 1 (4): 58 – 63.

Kateete, D.P., Kimani, N.C., Katabazi1, F.A., Okeng, A., Okee, M.S., Nanteza, A., Joloba, M.L. and Najjuka, F.C. 2010. Identification of *Staphylococcus aureus*: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. Clin. Microbiol. Antimicrob. 9: 23.

Loomba, P.S., Taneja, J. and Mishra, B. 2010. Methicillin and Vancomycin Resistant *S. aureus* in Hospitalized Patients. J. Glob. Infect. Dis. 2(3): 275–283.

Meshram, M., Ravikanth, K., Maini, S. and Rekhe, D.S. 2009. Treatment of Clinical Cases of Bacterial Enteritis in Goat with New Polyherbal Antidiarrhoeal formulation. Vet. World. 2(4):143-145.

Momin, M.A., Islam, M.A., Khatun, M.M., Rahman, M.M. and Islam, M.A. 2011. Characterization of bacteria associated with pneumonia in black bengal goats. Bangladesh Journal of Veterinary Medicine. 9(1): 67 – 71.

Monica, C. 1991. Medical Laboratory manual for Tropical countries. 11:60–63.

Mugalu, J.N.M., Kiguli, S. and Kaddu-Mulindwa, D.H. 2006. Aetiology, risk factors and immediate outcome of bacteriologically confirmed neonatal septicaemia in Mulago hospital, Uganda. Afr. Health. Sci. 6(2): 120-126.

OIE, 2000. Manual of Standards for Diagnostic Tests and Vaccines. Rev Sci Tech.

PAHO, 2001. Zoonoses and communicable diseases common to man and animals: Bacterioses and Mycoses. Scientific and technical publication no. 580, Washington, D.C. 20037 U.S.A., 1: 233-246.

Putturu, R., Thirtham, M. and Eevuri, T.R. 2013. Antimicrobial sensitivity and resistance of *Salmonella Enteritidis* isolated from natural samples. Vet. world. 6(4): 185-188.

Radostits, O.M., Gay, C.C., Hinchchif, K.W. and Constable, P.D. 2007. Veterinary medicine. In: A textbook of the Disease of cattle, sheep, pigs and goats. 10th ed. London: Ballier, Tindal. pp: 391-402.

Thaker, H.C., Brahmbhatt, M.N. and Nayak, J.B. 2013. Isolation and identification of *Staphylococcus aureus* from milk and milk products and their drug resistance patterns in Anand, Gujarat. Vet World. 6(1): 10-13.

Tsolis, R.M., Young, G.M., Solnick, J.V. and Baumler, A.J. 2008. From bench to bedside: stealth of enteroinvasive pathogens. Nat. Rev. Microbiol. 6: 883–892.

Turkyilmaz, S., Eskiizmirliler, S., Tunaligil, S., Bozdogan, B. 2013. Identifiation, characterization and molecular epidemiology of *Escherichia coli* isolated from lamb and goat kids with diarrhea. Act. Vet. Brno 82: 357–362.

Virpari, P.K., Nayak, J.B., Thaker, H.C. and Brahmbhatt, M.N. 2013. Isolation of pathogenic *Escherichia coli* from stool samples of diarrheal patients with history of raw milk consumption. Vet. World. 6(9): 659-663.

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