Multiplex PCR System for Rapid Identification of Bacterial Pathogens from Goats Presumed with Fever and/or Diarrhea

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June, 2018

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made.

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List of Abbreviations

Abbreviation	Elaboration
AMR	Antimicrobial Resistance
AST	Antimicrobial susceptibility test
C.	Campylobacter
C.	Clostridium
CI	Confidence interval
CLSI	Clinical and Laboratory Standards Institute
DLS	Department of livestock services
EDTA	Ethylene diamine tetra acetic acid
GDP	Gross domestic product
I	Intermediate
IUCN	International Union for Conservation of Nature
MRSA	Methicillin-resistant *Staphylococcus aureus*
mPCR	Multiplex Polymerase chain reaction
NHP	Non-human primate
OR	Odds ratio
PCR	Polymerase chain reaction
PPR	Peste des petits ruminants
R	Resistant
ROC	Receiver operating curve
S	Sensitive
S.	Staphylococcus
WHO	World Health Organization

Symbols

Symbols	Stands for
&	And
>	Greater than
<	Less than
˚C	Degree centigrade
≥	Greater than equal
≤	Less than equal
%	Percentage
µg	Microgram
$\chi 2$	Chi square
w/v	Weight/volume

Summary

Goat is one of the major livestock species, which play an important role in the economy of Bangladesh. However, outbreak of different infectious diseases in goats causes high mortality and economic losses due to lack of proper diagnosis and treatment. Conventional culture based methods for detecting pathogens are time consuming and laborious in compare to multiplex Polymerase Chain Reaction (mPCR), by which multiple pathogens can be detected at a time. The present study was aimed to perform faster molecular identification of multiple bacterial pathogens from whole blood of clinically infected goats. A total of 200 blood samples were collected from the goats that have fever and/or diarrhea at S. A. Quaderi Teaching Veterinary Hospital (SAQTVH) in Chittagong Veterinary and Animal Sciences University (CVASU) for the period of July-April 2017-2018. DNA was extracted and subsequently mPCR assay was performed for the screening of several bacterial pathogens (*Salmonella spp., Listeria monocytogenes, Bacillus cereus, Yersinia enterocolitica, Campylobacter jejuni, Campylobacter coli, Clostridium perfringens, Vibrio cholerae,* and *Staphylococcus aureus*). An antimicrobial susceptibility test (AST) against 10 antimicrobials for positive samples of each organism was conducted using the Kirby-Bauer Disc Diffusion Method on selective media. *Staphylococcus aureus*, *Clostridium perfringens, Listeria monocytogenes* and *Salmonella* spp. were detected from collected samples and their overall prevalence were 11.5%, 3.5%, 1% and 20.5%, respectively. Most common clinical signs were mild fever, nasal discharge, dyspnea and coughing (39.1%) for *Staphylococcus aureus*, diarrhea, convulsion, abdominal pain and incordination (57.1%) for *Clostridium perfringens*, fever, protrusion of tongue and incordination (100%) for *Listeria monocytogenes* and fever, anorexia, dehydration with mucus in stool (36.6%) for *Salmonella* spp infection in goats . Antimicrobial diagram represents *Staphylococcus aureus* showed resistance against Cefotaxime (74%), Cefixime (65%) and Tetracycline (65%) whereas highly sensitive against Amoxicillin (48%), Ciprofloxacilin (44%) and Gentamycin (44%). On the other hand Ampicillin (71%) and Gentamycin (71%) were highly resistant and Penicillin (57%) and Cefotaxime (57%) were highly sensitive against *Clostridium perfringens* infection. Penicillin (100%) and Cefixime (100%) were found sensitive against *Listeria*

monocytogenes infection in goats. *Salmonella* spp. showed resistance to Ampicillin (78%) and Amoxicillin (59%) but sensitive to Ciprofloxacin (54%). This study may explore frequent clinical signs and symptoms of some common infectious diseases in goats and also determine the potential risk factors, measuring strength of association. In conclusion, rapid diagnostic methods like multiplex PCR were effective for diagnosis of multiple pathogens.

Key Words: Goat, Infectious disease, Multiplex PCR, Prevalence, AMR

Chapter-1: Introduction

Livestock is one of the important sectors contributing the economy of Bangladesh. Total livestock population of Bangladesh is composed of 30.33 million goats, 23.44 million cattle, 14.54 million buffaloes and 3.17 million sheep (DLS, 2014). It is contributing about 1.54% of overall Gross Domestic Products (GDP), as well as 13.62% of agricultural GDP and 4.31% export earnings from leather and leather goods of total export (MOFL, 2018). Livestock sub-sector provides full time employment for 20% and part-time for 50% of the total population (Begum et al., 2011). This sector has huge potential for private sector investment in rural areas.

Small ruminants especially goat is very important in rural economy and nutrition and potentially using it as a tool for poverty reduction in Bangladesh. As far as known, goats were probably the first domesticated animals (Ershaduzzaman et al., 2007). There are about 300 breeds and varieties of goats domesticated in this subcontinent (Hirst, 2008). The majority of goat varieties are found in the tropics and sub-tropics. In Bangladesh, more than 90% of the total goat population are Black Bengal; remaining are Jamunapari and their crosses (Amin et al., 2001). Goat is ideally suited for poor people who have no ability to buy and rear large ruminants. As a result, goat husbandry is becoming an attractive activity among the poor women (Choudhury et al., 2013) under traditional scavenging system but still contributing to rural economy through income generation, women empowerment and hence rightly treated as an effective tool for the reduction of poverty. Goat is performing a variety of functions, displaying a unique ability to adapt and maintain themselves in harsh environment, dwarf-size animal produce a variety of products, mainly meat and skins, and contribute a major role in the national economy. Goat ranks second in position in terms of meat, milk, and skin production, representing about 28, 23, and 28 percent, respectively of total livestock in Bangladesh (Amin, 2000). Among the sources of animal protein, the meat of goat (chevon) is popular in Bangladesh irrespective to religion and caste (Islama et al., 2016).

However, different infectious diseases are the most important constraint to livestock development in Bangladesh. The impacts of diseases are multifaceted: loss to the farmers

through mortality, reduction of productivity, cost for disease management including treatment and sanitation, low quality of livestock products, disruption in the production cycle, market effects, culling, etc. These constrants seriously affect the livelihood of the poor farmers. Moreover, the climatic condition of Bangladesh is conducive to animal diseases. High density of animals and their seasonal aggregation particularly in the monsoon period aggravate the hazards. In goats, viral diseases like PPR, goat pox, contagious ecthyma and viral pneumonia, and bacterial diseases such as enterotoxaemia, tetanus, brucellosis, mastitis and metritis, mycotic diseases like ring worm infection, and rickettial infections like conjunctivitis are common causes for goat mortality in rural areas. Gastro-intestinal nematodiasis, fascioliasis and tape worm causes less mortality but cause severe depression in the growth and reproduction rate (Kashem et al., 2012). But the total disease complex is not clear, owing to the general lack of diagnostic and disease recording services in DLS.

SAQTVH of CVASU is the only seat of clinical learning of veterinary practices under close supervision of the concerned teaching staff members of the clinical departments. It is a busy clinic providing a veterinary service for the farm and companion animals, horses, poultry etc. While there are dedicated units like medicine, surgery, theriogenology, orthopaedics etc. are playing an important role in public service as well as animal welfare since 1996 (Parvez et al., 2014). Although diversified species and breeds of animals are coming to get treatment but goats are dominating among them. Among diseases, the highest prevalence was recorded as PPR (11.33%) and lowest was Babesiosis (0.40%). Occurrence of upper respiratory tract infection, pneumonia & diarrhea was recorded 8.74%, 5.61% and 5.36%, respectively in Chittagong (Nath et al., 2014).

For the diagnosis of diseases we are mainly depending on physical examinations and many others conventional methods of diagnosis. The conventional methods for detecting the bacterial pathogens present in infections are based on culturing the microorganisms on agar plates followed by standard biochemical identifications (Järvinen et al., 2009). These methods are usually inexpensive and simple but time consuming as they depend on the ability of the microorganisms to grow in different culture media such as pre-enrichment media, selective enrichment media and selective plating media (Ferroni et al.,

2010). It requires 2 to 3 days for preliminary identification and more than a week for confirmation of the species of the pathogens (Zhao et al., 2014). Moreover, these methods are laborious as they require too many procedures to be accomplished. Furthermore, false negative results may found due to viable but non-culturable (VBNC) pathogens (Lee et al., 2014). The failure of pathogen detection would increase the transmission risk of pathogens and treatment failure. Therefore, different rapid methods with high sensitivity and specificity have been developed to improve detection and identification of the pathogens. Rapid methods are also more time-efficient, labor-saving and able to reduce human errors (Mandal et al., 2011) . Researchers are still developing novel methods with improvements in terms of rapidity, sensitivity, specificity and suitability for *in situ* analysis and distinction of the viable cell (Law et al., 2015). One of the most commonly used molecular-based method for the detection of bacterial pathogens is multiplex polymerase chain reaction (mPCR). It offers a more rapid detection as compared to simple PCR through the simultaneous amplification of multiple gene targets. Although the basic principle of mPCR is similar to conventional PCR (Lee et al., 2014). However, these molecular methods based on nucleic acid amplification and hybridization aims to fasten the diagnostic procedures. In such methods, the pathogen is simultaneously detected and identified, which results in more rapid diagnoses than those obtained by conventional culturing methods and obviates the need for additional culture tests. Rapid diagnostics can also reduce the use of antimicrobial agents in addition to allowing a faster switch to the most optimum treatment, thus reducing both side-effects and costs (Barenfanger et al., 1999; Kerremans et al., 2007). However, in Bangladesh use of these rapid diagnostics kit were not established to diagnose the nonspecific diseases in livestock. Moreover, the practitioners are not aware enough of using specific antimicrobial to combat such kind of infectious diseases in field level. Therefore, the current study was aimed to confirm the specific causal agents causing nonspecific clinical signs and to assess the effectiveness of those rapid diagnostic methods to detect them. In addition, determine the status of antimicrobial using against those infectious diseases in goat.

The specific objectives of the present study were as follows:

- Confirmation of specific pathogens from goats showing non-specific clinical signs.
- To correlate the clinical signs and symptoms with specific causes which is not confirmed yet in goats at Teaching Veterinary Hospital (TVH) of Chittagong Veterinary and animal Sciences University (CVASU).
- To confirm the prevalence of those diseases and analyze their possible risk factors.
- To see the status of antimicrobial agents commonly using in SAQTVH against those diseases.

Chapter-2: Review of literature

The goat suffers with various diseases, which are caused by bacteria, viruses, parasites and other non-infectious agents. The diagnosis of the goat diseases only based on the clinical symptoms is most difficult, as many diseases resemble one another. The important clinical symptoms of common diseases have been given, only to help the farmers to detect the sick goat at the earliest stage. Treatment is not complete and many drugs may cause toxicity, in cases of the serious disease problem of the goat. The farmers may take some steps, as recommended, to prevent further deterioration in the condition of the animal, until it is brought under the supervision of a goat health specialist. It is observed that the seriousness can be prevented or minimized if timely preventive health care has been adopted in goat farming.

2.1. Salmonellosis in goats

Salmonella is an important human food-borne pathogen and is found in the intestinal tract of many animals. Although *Salmonella* can cause disease in animals, they are generally asymptomatic (Al-Habsi et al., 2018; Duffy, Barlow et al., 2009; Markey, Leonard et al., 2013). *Salmonella* is a genus of rod-shaped (bacillus) gram-negative bacteria of the family Enterobacteriaceae. *Salmonella* species are non-spore-forming, predominantly motile enterobacteria with cell diameters between about 0.7 and 1.5 µm, lengths from 2 to 5 µm, and peritrichous flagella (all around the cell body). They are chemotrops, obtaining their energy from oxidation and reduction reactions using organic sources. They are also facultative anaerobes, capable of generating ATP with oxygen ("aerobically") when it is available; or when oxygen is not available, using other electron acceptors or fermentation ("anaerobically") (Markey et al., 2013). *S. enterica* subspecies are found worldwide in all warm-blooded animals and in the environment. However, *Salmonella (S.) enterica* have been associated with diarrhoea, weight loss, lethargy and inappetance in goats, with sustained periods of stress identified as a risk factor for manifestation of disease in both goats and sheep (Bulgin & Anderson, 1981; Richards et al., 1989; Sharma et al., 2001). Outbreaks of acute diarrhea due to *S. enterica* (*S. Adelaide, S. Typhimurium, S. Muenchen and S. Singapore*) with 38% mortality rate have been reported in Australian rangeland goats (McOrist & Miller,

1981), but the epidemiology of *Salmonella* infections and specific risk factors for faecal carriage in goats are not well described. Isolation of *Salmonella* by growth culture medium followed by serotyping is considered for confirmation of *Salmonella*, but it is time consuming and labor intensive. Therefore techniques like PCR are increasingly being used for rapid detection and confirmation of *Salmonella* and considered as a very important diagnostic tool for detection of *Salmonella* invA targeting gene (Li et al., 2014). Most widely used DNA based technique is PCR, utilizing genus specific primers targeting various genes. For instance, invA gene (marker gene) has been introduced for the effective, rapid and accurate detection of *Salmonella* in foods of animal origin (Rahn et al., 1992)

2.2. Listeriosis in goats

Listeriosisis a life-threatening disease caused by the *Listeria monocytogenes* bacteria. *Listeria monocytogenes* is a facultative intracellular microorganism. It is a gram-positive, extremely antibiotic-resistant coccoid to bacillus shaped bacteria found in the environment (Low & Donachie, 1997). Spoiled forages and feed contaminated by *L. monocytogenes* are sources of contamination for goats. Listeriosis can infect animals and humans alike. The disease occurs worldwide and is widely distributed among different livestock species. *L. monocytogenesis* commonly found in the feces of infected birds, wild mammals, fish, crustaceans, insects, and in sewage (Johnson et al., 1996). *L. monocytogenes* can contaminate water, milk, cheese, fetal feces (meconium), adult feces, and soil. The infection enters through small wounds in the lips and oral and nasal mucosae and via the conjunctiva, although only immunosuppressed animals become ill. Asahi and others (1957) were the first to demonstrate experimentally that the infection can ascend from wounds in the lips and oral cavity along the trigeminal nerve. The experimental infection of dental pulp with listeria revealed that wounds caused by erupting or lost teeth provide important entry points for listeria organisms. The meningoencephalitic form of listeriosis has been known in sheep and goats for many years (Braun et al., 2002; Gerros, 1998)), and the two species show the same clinical signs. The clinical signs of listeriosis are due to the unilateral ascent of the infection along the trigeminal nerve. The main signs include central nervous system (CNS) disease with vestibular ataxia and unilateral cranial nerve deficits (Braun et al., 2002).

The diagnosis of listeriosis, based on the isolation of the bacteria can be difficult because of previous antibiotic treatment and a low number of bacteria (Le Monnier et al., 2011). Conventional assays used to identify *Listeria* species are time consuming (4–5 day processing) and labor intensive, depending on enrichment, selective media, agar isolation, and serological reactions (Amaglian et al., 2007; Bauwens et al., 2003; Liu, 2006). The *L. monocytogenes* hemolysin Listeriolysin O (LLO), encoded by hly, and Positive Regulatory Factor A (PrfA), a master transcriptional activator for hly and numerous other virulence genes, are key virulence determinants of *L. monocytogenes*, extensively characterized for their roles in host-pathogen interactions (Postollec et al., 2011). *PrfA*, a key transcriptional regulator, induces expression of virulence factors related to *L. monocytogenes* pathogenesis in infected host cells. When *L. monocytogenes* enters into host cells cytosol, *PrfA* is post-translationally activated (Xayarath et al., 2011). *PrfA* has been selected as for PCR assay targeting *L. monocytogenes*. its relative accuracy, specificity, and sensitivity were 96, 100, and 76.9%, respectively (Chen et al, 2017).

2.3. Bacillosis in goats

Bacillus cereus behaves as an opportunistic pathogen frequently causing gastrointestinal diseases, and it is increasingly recognized to be responsible for severe local or systemic infections (Drobniewski, 1993). It is a Gram-positive, spore-bearing rod that is widely distributed in the environment, namely soil, where spores persist under adverse conditions and can grow when readily decomposable matter is available. This motile bacterium is an aerobe or facultative anaerobe with large vegetative cells, typically organized in chains, ranging from 3–5 μm in length and 1 μm in width. *B. cereus* can grow over a wide temperature range (8–55 °C), but it is not well suited to tolerate low pH values (minimum 5–6) or water content (minimum water activity 0.95). Despite the fact that *B. cereus* can complete a full saprophytic life cycle, this bacterium may also behave as an opportunistic pathogen (Callegan et al., 1999; Robinson-Dunn, 2002). *B. cereus* is a type of bacteria that produces toxins.These toxins consists emetic toxin (ETE) and different enterotoxins: HBL, Nhe, and EntK etc (Senesi et al., 2010). The numbers of enterotoxins and their properties have also been debated for a long time. All these enterotoxins are cytotoxic and cell membrane active toxins that will make holes or channels in membranes (Granum & Lund, 1997; Kramer et al., 1989). *Bacillus cereus* has

a ubiquitous distribution in the environment. Although mastitis in animals is mostly due to *Staphylococci*, *Streptococci*, and *Escherichia coli*, *Bacillus cereus* may behave as an agent of mammary gland infection in cows and goats. Also, *Bacillus* species outside the *B. cereus* group seem to be potential pathogens for the mammalian udder, although many cases are merely reported as related to *Bacillus* spp. unfortunately (Savini, 2016).). One report from Nigeria cited that 7.5% of mastitis cases in goats were due to *B cereus* (Ajuwape et al., 2005). Clinical signs of *B cereus* mastitis in goat included acute onset of fever, anorexia, and lethargy, with considerably swollen affected quarters (Mavangira et al., 2013). Isolation of *B cereus* in goats with subclinical mastitis has also been reported (Kalogridou-Vassiliadou, 1991). Clinical cases of gangrenous mastitis also occur due to this bacterium but are considered rare (Gonzalez, 1996; Howell, 1972). The relatively low occurrence of *B cereus* mastitis in the face of a fairly wide host and environmental distribution of *Bacillus* spp has suggested that specific predisposing factors may be required for the development of clinical mastitis due to *B. cereus* (Watts, 1988).

2.4. Yersiniosis in goats

The genus *Yersinia* includes 11 species: *Y. pestis, Y. pseudotuberculosis, Y. enterocolitica, Y. frederiksenii, Y. intermedia, Y. kristensenii, Y. bercovieri, Y. mollaretii, Y. rohdei, Y. aldovae*, and *Y. ruckeri*. Among them, only *Y. pestis, Y. pseudotuberculosis*, and certain strains of *Y. enterocolitica* are of pathogenic importance for humans and certain warm-blooded animals, whereas the other species are of environmental origin and may, at best, act as opportunists (Fàbrega & Vila, 2012). However, *Yersinia* strains can be isolated from clinical materials, so have to be identified at the species level. *Yersinia enterocolitica* is a Gram-negative bacillus-shaped bacterium, belonging to the family Enterobacteriaceae. It is motile at temperatures of 22–29°C, but becomes nonmotile at normal human body temperature (Kapatral et al., 1996). *Yersinia enterocolitica* has been recognised as a cause of terminal ileitis and mesenteric adenitis in human beings since the 1930's and a number of domestic animals, including dogs, cats, cattle, sheep and pigs, have been suggested as possible sources for these infections (Slee & Button, 1990). *Y. enterocolitica* is widespread in nature, occurring in reservoirs ranging from the intestinal tracts of numerous mammals, avian species, cold-blooded species, and even from terrestrial and aquatic niches. Most environmental isolates are

avirulent; however, isolates recovered from porcine sources contain human pathogenic serogroups. In addition, dogs, sheep, wild rodents, and environmental water may also be a reservoir of pathogenic *Y. enterocolitica* strains (Fàbrega et al., 2012). *Y. enterocolitica* is a heterogeneous group of strains, which are traditionally classified by biotyping into six biogroups on the basis of phenotypic characteristics, and by serotyping into more than 57 O serogroups, on the basis of their O (lipopolysaccharide or LPS) surface antigen. Five of the six biogroups (1B and 2–5) are regarded as pathogens. However, only a few of these serogroups have been associated with disease in either humans or animals. Strains that belong to serogroups O:3 (biogroup 4), O:5,27 (biogroups 2 and 3), O:8 (biogroup 1B), and O:9 (biogroup 2) are most frequently isolated worldwide from human samples. However, the most important *Y. enterocolitica* serogroup in many European countries is serogroup O:3 followed by O:9, whereas the serogroup O:8 is mainly detected in the United States (Slee & Button, 1990).
A number of bioserotypes of *Yersinia enterocolitica* are known enteropathogens of human beings and animals (Swaminathan et al., 1982). Little is known about the relevance of *Y. enterocolitica* in goats. The first outbreak of *Y. enterocolitica* in a goat herd was reported from Norway (Krogstad et al., 1972). Some of the goats died while others developed only mild diarrhoea for a short time. Interestingly, some of the persons who had been in daily contact with the diseased goats had diarrhoea and abdominal pain. Antibodies against *Y. enterocolitica* type 2 were found in those patients (Winblad, 1967). However, attempts to provoke clinical disease in goats, experimentally infected with 109 cells of the strain isolated, failed (Krogstad et al., 1972). Slee and Button (1990) reported a number of *Y. enterocolitica* biotype 5 serotype O: 2, 3 infections in goats. Infected goats were usually young and showed diarrhoea, were in poor condition or emaciated. A direct transmission from goats to man has been found by (Krogstad et al., 1972). (Nikolaou et al., 2005) in 66% of 681 sera from goats in the German state of Lower Saxony anti-Yop/V-Ag antibodies. Serological and biochemical classification, however, are time consuming and are not generally available in routine laboratories. Alternative phenotypical tests, such as calcium-dependent growth at 37°C, Congo red binding (Prpic et al., 1983), pyrazinamidase testing (Kandolo & Wauters, 1985), autoagglutination testing, and serum resistance testing (Aulisio et al., 1983; Bhaduri et al., 1990; Farmer et

al., 1992) all have limited predictive value for the pathogenicity of *Y. enterocolitica* and *Y. pseudotuberculosis*. The tests are frequently ambiguous to read, and their outcome may be unreliable, since they depend on the presence and expression of (plasmid-borne) virulence genes and the virulence plasmid pYV can easily be lost depending on the culture conditions (Bhaduri et al., 1990). Therefore, differentiation of pathogenic strains should not rely solely on the expression or detection of the virulence plasmid but also on the detection of chromosomal virulence factors. Strains of the pathogenic biotypes 1B, 2, 3, and 4 were significantly more-frequently isolated from humans and animals than from food and environmental sources in comparison to the biotype 1A strains ($P < 0.001$), indicative of the less-pathogenic nature of biotype 1A. Of 53 animal isolates of *Y. enterocolitica,* 46 strains (87%) were isolated from pigs, which mainly belonged to the pathogenic biotypes. The other animal isolates were a biotype 2 from a goat, a biotype 4 from a dog, and 5 biotype 1A's from 3 birds, 1 cow, and 1 dog. All field strains were further serotyped for O: 3 and O: 9, which were shown to be the most important pathogenic serotypes in Switzerland (Thoerner et al., 2003).

2.5. Campylobacteriosis in goats

Campylobacter spp. are spiral, microaerobic, gram-negative bacteria that cause gastroenteritis in people and animals. Several *Campylobacter* spp. are zoonotic. Many domestic animals develop acute gastroenteritis after ingestion of *Campylobacter* spp. including dogs, cats, calves, sheep, pigs, ferrets, mink, monkeys, and several species of laboratory animals. *Campylobacter* spp. is spiral or curved rods that exhibit a characteristic corkscrew darting motility, mediated by a single polar flagellum. These are slow growing, with a generation time of ~90 min, fastidious, and require enriched medium and microaerobic conditions with increased CO_2 (3%–15% O_2, 3%–10% CO_2, 85% N_2) for growth (Dworkin, 2006). The family Campylobacteraceae consists of three genera, including *Campylobacter* and *Arcobacter* associated with animal and human diseases. Certain species are present commensally in animals as suspected reservoirs for human infections. The thermophilic *Campylobacter* spp., *C. jejuni*, or *C. coli* have the highest prevalence and disease impact (Lastovica & Atlas, 2008). *Campylobacter* species causing diseases in livestock include *C. jejuni* subsp *jejuni* (enteritis and abortion), *C. coli*, *C. mucosalis* (porcine enteritis), *C. upsaliensis*, *C. helveticus* (companion pet

enteritis), *C. hyointestinalis* subsp *hyointestinalis* (porcine and bovine enteritis), *C. sputorum* (abortions in sheep), and *C. fetus* subsp *fetus* (isolated from intestinal tracts of sheep and cattle, sporadic abortions). Certain species such as *C. jejuni*, *C. hyointestinalis*, and *C. fetus* possess closely related subspecies with different disease foci. Initially, *Arcobacter* spp were considered to be aerotolerant campylobacters and are implicated in reproductive disorders, mastitis, gastric ulcers, and/or diarrhea in livestock, including *A. cryaerophilus* (previously *C cryaerophila*), *A. skirrowii*, *A. thereius*, and *A. butzleri* (Lastovica, 2006). *Campylobacter jejuni* have been reported among healthy and diseased farm animals (Jiwa et al., 1994; Olubunmi & Ademiran, 1986; Raji et al., 2000). The majority (90%) of campylobacteriosis cases are caused by *Campylobacter jejuni* and *C. coli* (Dworkin, 2006). Lastovica & Allos, 2008 reported that Seventy-four (30%) samples were positive for Campylobacter. Four species were detected: *C. jejuni* (20%), *C. coli* (7%), *C. lari* (2%) and *C. hyointestinalis* (2%). The frequently isolated *Campylobacter* species from goats was *C. jejuni* with isolation rate of 62.1%. *Campylobacter* spp. was found in 34.6% of the examined samples. *C. jejuni* was isolated in 10.1% and *C. coli* in 26.7% of samples.

2.6. Vibriosis in goats

Vibrio cholerae is the causative agent of the diarrheal disease cholera in several species. It is a "comma" shaped Gram-negative bacteria with a single, polar flagellum for movement. There are numerous strains of *V. cholerae,* some of which are pathogenic and some of which are not. *V. cholerae* is a facultative aerobe and has a flagellum at one cell pole as well as pili (Heidelberg et al., 2000). *V. cholerae* can undergo respiratory and fermentative metabolism. When ingested, *V. cholerae* can cause diarrhoea and vomiting in a host within several hours to 2–3 days of ingestion. *V. cholerae* was first isolated as the cause of cholera by Italian anatomist Filippo Pacini in 1854, but his discovery was not widely known until Robert Koch, working independently 30 years later, publicized the knowledge and the means of fighting the disease (Pacini, 1854). *V. cholerae* enters to the body through ingestion of contaminated food or water. The bacterium enters the instestine, imbeds itself in the villi of absorptive intestinal cells, and releases cholera toxin. Cholera toxin (CT) is an enterotoxin made up of five B-subunits that form a pore to fits one A-subunit (Zhang et al., 1995). CT is made from filamentous phage gene, CTXφ.

A phage gene is also responsible for another virulence factor of *V. cholerae*, which is toxin co-regulated pilus (TCP), which acts as a receptor for CTXφ (Davis & Waldor, 2003). There are at least 72 other serogroups of *V. cholerae* that are called non-0:1 (Visser et al., 1991). Formerly these strains were called non-cholera vibrios (NCV) or non-agglutinable vibrios (NAG). Although the majority of the non-0:1 strains of *V. cholerae* isolated from the environment does not appear to be enteropathogenic (Baumann, 1984), many strains of *V cholerae* non-0:1 produce a toxin identical or very similar to cholera toxin (Arita et al., 1986; Honda et al., 1989). Other strains, which do not produce cholera toxin, are enteropathogenic to animals experimentally (Bisgaard et al., 1978). These strains do not cause cholera epidemics, but do cause individual cases and outbreaks of cholera-like diarrhoea in humans. These had been only rarely reported from places all over the world since 1960 but have been more frequently reported since 1972 (Kaper et al., 1979). An additional virulence factor such as intestinal adhesion of the bacteria is also important in producing disease. *V. cholerae* has been isolated from surface water (Embrey et al., 2004; Fraga et al., 2007) and the occurrence of *V. cholerae* in water sources can be linked to faecal pollution (Cox et al., 2005). Domestic and farm animals have been shown to be carriers of *V. cholerae* strains, contributing to their sustained presence in the area (Sanyal et al., 1974). Among the 193 currently recognised O serogroups of *V. cholerae*, only O1 and O139 have caused epidemics of cholera (Fraga et al., 2007). More than 95% of the strains belonging to serogroups O1 and O139 produce the CT, which is central to the disease process (Chakraborty et al., 2000).

Cholera, caused by *Vibrio cholerae*, is a severe epidemic diarrhoeal disease which continues to devastate many developing countries where socioeconomic conditions are poor, sanitary systems and public hygiene are rudimentary, and safe drinking water is not available (Chen et al., 2007). It is now believed that an aquatic environment is the natural reservoir for *V. cholerae* (Janda et al., 1988). Although it has been reported that farm animals may also carry the organisms, references are very scarce (Shewan, 1974). After an outbreak of cholera, isolated *V. cholerae* from the faeces of cattle, goats, dogs and chickens in Varanasi, India, where cholera has not been endemic (Sanyal et al., 1974). Some of the strains that were isolated from cattle and chickens were of the 0:1 type.

Although cows appeared to be infected for 8 months at most, *V. cholerae* was isolated only occasionally from goats. Clinical signs of disease were not observed in any of these animals. Under experimental conditions, however, toxigenic strains of both 0:1 and non-0:1 type are pathogenic for a variety of laboratory animals (Sanyal et al., 1974).

2.7. Clostridiosis in goats

The Gram-positive, anaerobic, spore-forming bacterium *Clostridium perfringens* is distributed ubiquitously throughout the environment, with a presence in soils, foods, sewage, feces, and the intestines of many healthy humans and animals (Doyle, 1989; McClane et al., 2006). This bacterium also ranks among the most common and important pathogens of humans and livestock (McClane et al., 2006; Rood, 2006). *C. perfringens* causes histotoxic infections, including gas gangrene (Clostridial myonecrosis), anaerobic cellulitis, and simple wound infections (Rood, 2006; Titball & Rood, 2002). It is also responsible for several human and animal diseases originating in the intestines; these illnesses typically manifest as enteritis or enterotoxemia, a condition where toxins produced in the intestines are absorbed into the circulation and then damage other internal organs such as the brain, lungs, or kidneys (McClane et al., 2006; Songer, 1996).

The *C. perfringens* species is a very heterogeneous group of organisms regarding their metabolic byproducts, toxins and pathogenic potential. For practical classification purposes, the species is divided into five types, from A to E, based on their ability to produce any of the four major lethal toxins (alpha, beta, epsilon and iota) (Niilo, 1980). Type A is the most frequently occurring *Clostridium* in mammals, birds and in the environment; it produces enteric disease generally mild, with minimal damage noted in the intestinal mucosa and, in addition to enteritis, it produces gas gangrene; in the Western United. States, it causes hemorrhagic abomasitis in young ruminants, often accompanied by severe diarrhea; in the Pacific Northwest of USA, a condition called yellow lamb is associated with *C. perfringens* type A (Zachary et al., 2013). Type D is perhaps the best known pathogenic *C. perfringens* type, being widely regarded as the causative organism of fatal enterotoxemia of sheep or “overeating disease”. It appears to have a worldwide distribution but is not a common intestinal commensal. It produces epsilon-toxin, an angiotoxin that damages endothelial cells, which is almost exclusively

responsible for the host pathology and subsequent death. The toxin is produced in the gut by abundantly growing bacterial cells and is triggered by some feeding factors and absorbed into the systemic circulation. The epsilon-toxin is resistant to digestive enzymes; in fact, these enzymes convert the freshly secreted less active prototoxin into the fully toxic form. Clinically, when large amounts of epsilon-toxin are produced in the gut, its absorption into the systemic circulation increases capillary permeability in many organs and tissues, including intestinal mucosa. This increases its absorption rate and consequently the systemic effects leading to extensive renal damage, hyperglycemia, hypertension and edema in various organs, including the brain (Niilo, 1980). Lesions of *C. perfringens* type D infection consist of multisystemic hemorrhages, particularly of serosal surfaces; pericardial effusion is present along with mild gastroenteritis (Zachary et al., 2013). Pulpy kidney, another common name for type D enterotoxemia, is derived from one of the hallmark lesions in affected sheep, a result of postmortem autolysis, which occurs rapidly in hyperemic, toxin-damaged tissue (Songer, 1996; Zachary et al., 2013).

Table 2.1: *Clostridium perfringens* toxinotypes

	Toxin produced			
Type	*Alpha*	*Beta*	*Epsilon*	*Iota*
A	+	–	–	–
B	+	+	+	–
C	+	+	–	–
D	+	–	+	–
E	+	–	–	+

The classification of *C. perfringens* isolates into toxigenic types has been traditionally performed by sero-neutralization with mice or guinea-pigs (McDonel, 1986; Sterne et al., 1975). However, these methods are time consuming, expensive and raise an ethical concern due to the use of experimental animals. Therefore, during the last decade these methods have been largely replaced by PCR-based detection. Different toxin types of *C.*

perfringens strains have also been screened with commercial enzyme-linked immunosorbent assay kits. Moreover, a DNA microarray method has been recently published for detecting the presence of toxin genes from *C. perfringens* isolates (Al-Khaldi et al., 2004). Heikinheimo et al., also chose PCR protocol published by Meer and Songer (1997) in order to determine the presence of major toxin genes (cpa, cpb, etx and iA) and Cpe gene from *C. perfringens* isolates.

2.8. Staphylococcosis in goat

Staphylococcus aureus is a gram-positive round-shaped bacterium that is a member of the firmicutes, and it is a member of the normal flora of the body, frequently found in the nose, respiratory tract, and on the skin of both humans and animals. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen (Masalha et al., 2001). Although *S. aureus* is not always pathogenic (and can commonly be found existing as a commensal), it is a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins, and the expression of a cell-surface protein that binds and inactivates antibodies (Klimešová et al., 2017). *Staphylococcus aureus* is involved in a wide variety of diseases in humans and animals and its pathogenicity is mainly related to a combination of toxin-mediated virulence, invasive capacity, and antibiotic resistance. In 2009, the European Food Safety Authority underlined the increasing concern for Public Health represented by the presence of methicillin-resistant *S. aureus* (MRSA) in food producing animals, and recommended that further work should be performed on sampling, detection and quantification of MRSA carriage in both humans and animals, as well as on the contamination of food and the environment (Vanderhaeghen et al., 2010). *S. aureus* is also considered a major food borne pathogen (Hennekinne et al., 2010). Some strains are able to produce enterotoxins within foodstuff, causing staphylococcal food-poisoning (SFP), (Argudín et al., 2010). Recently published work has shown that 3 percent of all animals are infected with *S. aureus* (Schukken et al., 2009). However, *S. aureus* represents 10 to 12 percent of all clinical mastitis infections (Tenhagen et al., 2009). *S. aureus* bacteria produce toxins that

destroy cell membranes and can directly damage milk-producing tissue. White blood cells (leukocytes) are attracted to the area of inflammation, where they attempt to fight the infection. Initially, the bacteria damage the tissues lining the teats and gland cisterns within the quarter, which eventually leads to formation of scar tissue (Trinidad et al., 1990). The bacteria then move up into the duct system and establish deep-seated pockets of infection in the milk secreting cells (alveoli). This is followed by the formation of abscesses that wall-off the bacteria to prevent spread but allow the bacteria to avoid detection by the immune system. During infection, destruction of alveolar and ductal cells reduces milk yield (Petersson-Wolfe et al., 2010). These damaged cells may combine with leukocytes and clog the milk ducts that drain the alveolar areas, contributing to further scar tissue formation, occlusion of ducts, and decreased milk production. *Staphylococcus aureus* was involved in 37% of subclinical cases of mastitis in dairy goats. Though most cases of *S. aureus* mastitis are subclinical causes but it also causes one of the most common types of chronic mastitis (Roberson, et al., 1994). Usually, the etiological diagnosis is dependent on isolation of the bacterium from the focus of infection or in blood cultures. In some cases, access to the focus may be difficult or dangerous or cultural confirmation may be hampered by ongoing antimicrobial therapy. Also, serological assays for *S. aureus* infections are of limited value because of insufficient diagnostic sensitivity and specificity (Verbrugh et al., 1983), although promising results have been reported recently (Brakstad et al., 1989; Julander, et al., 1983; Verbrugh et al., 1986). Consequently, it would be desirable to find methods which could supplement the cultural and serological methods, notably, alternative methods which could secure a rapid diagnosis of *S. aureus* infection. This purpose may be achieved by techniques which enable detection of *S. aureus* nucleic acids in clinical specimens. Nucleic acid amplification by PCR has applications in many fields of biology and medicine, including the detection of viruses, bacteria, and other infectious agents. *S. aureus* strains produce an extracellular thermostable nuclease (thermonuclease [TNase]) with a frequency similar to that at which they produce coagulase (Madison et al., 1983). The TNase is a protein with a molecular mass of 17,000 Da. It is an endonuclease, degrading both DNA and RNA, and the enzymatic activity can resist 100^0C for at least 1 h (Lachica et al., 1972). The TNase protein has been well characterized , and its gene, the

nuc gene, has been cloned and sequenced (Kovacevic et al., 1985). An enzymatic test for TNase production is used in many laboratories for the identification of *S. aureus* isolates (R. Lachica et al., 1971). However, TNase activity is not specific for *S. aureus* (Gudding, 1983). (Brakstad et al., 1992) recognized sequences of the *S. aureus* nuc gene, which encodes the TNase produced by these bacteria.

Chapter-3: Materials and methods

3.1. Study design and sampling

The study was conducted at S. A. Quaderi Teaching Veterinary Hospital (SAQTVH) in Chittagong Veterinary and Animal Sciences University (CVASU) during the period of July 2017-April 2018. Two hundred (n=200) blood samples were collected from the goats having fever and/or diarrhea. Samples were collected from jugular vein. Collected samples were placed in a vacutainer (5ml) containing ethylenediaminetetraacetic acid (*EDTA*), and transported with 4^0C cool box to the Poultry Research and Training Centre (PRTC), CVASU for further analysis. All the molecular investigations of this study were conducted in PRTC, CVASU.

3.2. Questionnaire used for sample collection

A pre-designed questionnaire was used to collect relevant information such as species, age, and sex, diarrheic or not, antibiotic used or not etc. from sampled goat. Attempt was made to enlist the antimicrobials used for that specific case. Recovery after using the drugs against infection was noted during the study.

Figure-3.1: Geographical location of the sampling sites

3.3. DNA extraction from blood samples

DNA was extracted from collected blood samples using DNA extraction kit (K-3000, GeNet Bio, Korea) according to the method described elsewhere (Hansen et al., 2009). Briefly Protinase K solution (20 µl, 20 mg/ml) was added to a 1.5 ml microcentrifuge tube. Then 200 µl of whole blood sample was transferred to the microcentrifuge tube. Two hundred micro liters of buffer GB was added to the sample and mixed by vortexing for 15 sec and incubated at 56^0 c for 10 minutes (min). After that 200 µl absolute ethanol was added and again mixed by vortexing for 15 sec. Tube was then briefly spin down to get the drops clinging under the lid. Afterwards the lysate was carefully transferred into the upper reservoir of the spin column (fit in a 2ml tube) without wetting the rim and centrifuged at 10000 rpm for 1 min. The spin column was transferred to a new 2ml collection tube for filtration. Buffer GW1 (500 µl) was added to the spin column and centrifuged at 10000 rpm for 1min. After centrifugation, the flow through was discarded and the spin column was transferred to a new 2 ml collection tube. Again 500 µl of buffer GW1 was added to the spin column and centrifuged at 10000 rpm for 1min. After that, the flow through was discarded and the spin column was resembles with its collection tube. Furthermore for complete removal of ethanol it was centrifuged once more at 12000 rpm for 2 min and ensures that there was no droplet clinging to the bottom of collection tube. For elution the spin column was transferred to a new 1.5 ml tube then 200 µl of buffer GE was added onto spin column and wait for 1 min at room temperature. Finally DNA was eluted by centrifugation at 10000 rpm for 1min.

3.4. Multiplex PCR (mPCR) reactions

Multiplex Polymerase Chain Reaction (mPCR) was conducted according to the instruction given by manufacturing company (EB-1000, GeNet Bio, Korea).The primer sequences used for the mPCR are shown in **Table 3.1.**

Table 3.1: Primer sequence of bacterial toxin genes

Bacteria	Toxins	Primer sequence	Size
Lambda DNA	lambda-F	CGCGAATATGCCGGTTATCA	1000bp
	lambda-R	CACGGAGTAGCCGTTATCCGT	
Salmonella spp.	invA-F	TCATATTACGCACGGAAACACGTTC	100bp
	invA-R	CCTGATTTACTTAAAGAAGTGCTCAG	
Lysteria monocytogenes	prfA-F	GGAGTTTCTTTACCATACACATAGGTC	150bp
	prfA-R	TCTTACGCACTTTTTCTATGTTTTCCAAA	
Bacillus cereus	hblC-F	CTCTCGCAACACCAATCGTTCA	200bp
	hblC-R	CCATTCCTTCATATCTTGTTTGATTAG	
	bceT-F	TTCAGTTCAAAGAAGCATGGACGAAAG	
	bceT-R	ATGCTGACGAGCTACATCCATAATGACT	
	nheA-F	ACAGGGTTATTGGTTACAGCAGTATC	
	nheA-R	TCTGGCTGTTGCAAAATAAYTAATCC	
	entFM-F	TGTTCGTTCAGGTGCTGGTACAGG	
	entFM-R	ACTGTGTAAGTACCWGTTCCTTGTTGAA	
	cytK-F	AGGGATTGGGTAGTTATCAATAGG	
	cytK-R	TCGGGCAAAATGCAAAAACACATACG	
	CER-F	GGGACCAAGAAACGAAAAAGAAGCA	
	CER-R	AGTTCAGCAATCGTTTGATACTGAAAG	
Yersinia enterocolitica	inv(Y)-F	GGCAAATCAGGAAGTAAAACACTGG	250bp
	inv(Y)-R	TGTCATAGAAAGTGTTAAAGCCATAC	
Campylobacter jejuni	hipO-F	TCTGGAGCACTTCCATGACCACC	300bp
	hipO-R	TTGCGGTCATGATGGACATACTAC	
Campylobacter coli	glyA-F	TCAAGGCGTTTATGCTGCACTTTTAA	
	glyA-R	GCAATGTCTGCAAAAAGATAAGCTCCAAC	
Clostridium perfringens	cpe-F	TGGATTTGGAATAACTATAGGAGAAC	400bp
	cpe-R	AGTCCAAGGGTATGAGTTAGAAGAACG	
	cpb2-F	AGCAATAAGTCCAATGAAAGCAAGTGC	
	cpb2-R	ACAAACTTGAGTTCTAAATGATGGTGT	
Vibrio cholera	hly-F	AGCAGAGATGCAAGCCCAATTCAG	500bp
	hly-R	TGGCTCCAAACTGACGATAACCGAG	
Vibrio vulnificus	vvha-F	GGGTATTTGATAAGACGAAGTTCAA	
	vvha-R	CTAAGTTCGCACCACACTGTTCG	
Vibrio parahaemolyticus	tlh-F	TCGCACCAGCTACTCGAAAGATG	
	tlh-R	CAACCCCTGTTAGCGCGATGTATT	
Staphylococcus aureus	nuc-F	GTGCTGGCATATGTATGGCAAT	658bp
	nuc-R	CTGAATCAGCGTTGTCTTCGC	

The mPCR reactions were conducted with a final volume of 20 µl. Proportions of different reagents used for mPCR of different genes are given in **Table 3.2.**

Table 3.2: Reagents used for mPCR amplifications of the genes

Serial No	Name	Manufacturer
1	Master Mix	Genet Bio
2	Molecular marker	Genet Bio
3	Ethidium bromide solution (1%)	Fermantas
4	Electrophoresis buffer 50x TAE	Fermantas
5	Agarose powder	Seakem® Le agarose-Lonza

The PCR reaction was run in a thermocycler (Applied Biosystem, 2720 thermal cycler, Singapore) following the cycling conditions mentioned in **Table 3.3.**

Table 3.3: Contents of each reaction mixture of mPCR assay

Components	Volume
2 Multi HS Prime Taq Premix	10µl
Primer Mixture	5µl
Template DNA	5µl
Total	20µl

Table 3.4: Cycling conditions used during mPCR for detection of bacterial genes

Step	Temperature	Time	Cycle
UDG reaction	50^0C	3 min	1
Pre-denaturation	95^0C	10 min	1
Denaturation	95^0C	30 sec	
Annealing	60^0C	20 sec	35
Extension	72^0C	1 min	
Final Extension	72^0C	5 min	1
Store	4^0C	∞	-

The mPCR protocol followed by agarose gel electrophoresis technique was adopted for the detection of bacterial genes from whole blood as described by (Henegariu et al., 1997) . A total volume of 20 µl was used for mPCR assay by maintaining the cycling conditions that are shown in **Table 3.4.**

3.5. Visualization of mPCR products by Agar Gel Electrophoresis

Agarose gel (1.5 %, W/V) was used to visualize the PCR product. Agarose powder (0.75 gm) and 50 ml of 1X TAE buffer was mixed thoroughly in a conical flask and boiled in a microwave oven until dissolved. Then the mixture was cooled to 50°C using a rocker and 0.05 µl/ml of ethidium bromide was added to the agarose solution. The agarose solution containing was then poured into the gel casting tray which was assembled by sealing the ends of gel chamber with tape and placed appropriate number of combs in gel tray. The gel tray was for solidification at room temperature then combs were removed and the gel was shifted into an electrophoresis tank filled with 1X TAE buffer for further use. An amount of 7 µl of PCR product and 1 kb control DNA marker was loaded into a gel hole to compare the amplicons size of a gene product and the electrophoresis was run at 110 volts and 80mA for 30 minutes. Let the samples goes down to the target level and then the gel was immediately placed to the UV trans-illuminator in the dark chamber for image viewing and documentation system. Finally the image was visualized on the monitor followed by the printed as well as saved electronically.

3.6. Antimicrobial resistance profile testing of bacteria

Positive samples were sub-cultured on blood agar and incubated at 37° C for 24 hours to obtain a pure growth. Using sterile inoculating loop 3 or 4 individual colonies from the blood agar were transferred into a tube containing 3 ml of sterile phosphate buffer saline solution (PBS, 0.85% w/v NaCl solution). Emulsification of the inoculum was done to avoid clumping of the cells inside test tube using vortex machine. Then the bacterial suspension was adjusted to the turbidity of 0.5 McFarland standards (equivalent to growth of 1-2×10^8CFU/ml). Within 15 minutes of preparing the inoculums, a pre-sterile cotton swab was dipped into the inoculums and rotated against the side of the tube with

firm pressure to remove excess fluid. Swab was streaked over the entire dry surface of Mueller Hinton agar for three times rotating the plate approximately at 60^0 F. After 15 minutes of inoculation, discs were placed on the agar surface using a sterile forceps. Agar plates were incubated at 37°C for 18 hours after dispensing all the discs on it. Size of zone of inhibition (in mm) around a disc including the diameter of the disc was measured using a ruler and the result was interpreted according to CLSI, 2011 (Pa, 2006). The panel of antibiotics used for different bacterial species along with the size of zone of inhibition of them to be considered as a resistant (R), intermediately sensitive (I), sensitive (S) against the tested isolate are shown in **Table 3.5.**

Table 3.5: Panel of antibiotics, their concentration and zone diameter interpretative standards for different bacteria (CLIS, 2011).

Name of Bacteria	Name of Antimicobial agent(µg)	Disk content(µg)	Interpretation of zone diameters (mm)		
			R≤	I	S≥
Salmonella spp.	Penicillin	6	-	-	-
	Ampicillin	10	14	-	15
	Amoxicillin	25	14	-	15
	Cefotaxime	30	19	-	20
	Gentamycin	10	16	17-19	20
	Ciprofloxacin	5	16	17-19	20
	Tetracycline	3	19	20-23	24
	Sulfamethoxazole	300	13	14-16	17
Lysteria monocytogenes	Penicillin	6	28	20-27	19
	Ampicillin	10	20	-	19
	Amoxicillin	25	-	-	-
	Cefotaxime	30	-	-	-
	Gentamycin	10	-	-	-
	Ciprofloxacin	5	-	-	-
	Tetracycline	3	19	15-18	14
	Sulfamethoxazole	300	16	11-15	10
Clostridium perfringens	Penicillin	6	12.5	1.6-6.2	0.8
	Ampicillin	10	-	-	-
	Amoxicillin	25	-	-	-
	Cefotaxime	30	-	-	-
	Gentamycin	10	-	-	-
	Ciprofloxacin	5	-	-	-
	Tetracycline	3	12.5	3.1-6.2	1 .6
	Sulfamethoxazole	300	-	-	-
Staphylococcus aureus	Penicillin	6	24	-	25
	Ampicillin	10	25	-	26
	Amoxicillin	25	-	-	-
	Cefotaxime	30	21	-	22
	Gentamycin	10	-	-	-
	Ciprofloxacin	5	13	-	14
	Tetracycline	3	19	-	20
	Sulfamethoxazole	300	19	-	20

R= Resistant, I=Intermidiate S=Sensitive, (-) = no established value found

3.7. Statistical evaluation

Data were entered into Microsoft Office Excel 2013 and then exported to STATA-13 (StataCorp 4905, Lakeway Drive, College Station, Texas 77845, USA) for epidemiological analysis.

3.7.1. Descriptive analysis

Distribution of goats was presented according to the locations and quantities of the group, population size, sample size, age and sex variables. Prevalence of different microorganisms was calculated using positive samples divided by the total number of samples tested and the results were expressed as a percentage with 95% confidence interval (CI). Prevalence of microorganisms according to the season, sexes and age were calculated with 95% CI too. Antimicrobial susceptibility testing was done and the percentage of susceptibility was calculated according to the resistance, intermediate and sensitive antimicrobials. Percentages of different antimicrobials were presented as a chart.

3.7.2. Risk factor analysis

Based on data collection, goat samples were grouped according to the seasons they were collected from (summer and winter), breed (Black-Bengal, Jamunapari and Cross), source (family and farm), age (adult, sub-adult, juvenile and young), sex (male and female), and vaccination (yes and no). Chi-square test was done to identify significant risk factors.

3.7.3. Logistic regression model

For *Staphylococcus aureus* and *Clostridium perfringens*, variables- Breed, sex and age (p<0.3) was forwarded to logistic regression model after chi-square test. In case of *Salmonella* spp., sex and age were dropped and source was added. Logistic regression was omitted in case due to low prevalence. After adjusting the factor with each other *Staphylococcus aureus* (breed, sex, age and BCS), *Clostridium perfringenes* (breed, sex and age), and *Salmonella* spp. (breed, source, BCS and vaccination), were found to be a

significant risk factor. Confounder was checked by observing the variation in the coefficient. If the variation was greater than 10%, then the factor was considered as a confounder. The validity of the model was checked. The model was valid by Receiver operating curve (ROC) and goodness of fit test (lfit) (Dohoo, Martin, & Stryhn, 2003). The results were expressed as OR, 95% CI and P value.

Chapter-4: Results

4.1. Confirmation of pathogens by mPCR

Toxin genes of different bacteria were detected through agar gel electrophoresis after mPCR reactions. From 200 samples we were able to identify *Staphylococcus aureus*, *Clostidium perfringenes*, *Listeria monocytogenes and Salmonella* spp. by observing their band size described in manufacturer protocol (EB-1000, GeNet Bio, Korea). The amplicons of *nuc* genes of Staphylococcus *aureus* produced a band of 658 bp (**Figure 4.1**) whereas amplicons of *Clostridium perfringenes, Listeria monocytogenes and Salmonella* spp. produced 400 bp (**Figure 4.2**), 150 bp (**Figure 4.3**) and 100 bp (**Figure 4.4**) respectively.

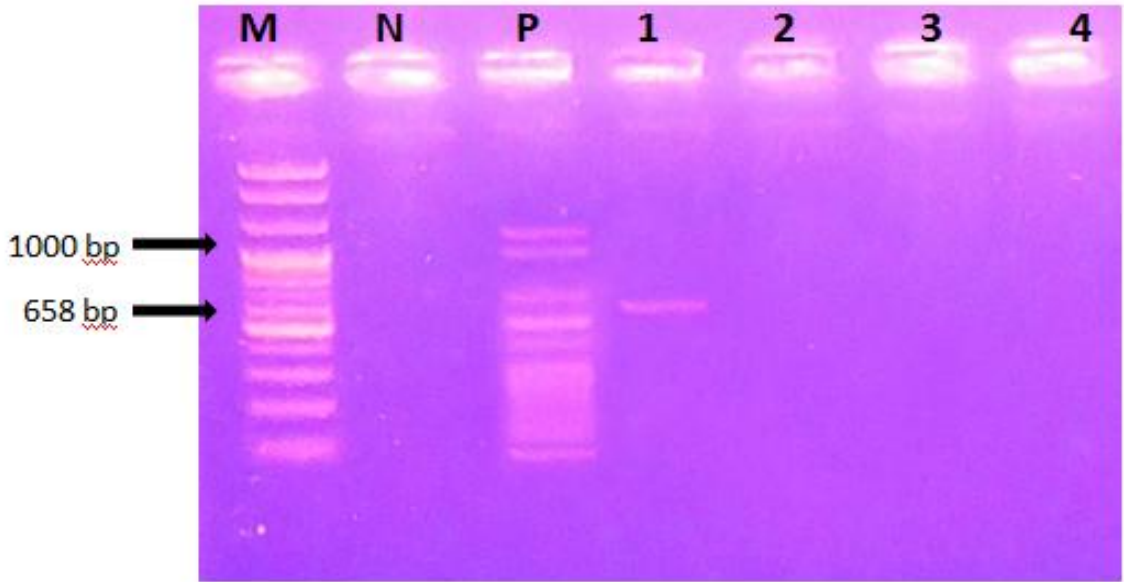

Figure 4.1: Result of mPCR assay for *nuc gene* of *Staphylococcus aureus* identify from the samples; Lane M: 1 Kb DNA marker; Lane N: Negative control; Lane P: Control DNA; Lane 1: *Staphylococcus aureus* gene-sized (658 bp) amplicon.

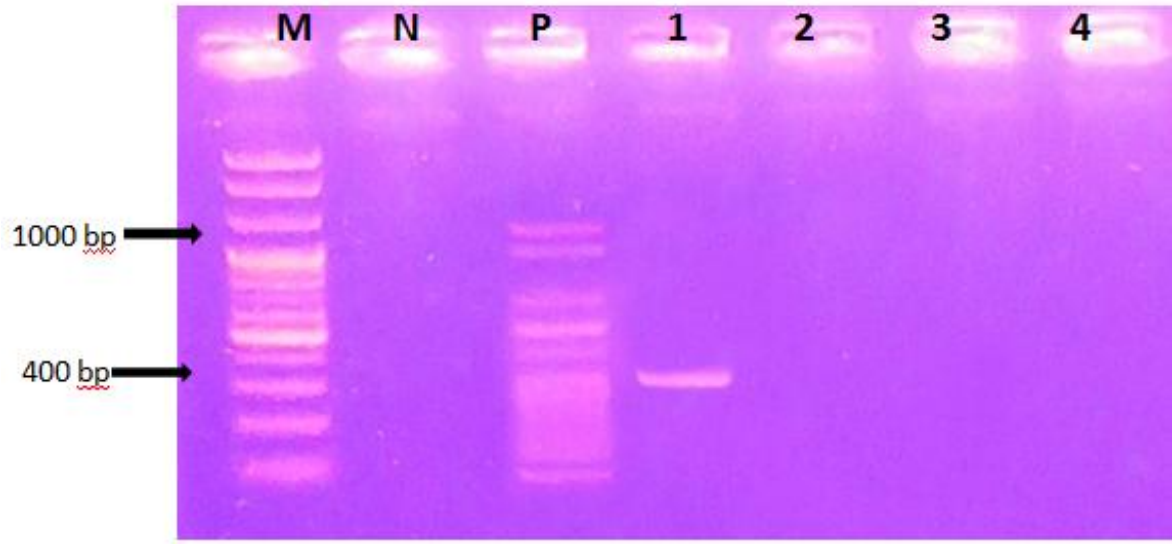

Figure 4.2: Result of mPCR assay for *cpe* and *cpb2 gene* of *Clostidium perfringenes* identify from the samples; Lane M: 1 Kb DNA marker; Lane N: Negative control; Lane P: Control DNA; Lane 1: *Clostidium perfringenes* gene-sized (400 bp) amplicon.

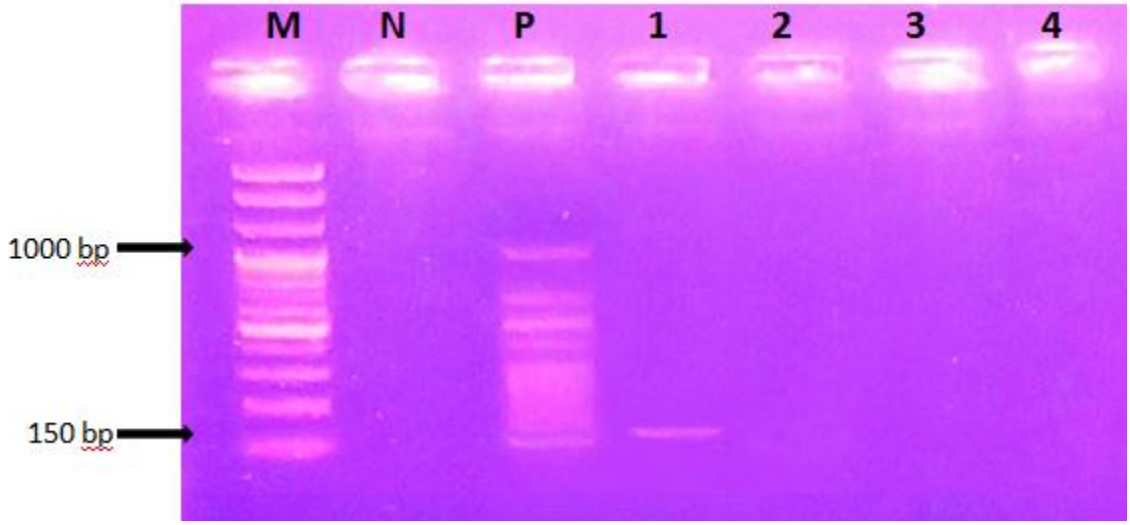

Figure 3: Result of mPCR assay for *prfA gene* of *Listeria monocytogenes* identify from the samples; Lane M: 1 Kb DNA marker; Lane N: Negative control; Lane P: Control DNA; Lane 1: *Listeria monocytogenes* gene-sized (150 bp) amplicon.

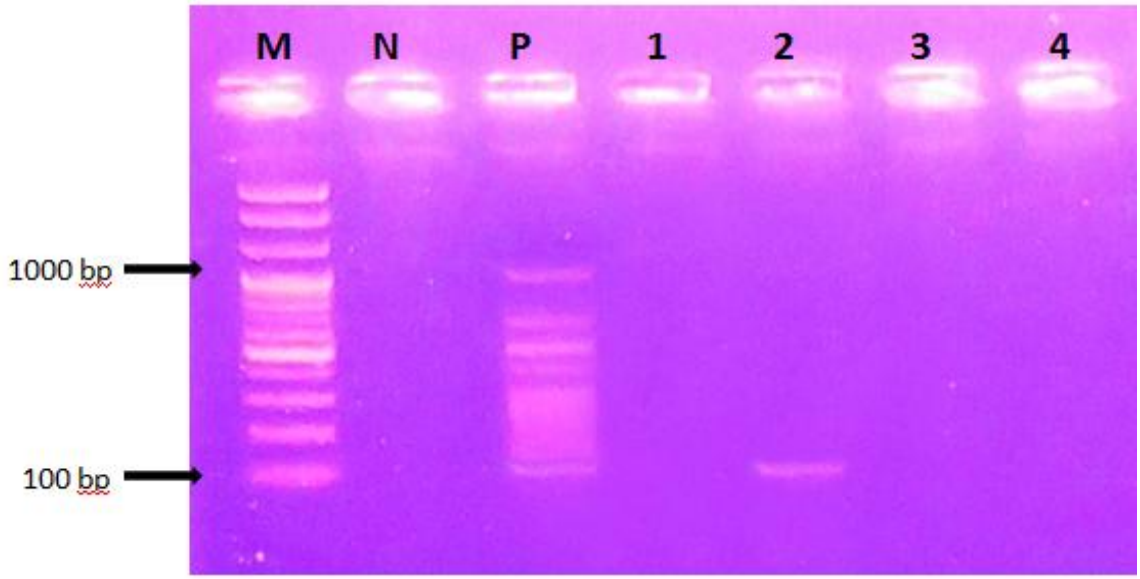

Figure 4: Result of mPCR assay for invA *gene* of *Salmonella spp.* identify from the samples; Lane M: 1 Kb DNA marker; Lane N: Negative control; Lane P: Control DNA; Lane 2: *Salmonella* spp. gene-sized (100 bp) amplicon.

4.2. Prevalence of the different microorganisms

A total of 200 samples were collected from goat patient at SAQTVH, CVASU. Goats having fever and/or diarrhea coming from different parts of Chittagong metropolitan areas were selected for sampling. Among them, 23 (11.5%; 95% CI 7.4% - 16.7%) were *Staphylococcus aureus*, 07 were (3.5%; 95% CI 1.4% - 7.1%) *Clostidium perfringenes*, 2 (1%; 95% CI 0.1% - 3.6%) and 41 (20.5%; 95% CI 15.1% - 26.8%) were confirmed as, *Listeria monocytogenes and Salmonella* spp. respectively. **(Table 4.1)**

Table 4.1: Prevalence of microorganisms confirmed by mPCR

Name of the microorganisms	Positive (n)	prevalence	95% CI
Staphylococcus aureus	23	11.5%	7.4-16.7
Vibrio cholera	0	0	-
Clostidium perfringens	7	3.5%	1.4-7.1
Camphylobacter jejuni	0	0	-
Yersinia enterocolitica	0	0	-
Bacillus cereus	0	0	-
Listeria monocytogenes	2	1%	0.1-3.6
Salmonella spp.	41	20.5%	15.1-26.8

4.3. Frequency distribution of affected systems of goat

The frequency distribution of affected body systems were measured to see the distribution of pathogens causing diarrhea and/or fever in goat. The respiratory (40.5%) and digestive system (38%) were more frequently affected in compare to the other systems which were shown in **Table 4.2.**

Table 4.2: Frequency distribution of system affected

Syestem	Frequency	Percentage
Digestive	76	38%
Respiratory	81	40.5%
Urinary	20	10%
Nervous	6	3%
Muscular	4	2%
Integumentary	9	4.5%
Reproductive	4	2%
Total	200	100%

4.4. AMR and associated factors influencing microbial infection in goat

AMR and related clinical signs and certain potential risk factors (age, sex, breed and seasons) were taken into consideration to see the integration of prevalence of infection of different microorganism in goats in this area.

4.4.1. AMR and associated factors influencing *Staphylococcus aureus* infection in goat

4.4.1.1. Frequency of different clinical signs in *Staphylococcus aureus* infected goat

Major clinical signs were recorded while collecting samples from goat having diarrhea and/or fever. The *Staphylococcus aureus* infected goats were showing variable clinical signs and symptoms. Percentages of major clinical signs were calculated which may will help to diagnose *Staphylococcus* infected goat in field level. Mild fever, nasal discharge, dyspnea and coughing together were recorded as the most common clinical signs of *Staphylococcus aureus* infection (39.1%), goats with parturition history having fever, lethargy, dehydration, and loss of appetite were second common (34.8%) followed by mild fever, dry nose and wheezing (17.4%) and fever, sneezing, foaming at the mouth (8.7%) (**Table 4.3**). From these findings we can come into conclusion that, goat infected with *Staphylococcus aureus* may shows common clinical signs of fever and respiratory distress like coughing, dyspnea and nasal discharge.

Table 4.3: Frequency distribution of symptoms of *Staphylococcus aureus* infection in goats

Clinical signs and symptoms	N	%
Mild fever, nasal discharge, dyspnea and coughing	09	39.1
Fever, lethargy, dehydration, loss of appetite and parturition history	08	34.8
Mild fever, dry nose and wheezing	04	17.4
Fever, sneezing, foaming at the mouth	02	8.7

4.4.1.2. Univariate and multivariate association between *Staphylococcus aureus* and selected variables

The prevalence of *Staphylococcus aureus* was 16.8% which was significantly highest in females ($P \leq 0.01$) whereas the lowest prevalence found among the males (3.7%). *Staphylococcus aureus* was significantly more prevalent (33.3%) among the adult goats ($P \leq 0.01$) than that of young (9.8%) and juvenile (4.3%). In case of breed, the highest prevalence (14.3%) of *Staphylococcus aureus* was found in the Jamnapari breed of goats ($P=0.02$) whereas the prevalence was 7.1% in Cross breeds and 4% in Black Bengal goats. *Staphylococcus aureus* was more prevalent (18.5%) in goats ($P=0.09$) with good body condition score (BCS) compare to the fair and poor body condition scored goats. Among fair and poor BCS goats were 9.8% and 4.3% prevalent respectively. **(Table 4.4).**

The significant variables (sex, breed and age; $p \leq 0.3$) identified through univariate chi-square analysis were forwarded to the logistic regression model. After adjustment of the factors each other through the model, the odds of prevalence of *Staphylococcus aureus* was significantly higher in good BCS (OR=3.6; CI: 0.9-13.2, $P=0.05$) and fair BCS goats (OR=2.4; CI: 0.6-8.6) than that of poor BCS goats. In case of sex, the OR of prevalence were 2.6 times higher in females than the males. On the other hand, the OR of prevalence was higher in adult (OR=6; CI: 1.5-24.5, P=0.01) and young (OR=1.8; CI: 0.5-6.9) than that of juvenile goats **(Table 4.4)**.

Table 4.4: Frequency distribution of *Staphylococcus aureus* in goats

Variables	Categories	*Staphylococcus aureus*			Multiple logistic regression		
		n (%)	95% CI	*P* (χ^2-test)	OR	95% CI	*P*
Season	Summer (80)	11(13.7%)	7.1-23.3	0.42			
	Winter (120)	12 (10%)	5.3-16.8				
Breed	Black Bengal(25)	1 (4%)	0.1-20.3	0.02	1		
	Jamunapari (133)	19(14.3%)	8.8-21.4		1.6	0.2-14.2	0.67
	Cross (42)	3 (7.1%)	1.5-19.5		1.5	0.1-18.1	0.71
Sex	Male (81)	3 (3.7%)	0.7-10.4	**<0.01**	1		
	Female (119)	20(16.8%)	10.6-24.7		2.6	0.6-10.9	0.20
Source	Family (146)	15(10.3%)	5.8-16.4	0.37			
	Farm (54)	8 (14.8%)	6.6-27.1				
Age	Juvenile (0 days-1 year) (93)	4 (4.3%)	1.2-10.6	**<0.01**	1		
	Young (1 year- 2 years) (71)	7 (9.8%)	4.1-19.3		1.8	0.5-6.9	0.39
	Adult (> 2 years) (36)	12(33.3%)	18.6-50.9		6	1.5-24.5	**0.01**
BCS	Poor -1 (69)	4 (5.8%)	1.6-14.2	0.09	1		
	Fair-2 (77)	9 (11.7%)	5.5-21.1		2.4	0.6-8.6	0.18
	Good-3 (54)	10(18.5%)	9.3-31.4		3.6	0.9-13.2	**0.05**
Vaccination	Yes (14)	1 (7.1%)	0.2-33.8	0.59			
	No (186)	22(11.8%)	7.6-17.4				

4.4.1.3. Antimicrobial resistance pattern of *Staphylococcus aureus* in goats

To observe the antimicrobial resistance pattern, cultural sensitivity test was performed against 10 different antimicrobials. Cefotaxime (74%) followed by Cefixime (65%), Tetracycline (65%) and Penicillin (61%) showed the highest resistance against *Staphylococcus aureus* in goats. On the other hand, Gentamycin (13%), Amoxicillin (13%), and Ciprofloxacin (18%) showed the lowest resistance.

In case of the sensitivity of antimicrobials, Amoxicillin (48%), Ciprofloxacin (44%), and Gentamicin (44%) were showing highest sensitivity whereas Cefixime (13%), Cefotaxime (13%), Doxycycline (13%) and Tetracycline (13%) showed lowest sensitivity among all drugs against *Staphylococcus aureus* infection in goats (**Fig. 4.5**).

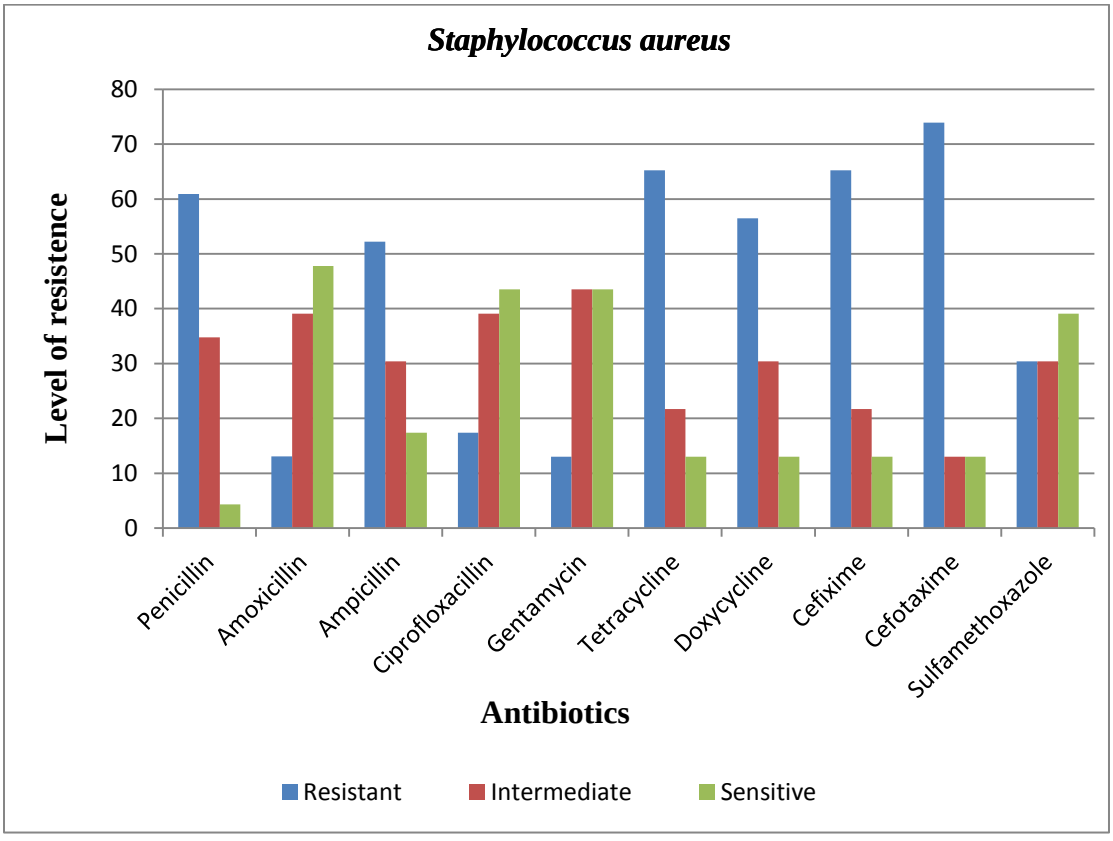

Figure 1.5: Antimicrobial resistance pattern of *Staphylococcus aureus*

4.4.2. AMR and associated factors influencing *Clostridium perfringens* infection in goat

4.4.2.1. Frequency of different clinical signs in *Clostridium perfringens* infected goat

Diarrhea, convulsion, abdominal pain and incordination (57.1%) were the most frequently encountered clinical signs of *Clostridium perfringens* infection in goats. Other commonly found signs presented by *Clostridium perfringens* infected fever, diarrhea and convulsion (28.6%) and anorexia, diarrhea with blood and dehydration (14.3%), **Table 4.5.** From these findings we can say that, goat infected with *Clostridium perfringens* may shows common clinical signs of fever, diarrhea and convulsion. Incordination and abdominal pain may also found.

Table 4.5: Frequency distribution of symptoms of *Clostridium perfringens* infection in goats

Clinical signs and symptoms	N	%
Diarrhea, convulsion, abdominal pain and incordination	04	57.1
Fever, diarrhea and convulsion	02	28.6
Anorexia, diarrhea with blood and dehydration	01	17.1

4.4.2.2. Univariate and multivariate association between *Clostridium perfringens* and selected variables

In risk factor analysis, no significant differences were observed between summer and winter season in prevalence of *Clostridial* infection in goat. However, the prevalence in winter is somewhat higher (4.2% with CI: 1.4-9.5) than summer (2.5% with CI: 0.3-8.7). Within 3 breeds, prevalence of *Clostridium perfringens* was significantly high in the cross breeds (11.9%, 95% CI: 3.9-25.6, $P<0.01$) than that of Jamunapari and Black Bengal. Male was most prevalent in *Clostridium perfringens* (7.4% with CI: 2.8-15.4, $P=0.01$) in comparison with females (0.8% with CI: 0.1-4.6). There was no significant difference in age and BCS but a higher percentage of positive in juvenile (5.4% with CI:

1.8-12.1) than other age group and goat with poor BCS showed 4.3% prevalence of *Clostridium perfringens* **(Table 4.6).**

Through the Logistic regression model, factors like breed, sex, season and age influencing the prevalence of *Clostridium perfringens* infection were compared among the same group. Prevalence was significantly higher (5 times) in cross breeds (OR=4.9, CI: 0.8-28.7, *P*=0.08), than Jamunapari and Black Bengal. In case of sex, male goats (OR=5.6, CI: 0.6-54.1, *P*=0.13) was high on prevalence of *Clostridium perfringens* than the females **(Table 4.6).**

4.6: Frequency distribution of *Clostridium perfringens* in goats

Variables	Categories	*Clostridium perfringens*			Multiple logistic regression		
		n (%)	95% CI	*P* (χ^2-test)	OR	95% CI	*P*
Season	Summer (80)	2 (2.5)	0.3-8.7	0.53			
	Winter (120)	5 (4.2)	**1.4-9.5**				
Breed	Black Bengal(25)	0	0	**<0.01**			
	Jamnapari (133)	2 (1.5%)	0.2-5.3		1		
	Cross (42)	5 (11.9%)	3.9-25.6		4.9	0.8-28.7	0.08
Sex	Female (119)	1 (0.8%)	0.1-4.6	**0.01**	1		
	Male (81)	6 (7.4%)	2.8-15.4		5.6	0.6-54.1	0.13
Source	Family (146)	5 (3.4%)	1.1-7.8	0.92			
	Farm (54)	2 (3.7%)	0.5-12.7				
Age	Juvenile (0 days-1 year) (93)	5 (5.4%)	1.8-12.1	**0.30**			
	Young (1 year- 2 years) (71)	2 (2.8%)	0.3-9.8				
	Adult (> 2 years) (36)	0	0				
BCS	Poor-1 (69)	3 (4.3%)	0.9-12.2	0.84			
	Fair- 2 (77)	2 (2.6%)	0.3-9.1				
	Good-3 (54)	2 (3.7%)	0.5-12.7				
Vaccination	Yes (14)	0	0	0.46			
	No (186)	7 (3.7%)	1.5-7.6				

4.4.2.3. Antimicrobial resistance pattern of *Clostridium perfringens* in goats

Like *Staphylococcus aureus*, antimicrobial resistance pattern was also investigated for *Clostridium perfringens***.** Present study revealed that, Amphicillin (71%), Gentamycin (71%) followed by Amoxicillin (57.1%) and Ciprofloxacin (57.1%) were highly resistant against *Clostridium perfringens* in goats. Whereas, Penicillin (14%), Tetracycline (14.3%), and Cefotaxime (14%) showed lowest resistance against *Clostridium perfringens.* Penicillin (57%), Cefotaxime (57%) followed by Tetracycline (43%) and Cefixime (43%) were highly sensitive among all the tested antibiotics against *Clostridium perfringens.* Gentamycin was found least sensitive among all the antibiotics tested against this organism.

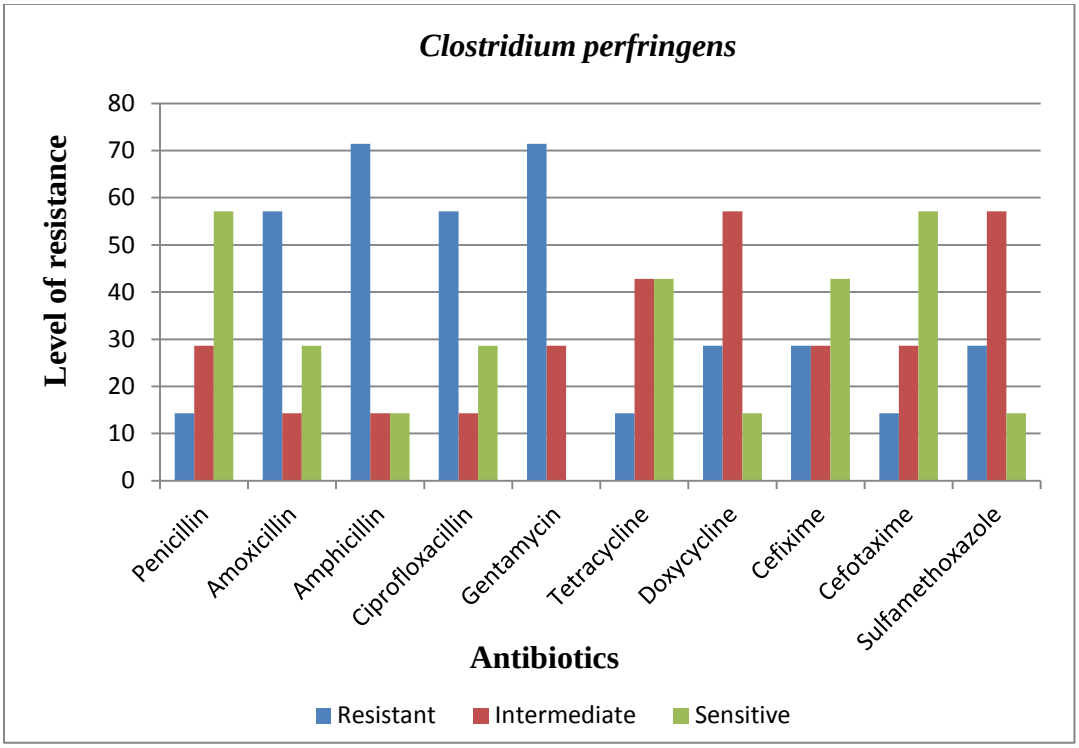

Figure 2.6: Antimicrobial resistance pattern of *Clostridium perfringens*

4.4.3. AMR and associated factors influencing *Listeria monocytogenes* infection in goat

4.4.3.1. Frequency of different clinical signs in *Listeria monocytogenes* infected goat

In present study, only two cases of *Listeria monocytogenes* were identified out of 200 patients. In both the cases clinical signs were similar like fever, protrusion of tongue and incordination. **(Table 4.7)**

Table 4.7: Frequency distribution of symptoms of *Listeria monocytogenes* infection in goats

Clinical signs and symptoms	N	%
Fever, protrusion of tongue and incordination	02	100

4.4.3.2. Univariate association between *Listeria monocytogenes* and selected variables

Association between *Listeria monocytogenes* and selected variables were measured to see the relation of listeria infection with different risk factors. Due to the low prevalence multiple logistic regressions was not possible. Prevalence of listeria infection found higher in winter (1.7% with CI: 0.2-5.9) than that of summer. Prevalence of listeriosis is significantly higher in farm animals (3.8% with CI: 0.4-13.2) compare to the animals that come from family source. No other significant relation was found among other variables (**Table 4.8).**

Table 4.8: Frequency distribution of *Listeria monocytogenes* in goats of Chittagong

Variables	Categories	*Listeria monocytogenes*		
		n (%)	95% CI	*P* (χ^2-test)
Season	Winter (120)	2 (1.7%)	0.2-5.9	0.24
	Summer (80)	0	-	
Breed	Jamunapari (133)	2 (1.5%)	0.1-5.3	0.6
	Black bengal (25)	0		
	Cross (42)	0		
Sex	Female (119)	0		0.08
	Male (81)	2 (2.5%)	0.3-8.6	
Source	Farm (54)	2 (3.8%)	0.4-13.2	**0.02**
	Family (146)	0		
Age	Juvenile (0 days-1 year) (93)	1 (1.1%)	0.03-5.8	0.78
	Young (1 year- 2 years) (71)	1 (1.4%)	0.04-7.6	
	Adults (> 2 years) (36)	0		
BCS	Poor-1 (69)	2 (2.9%)	0.3-10.1	0.15
	Fair-2 (77)	0		
	Good-3 (54)	0		
Vaccination	Yes (14)			0.69
	No (186)	2 (1.1%)	0.1-3.8	

4.4.3.3. Antimicrobial resistance pattern of *Listeria monocytogenes* in goats

AMR pattern of *Listeria monocytogenes* showed higher resistance against Amoxicillin and Ampicillin (100%), followed by Doxycycline, Cefotaxime and Sulfamethoxazole (50%). However, Penicillin and Cefixime (100%) showed highly sensitive against *Listeria monocytogenes* in goats.

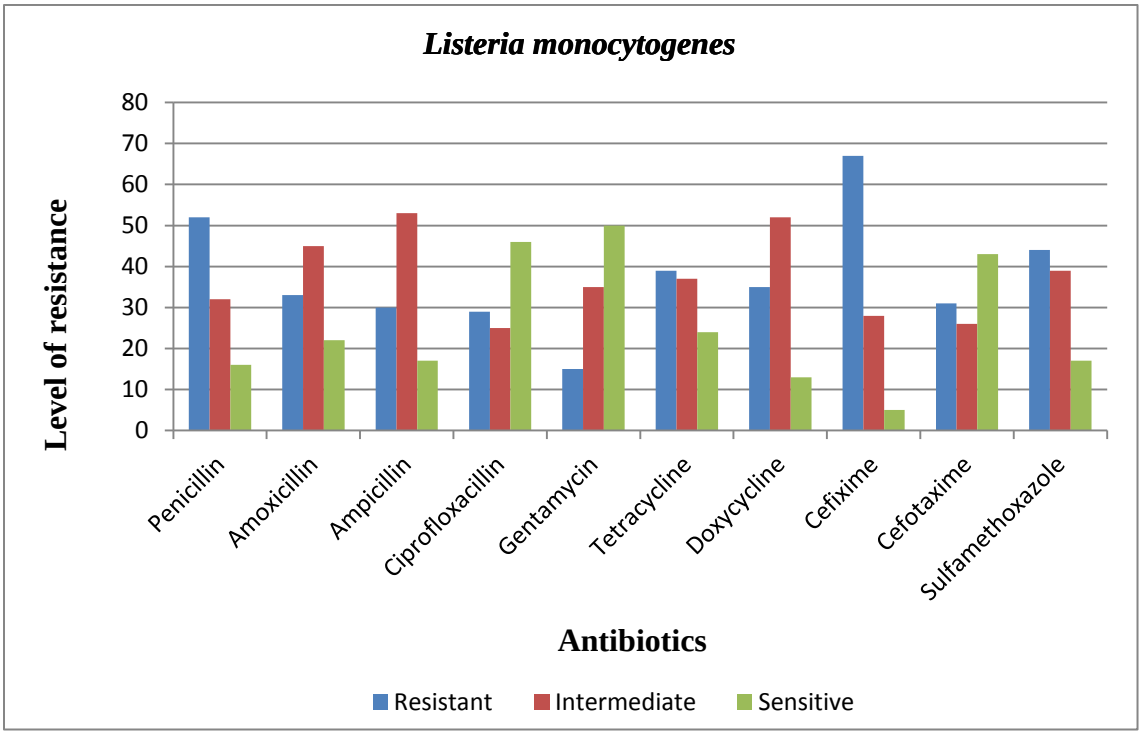

Figure 3.3: Antimicrobial resistance pattern of *Listeria monocytogenes*

4.4.4. AMR and associated factors influencing *Salmonella* spp. infection in goat

4.4.4.1. Frequency of different clinical signs in *Salmonella* spp. infected goat

In case of salmonellosis in goat, fever, anorexia, dehydration and mucus in feces (36.6%) were most prevalent clinical signs. Profuse watery foul smelling diarrhea, anorexia and dehydration (29.3%) and high fever, lethargy and yellow to greenish-brown diarrhea (17.1%) were also recorded. From these studies, we can say that, fever and diarrhea is common in salmonella infected goat. Diarrhea could be different color with foul smelling, blood may or may not be present (table 4.9).

Table 4.9: Frequency distribution of symptoms of *Salmonella* spp. infection in goats

Clinical signs and symptoms	N	%
Fever, anorexia, dehydration and mucus in stool	15	36.6
Profuse, watery foul smelling diarrhea, anorexia and dehydration	12	29.3
High fever, lethargy and yellow to greenish-brown diarrhea	07	17.1
Mild fever and blood streaked diarrhea	04	9.7
Mild fever, lethargy, gaseous stomach and diarrhea	03	7.3

4.4.4.2. Univariate and multivariate association between *Salmonella* spp. and selected variables

Significant differences in the prevalence of *Salmonella* spp. among the different breeds of goats were determined **(Table 4.10)**. Cross breeds (57.1%; 95%CI: 40.9-72.3) were found to be significantly high prevalent to *Salmonella* spp. than Black Bengal (44%; 95%CI: 24.4-65.1) and Jamunapari (4.5%; 95%CI: 1.7-9.6) breeds of goat. The prevalence of *Salmonella* spp. was significantly higher (P=0.02) in goats with good BCS (28.6%; 95%CI: 18.8-40%) than others. The goats from the family sources (23.9%) were more prevalent than that of goats from farm (11.1%). In case of age, adult goats were significantly more prevalent (27.8%; 95% CI: 14.2-45.2) than sub-adult and juvenile. Although there was no significant difference, male was highly prevalent (23.5%) than female.

The significant variables from univariate analysis (breed, source and BCS) were transferred to the logistic regression model. The prevalence of *Salmonella* spp. was 27.3 times higher in cross breeds and 22.8 times higher in Black Bengal goats than the Jamnapari. The odds of prevalence of *Salmonella* spp. was significantly higher in goats with good BCS (OR=5.7, CI: 1.5-21.5, P=0.01) than the fair and poor BCS goats. The prevalence of *Salmonella* spp. was 3.1 times higher in goats reared in family than the goats reared in farms **(Table 4.10).**

Table 4.10: Frequency distribution of *Salmonella* spp. in goats

Variables	Categories	*Salmonella* spp.			Multiple logistic regression		
		n (%)	95% CI	*P* (χ^2-test)	OR	95% CI	*P*
Season	Winter (120)	21 (17.5%)	11.2-25.5	0.19	1		
	Summer (80)	20 (25%)	15.9-35.9		1.8	0.7-4.4	0.24
Breed	Jamnapari (133)	6 (4.5%)	1.7-9.6	**<0.01**	1		
	Black bengal (25)	11 (44%)	24.4-65.1		22.8	6.5-79.9	**<0.01**
	Cross (42)	24 (57.1%)	40.9-72.3		27.3	9.1-81.8	**<0.01**
Sex	Female (119)	22 (18.5%)	11.9-26.6	0.39			
	Male (81)	19 (23.5%)	14.7-34.2				
Source	Farm (54)	6 (11.1%)	4.2-22.6	**0.04**	1		
	Family (146)	35 (23.9%)	17.3-31.7		3.1	0.9-9.5	**0.05**
Age	Juvenile (0 days-1 year) (93)	18 (19.4%)	11.9-28.8	0.48			
	Young (1 year- 2 years) (71)	13 (18.3%)	10.1-29.3				
	Adult (> 2 years) (36)	10 (27.8%)	14.2-45.2				
BCS	Poor-1 (54)	5 (9.3%)	3.1-20.3	**0.02**	1		
	Fair-2 (69)	14 (20.3%)	11.6-31.7		2.2	0.5-8.4	0.26
	Poor-3 (77)	22 (28.6%)	18.8-40		5.7	1.5-21.5	**0.01**
Vaccination	Yes (14)	7 (50%)	23.1-76.9	**<0.01**	6	1.2-31.3	**0.03**
	No (186)	34 (18.3%)	13-24.6		1		

4.4.4.3. Antimicrobial resistance pattern of *Salmonella* spp. in goats

In antimicrobial resistance and sensitivity test, Ampicillin (78%), Amoxicillin (59%), and Penicillin (56%) were found to be highly resistance among all drugs whereas Cefotaxime (12%) was lowest resistant to *Salmonella* spp. Ciprofloxacillin showed the highly sensitive (54%) and Penicillin (2%) showed the least sensitive among all drugs. Doxycycline (51%) showed moderately resistance against *Salmonella* spp. followed by Gentamycin (42%) and Penicillin (42%).

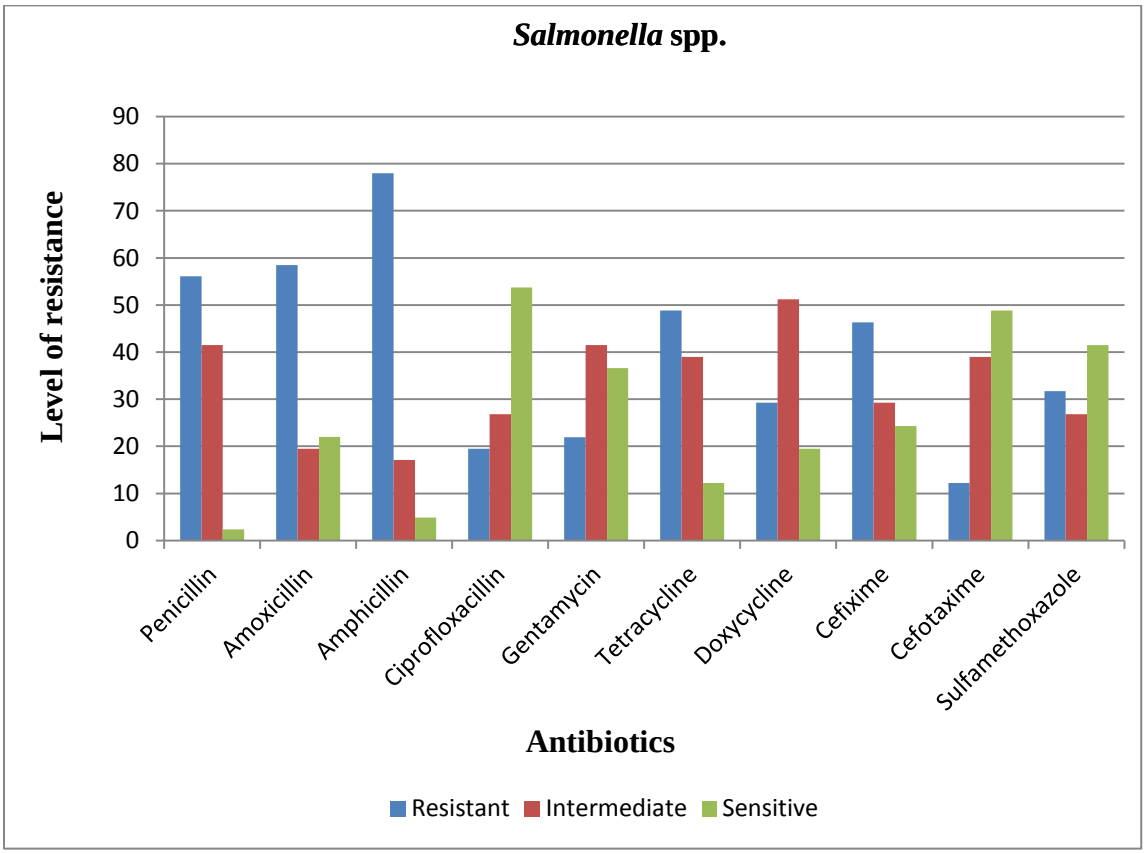

Figure 4.8: Antimicrobial resistance pattern of *Salmonella* spp.

Chapter-5: Discussion

In developing country like Bangladesh, livestock plays an essential role in food security, poverty alleviation and indeed a token of people's livelihoods (Herrero et al., 2013). However, its productivity is sometimes threatened by a number of infectious and transboundary Animal diseases (TADs). Infectious diseases of animals are causing major economic losses of farmers inflicted by hampering production. Moreover, direct losses result from death and from decreased production of food products, such as meat, milk and eggs. Indirect losses include legislation of restriction in national and international movement of animals and animal products. Furthermore, many infectious diseases are zoonotic in nature which increases the significance of it both animal and human health. Therefore, importance of rapid diagnosis became a key concern for the treatment, prevention and control of these infectious diseases.

In the present study, confirm diagnosis of some infectious diseases having nonspecific clinical signs like diarrhea and/or fever were done using rapid molecular diagnosis kit to establish their diagnostic protocol in goat in SAQTVH, CVASU. These were *Staphylococcus aureus, Clostridium perfringens, Listeria monocytogenes, Salmonella* spp. Overall proportionate prevalence of *Staphylococcus aureus* infection in goats of Chittagong Metropoliton area was found to be 11.5%. Similar study was conducted by Zaman et al. (2018) in SAQTVH and reported as 14% prevalence of *Staphylococcus aureus* in goat . Another group in other parts of Bangladesh reported somewhat higher prevalence (26%) of *Staphylococcus aureus* (Momin et al., 2012). Researcher reported variable results in other parts of the world such as 43.24% in China (Zhou et al., 2017), 44% in Egypt (Saleh et al., 2014), 39.7% in Nigeria (Gulani et al., 2016) and 30% in West African dwarf goats (Emikpe et al., 2009). This variation may be due to different geographical location and environmental condition.

The present study determined sex as a potential risk factor for *Staphylococcus aureus* infection in goats (OR= 2.6, female versus male) which is coincide with the findings of Zaman et al. (2018) in Bangladesh. However, this findings differs from an Nigerian where the researchers find more prevalence of *Staphylococcus aureus* infection in males

compare to the females (Gulani et al., 2016). This current study also identified age as a significant risk factor (OR= 6, adult versus young and juveniles) which is also similar to the previous findings (Zaman et al., 2018).

Different types of clinical signs were noticed in this study in case of *Staphylococcus aureus* infection in goats. Common clinical signs encountered like mild fever, nasal discharge, dyspnea and coughing whose are obvious as it causes respiratory infection. These clinical signs also matches with findings reported in earlier report (Momin et al., 2012). *Staphylococcus aureus* is also responsible for the sub-clinical mastitis in goats where the clinical signs are limited (Mishra et al., 2018). This study also reported some clinical signs like fever, lethargy, dehydration, loss of appetite in recently parturated goats that may be due to sub-clinical mastitis.

The antimicrobial susceptibility test showed most of the *S. aureus* isolates were resistant to a majority of antibiotics and the sensitivity rates below 40%, with exceptions of Amoxicillin (48%), Ciprofloxacillin (44%) and Gentamycin (44%). *S. aureus* showed relatively high resistance to Penicillin, Amphicillin, Tetracycline, Doxycycline, Cefixime and Cefotaxime which is near to the resistance pattern of *S. aureus* isolated from sheep and goat in Chaina and Spain (Porrero et al., 2012; Zhou et al., 2017). Present study reveals Amoxicillin, Ciprofloxacin and Gentamycin relatively sensitive and Cefotaxime, Tetracycline and Cefixime resistant against *S. aureus* which is similar to the findings reported previously (Klimešová et al., 2017; Nathawat et al., 2013).

Clostridium perfringens toxinotypes are responsible for enterotoxemia in goat. In this study, goat patient at SAQTVH from different regions of Chittagong Metropolitan area were screened for the presence of *C. perfringens* type D. Our findings revealed that 7 (3.5%) out of 200 goats were positive for *C. perfringens* by PCR amplification. In accordance with our study, a higher prevalence of *C. perfringens* in goats of India (60%) and Pakistan (66.5%) has been recorded (Nazki et al., 2017). In this study, we spot sex as a key risk factor for *Clostridium perfringens* in goats (OR= 5.6 male versus female) which contradict to the findings of (Ajaz-ul-Haq et al., 2016) where they found more prevalence (15%) in female than male goats. In case of age, this study recognize juvenile and young goats are more susceptible to *Clostridium perfringens* infection than the adult

goats which is supported by the findings previously reported (Ajaz-ul-Haq et al., 2016; Radostits et al., 2007).

Most frequent clinical signs of *Clostridium perfringens* infected goats were diarrhea, convulsion, abdominal pain and incordination which is supported by previous findings where they observed neurological sign along with abdominal discomfort and diarrhea (Uzal et al, 2016).

The beta-lactams are commonly used for the treatment of *Clostridium perfringens* associated diseases. In our study we also found *Clostridium perfringens* as susceptible to Penicillin (57%), which is in consistent with some previous findings (de Oliveira Júnior et al., 2016). Gentamycin (71%) and Ciprofloxacin (57%) found to be highly resistant against *Clostridium perfringens,* which is similar to the finding were described earlier (Osman & Elhariri, 2013).

Prevalence of listeriosis has not been well reported in goats of Bangladesh. Therefore, we aimed to determine the prevalence of *Listeria monocytogenes* in goats. The overall prevalence of *L. monocytogenes* in the present study was 1% which is similar to some of the studies conducted at National and International levels where they recorded the prevalence of *Listeria monocytogenes* in small ruminants is lower than other infectious causes (KALENDER, 2003; Nath et al., 2014; Yadav & Roy, 2009). Studies from India reported higher prevalence (16.66%) of *Listeria monocytogenes* in goats which may be due to inadequate hygienic condition and low ambient temperature during the period of sampling and processing (Hilal, 2016).

Due to the low prevalence it is difficult to identify the risk factors for *Listeria monocytogenes* in goats. In this present study both the positive cases were found in winter and in goats that were reared in farms where silage was supplied. This may be due to the organism is more prevalent in winter and transmitted through silage (Fentahun & Fresebehet, 2012). However, this is difficult to come into conclusion with this sample size and identified case. Further study may necessary for detail report.

Clinical manifestations of invasive listeriosis in ruminants are usually severe. In this study we found protrusion of tongue and incordination in listeria affected goats

supporting the findings of encephalitic listeriosis in small ruminants (Campero et al., 2002)

Antibiogram study of *Listeria monocytogenes* isolates exhibited highly sensitivity against Penicillin and Cefixime and resistance against Amoxicillin and Amphicillin which contradict with one study (Sarangi et al., 2012) but in consistent with another (Vaidya et al., 2018) . Present study showed, Ciprofloxacin, Doxycycline, and Tetracycline are intermediately sensitive whereas (Hilal, 2016) spot 100% sensitivity of Ciprofloxacin against *Listeria monocytogenes*.

The overall prevalence rate of *Salmonella* spp in goats of Chittagong Metropolitan area was recorded as 20.5% which is significant and should not be overlooked, because of the public health significance and the possibility of dissemination of diseases in man, animals and birds. The prevalence rate of *Salmonella* spp in goats of this study is near to the findings of some other studies conducted in Bangladesh as well as in abroad (Ferede et al., 2015; Sahaet al., 2014; Zaman et al., 2018). On the other hand, very low prevalence (0.1%) in adult diarrheic goats was also reported (Mahmood et al., 2014).

This study recognized breed as a potential risk factor for the infection of salmonella in goat (OR= 27.3, cross versus Jamnapari) and (OR= 22.8, Black Bengal versus Jamnapari). High prevalence of *Salmonella* spp in Black Bengal and Cross breeds in compare to Jamnapari was also identify previously (Zaman et al., 2018). In this study, prevalence of *Salmonella* spp was somewhat higher in adult goats compare to the young which is supported by Zaman et al. (2018) but in contrast to the findings of another study (Saha et al., 2014) . This study also identified the source of animals as a potential risk factor (OR= 3.1, familly versus farm), family livestock are much more susceptible to salmonellosis due to poor hygienic management in the family compare to farm which is similar to the findings previously reported (Chandra et al., 2006).

Salmonellosis is one of important disease that causes diarrhea in goats. Three common conditions caused by *Salmonella* are gastroenteritis, enteric fever, and bacteremia. In this study, fever, anorexia, dehydration and mucus in feces were most commonly encountered in goat infected with salmonella but there were few other signs that were also observed.

Clinical signs recorded in this study is accordance with signs mentioned by Blood and Radostits (Radostits et al., 2007)

All the Salmonella isolates were tested against ten antibiotics of different groups. Highest sensitivity of Ciprofloxacin indicates that fluoroquinolone still be the first choice of salmonella infected patient. Chloramphenicol was also suggested as a drug of choice in salmonellosis in goats (Chandra et al., 2006) . In this study Salmonellae isolates were highly sensitive to Ciprofloxacin, Cefotaxime and Sulfamethoxazole, intermediately sensitive against Gentamycin and Doxycycline and resistant against Penicillin, Amoxicillin and Tetracycline which is close to the several findings reported in home and abroad (Ferede et al., 2015; Saha et al.,, 2013).

Chapter-6: Conclusion

Infectious diseases are important constraint for development of goat farming in Bangladesh. In this study, overall prevalence of *Staphylococcus aureus*, *Clostridium perfringens*, *Listeria monocytogenes* and *Salmonella* spp. In goat were recorded as 11.5%, 3.5%, 1% and 20.5%, respectively. Females were found to be more susceptible to *Staphylococcus aureus* infection in goats than male. Male goats and Cross breeds acted as potential risk factors and significantly higher contribution to the occurrence of *Clostridium perfringens* infecton. Black Bengal and Cross breeds along with family goats had significantly higher contribution to the occurrence of salmonellosis in goats. Ciprofloxacin found sensitive against *Staphylococcus aureus* (44%) and *Salmonella* spp. (54%) but resistant against *Clostridium perfringens* (57%). Penicillin showed sensitive against *Clostridium perfringens* (57%) and *Lysteria monocytogenes* (100%) infection in goats whereas resistant against *Staphylococcus aureus* (62%) and *Salmonella* spp. (56%). Amoxicillin was highly sensitive against *Staphylococcus aureus* (48%) and resistant against *Salmonella* spp. (59%). So we can say that, rapid diagnostic methods like multiplex PCR were effective for confirmation of specific pathogens from goats showing non-specific clinical signs. This present study also able to identify some potential risk factors responsible for those disease and antimicrobials that were effective against those organisms.

Chapter-7: Recommendation

- Due to time and resource limitation we conducted the study in small scale. In future the study can be conducted involving higher sample size.
- The current study proposes some common signs for the diagnosis of staphylococcosis, clostridiosis, listeriosis and salmonelosis in goats which is based on the molecular detection of organisms.

Chapter-8: References

Ajaz-ul-Haq, Muhammad Kamran Taj, Taj, Imran, Arif, Sana, Ahmed, Ashfaq, Muhammad, Ghulam, Ahmed, Zaheer. Samad, Abdul (2016). Isolation of *Clostridium perfringens* from Goats and Sheep of the Khuzdar district of Balochistan, Pakistan.

Ajuwape, ATP, Roberts, AA, Solarin, OO, & Adetosoye, AI. (2005). Bacteriological and haematological studies of clinical mastitis in goats in Ibadan, OYO State, Nigeria. *Small Ruminant Research, 60*(3), 307-310.

Al-Habsi, Khalid, Yang, Rongchang, Abraham, Sam, Ryan, Una, Miller, David, & Jacobson, Caroline. (2018). Molecular characterisation of Salmonella enterica serovar Typhimurium and Campylobacter jejuni faecal carriage by captured rangeland goats. *Small Ruminant Research, 158*, 48-53.

Amagliani, G, Giammarini, C, Omiccioli, E, Brandi, G, & Magnani, M. (2007). Detection of *Listeria monocytogenes* using a commercial PCR kit and different DNA extraction methods. *Food Control, 18*(9), 1137-1142.

Amin, MR. (2000). *Genetic Improvement of Production Traits in Bangladesh Goats by Selective Breeding and Crossbreeding.* Ph. D. Thesis, Department of Animal Breeding and Genetics, Bangladesh Agricultural University, Mymensingh.

Amin, MR, Husain, SS, & Islam, ABMM. (2001). Reproductive peculiarities and litter weight in goats.

Argudín, María Ángeles, Mendoza, María Carmen, & Rodicio, María Rosario. (2010). Food poisoning and *Staphylococcus aureus* enterotoxins. *Toxins, 2*(7), 1751-1773.

Arita, M, Takeda, T, Honda, T, & Miwatani, T. (1986). Purification and characterization of *Vibrio cholerae* non-O1 heat-stable enterotoxin. *Infection and immunity, 52*(1), 45-49.

Aulisio, CC, Hill, WALTER E, Stanfield, JOHN T, & Sellers, RL. (1983). Evaluation of virulence factor testing and characteristics of pathogenicity in Yersinia enterocolitica. *Infection and immunity, 40*(1), 330-335.

Barenfanger, Joan, Drake, Cheryl, & Kacich, Gail. (1999). Clinical and financial benefits of rapid bacterial identification and antimicrobial susceptibility testing. *Journal of clinical microbiology, 37*(5), 1415-1418.

Baumann, Paul. (1984). Family II. Vibrionaceae Veron 1965, 5245. *Bergey's manual of systematic bacteriology*, 516-550.

Bauwens, Luc, Vercammen, Francis, & Hertsens, Anja. (2003). Detection of pathogenic *Listeria* spp. in zoo animal faeces: use of immunomagnetic separation and a chromogenic isolation medium. *Veterinary microbiology, 91*(2-3), 115-123.

Begum, IA, Alam, MJ, Buysse, J, Frija, A, & Van Huylenbroeck, G. (2011). A comparative efficiency analysis of poultry farming systems in Bangladesh: A Data Envelopment Analysis approach. *Applied Economics, 44*(44), 3737-3747.

Bhaduri, SAUMYA, Turner-Jones, CAROLYN, Taylor, MARYANN M, & Lachica, R VICTOR. (1990). Simple assay of calcium dependency for virulent plasmid-bearing clones of Yersinia enterocolitica. *Journal of clinical microbiology, 28*(4), 798-800.

Bisgaard, M, Sakazaki, R, & Shimada, T. (1978). Prevalence of non-cholera vibrios in cavum nasi and pharynx of ducks. *Acta Pathologica Microbiologica Scandinavica Section B Microbiology, 86*(1-6), 261-266.

Brakstad, Odd G, Aasbakk, Kjetill, & Maeland, Johan A. (1992). Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. *Journal of clinical microbiology, 30*(7), 1654-1660.

Brakstad, Odd G, Maeland, Johan A, & Wergeland, Heidrun I. (1989). Serum antibodies to a *Staphylococcus aureus* thermonuclease preparation in healthy persons and

patients with bacteremia. *Serodiagnosis and Immunotherapy in Infectious Disease, 3*(3), 201-210.

Braun, U, Stehle, C, & Ehrensperger, F. (2002). Clinical findings and treatment of listeriosis in 67 sheep and goats. *Veterinary Record, 150*(2), 38-41.

Bulgin, MS, & Anderson, BC. (1981). Salmonellosis in goats. *Journal of the American Veterinary Medical Association, 178*(7), 720-723.

Callegan, Michelle C, Booth, Mary C, Jett, Bradley D, & Gilmore, Michael S. (1999). Pathogenesis of gram-positive bacterial endophthalmitis. *Infection and immunity, 67*(7), 3348-3356.

Campero, Carlos Manuel, Odeón, Anselmo Carlos, Cipolla, Ana Libertad, Moore, DP, Poso, María A, & Odriozola, E. (2002). Demonstration of *Listeria monocytogenes* by immunohistochemistry in formalin-fixed brain tissues from natural cases of ovine and bovine encephalitis. *Journal of Veterinary Medicine, Series B, 49*(8), 379-383.

Chakraborty, Soumen, Mukhopadhyay, Asish K, Bhadra, Rupak Kumar, Ghosh, Amar Nath, Mitra, Rupak, Shimada, Toshio, . Colwell, Rita R. (2000). Virulence genes in environmental strains of *Vibrio cholerae*. *Applied and environmental microbiology, 66*(9), 4022-4028.

Chandra, Mudit, Singh, BR, Shankar, Hari, Agarwal, Meenu, Agrawal, Ravi Kant, Sharma, Gautam, & Babu, N. (2006). Study on prevalence of Salmonella infection in goats. *Small Ruminant Research, 65*(1-2), 24-30.

Chen, Jin-Qiang, Healey, Stephanie, Regan, Patrick, Laksanalamai, Pongpan, & Hu, Zonglin. (2017). PCR-based methodologies for detection and characterization of *Listeria monocytogenes* and Listeria ivanovii in foods and environmental sources. *Food Science and Human Wellness, 6*(2), 39-59.

Chen, Yuansha, Johnson, Judith A, Pusch, Gordon D, Morris, J Glenn, & Stine, O Colin. (2007). The genome of non-O1 *Vibrio cholerae* NRT36S demonstrates the

presence of pathogenic mechanisms that are distinct from those of O1 Vibrio cholerae. *Infection and immunity*, *75*(5), 2645-2647.

Choudhury, MP, Sarker, SC, Islam, F, Ali, A, Bhuiyan, AKFH, Ibrahim, MNM, & Okeyo, AM. (2013). Morphometry and performance of Black Bengal goats at the rural community level in Bangladesh. *Bangladesh Journal of Animal Science*, *41*(2), 83-89.

Cox, Peter, Griffith, Merran, Angles, Mark, Deere, Daniel, & Ferguson, Christobel. (2005). Concentrations of pathogens and indicators in animal feces in the Sydney watershed. *Applied and environmental microbiology*, *71*(10), 5929-5934.

Daube, Georges, China, Bernard, Simon, P, Hvala, K, & Mainil, J. (1994). Typing of *Clostridium perfringens* by in vitro amplification of toxin genes. *Journal of Applied Microbiology*, *77*(6), 650-655.

Davis, Brigid M, & Waldor, Matthew K. (2003). Filamentous phages linked to virulence of *Vibrio cholerae*. *Current opinion in microbiology*, *6*(1), 35-42.

de Oliveira Júnior, Carlos Augusto, Silveira Silva, Rodrigo Otávio, Diniz, Amanda Nadia, Sadanã Pires, Prhiscylla, Masiero Salvarani, Felipe, Antunes de Assis, Ronnie, & Faria Lobato, Francisco Carlos. (2016). Antimicrobial susceptibility of *Clostridium perfringens* isolated from domestic and wild animal species in Brazil. *Semina: Ciências Agrárias*, *37*(1).

Dohoo, I, Martin, W, & Stryhn, H. (2003). Screening and diagnostic tests. *Veterinary epidemiologic research*, 85-120.

Doyle, MP. (1989). Foodborne Bacterial Pathogens. Marcel Decker. *Inc. New York and Basel*, 464-513.

Drobniewski, Francis A. (1993). Bacillus cereus and related species. *Clinical microbiology reviews*, *6*(4), 324-338.

Duffy, L, Barlow, R, Fegan, N, & Vanderlinde, P. (2009). Prevalence and serotypes of Salmonella associated with goats at two Australian abattoirs. *Letters in applied microbiology, 48*(2), 193-197.

Dworkin, Martin. (2006). *The Prokaryotes: Vol. 6: Proteobacteria: Gamma Subclass*: Springer Science & Business Media.

Embrey, Martha, Hunter, Paul, Chalmers, Rachel, Sellwood, Jane, & Wyn-Jones, Peter. (2004). *Microbiology of Waterborne Diseases*: Elsevier Science & Technology.

Emikpe, BO, Oyero, OG, & Akpavie, SO. (2009). Isolation and antibiogram of aerobic nasal bacterial flora of apparently healthy West African dwarf goats. *Revue d'elevage et de Medecine Veterinaire des Pays Tropicaux, 62*(1), 17-21.

Ershaduzzaman, M, Rahman, MM, Roy, BK, & Chowdhury, SA. (2007). Studies on the diseases and mortality pattern of goats under farm conditions and some factors affecting mortality and survival rates in Black Bengal kids. *Bangladesh Journal of Veterinary Medicine, 5*(1), 71-76.

Fàbrega, Anna, & Vila, Jordi. (2012). Yersinia enterocolitica: pathogenesis, virulence and antimicrobial resistance. *Enfermedades infecciosas y microbiologia clinica, 30*(1), 24-32.

Farmer, JJ, Carter, GP, Miller, VL, Falkow, S, & Wachsmuth, IK. (1992). Pyrazinamidase, CR-MOX agar, salicin fermentation-esculin hydrolysis, and D-xylose fermentation for identifying pathogenic serotypes of *Yersinia enterocolitica*. *Journal of clinical microbiology, 30*(10), 2589-2594.

Fentahun, Tewodros, & Fresebehat, Atsedewoyne. (2012). Listeriosis in small ruminants: a review. *Adv Biolog Res, 6*, 202-209.

Ferede, Beshatu, Desissa, Fanta, Feleke, Aklilu, Tadesse, Getachew, & Moje, Nebyu. (2015). Prevalence and antimicrobial susceptibility of Salmonella isolates from apparently healthy slaughtered goats at Dire Dawa municipal abattoir, Eastern Ethiopia. *Journal of Microbiology and Antimicrobials, 7*(1), 1-5.

Ferroni, Agnès, Suarez, Stéphanie, Beretti, Jean-Luc, Dauphin, Brunhilde, Bille, Emmanuelle, Meyer, Julie, . Nassif, Xavier. (2010). Real-time identification of bacteria and Candida species in positive blood culture broths by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *Journal of clinical microbiology, 48*(5), 1542-1548.

Fraga, S González, Pichel, Mariana, Costagliola, M, Cecilia, M, Jurquiza, Verónica, Peressutti, S, . Tracanna, BC. (2007). Environment and virulence factors of Vibrio cholerae strains isolated in Argentina. *Journal of applied microbiology, 103*(6), 2448-2456.

Gerros, TC. (1998). Recognizing and treating listeriosis in dairy goats. *Veterinary medicine (1985)(USA)*.

Gonzalez, Ruben N. (1996). *Prototheca, yeast, and Bacillus as a cause of mastitis.* Paper presented at the National Mastitis Council (US). Meeting (USA).

Granum, Per Einar, & Lund, Terje. (1997). Bacillus cereus and its food poisoning toxins. *FEMS microbiology letters, 157*(2), 223-228.

Gudding, ROAR. (1983). Differentiation of staphylococci on the basis of nuclease properties. *Journal of clinical microbiology, 18*(5), 1098-1101.

Gulani, Isa Adamu, Geidam, Yaqub Ahmed, Adamu, Lawan, Lawal, JR, & Abadam, Falmata Ali. (2016). Prevalence and phenotypic detection of methicillin resistance *Staphylococcus aureus* between ruminants butchered for humanoid intake and animal handlers in Maiduguri, Nigeria. *Journal of Advanced Veterinary & Animal Research, 3*(2).

Hansen, Wendy LJ, Bruggeman, Cathrien A, & Wolffs, Petra FG. (2009). Evaluation of new preanalysis sample treatment tools and DNA isolation protocols to improve bacterial pathogen detection in whole blood. *Journal of clinical microbiology, 47*(8), 2629-2631.

Heidelberg, John F, Eisen, Jonathan A, Nelson, William C, Clayton, Rebecca A, Gwinn, Michelle L, Dodson, Robert J, . Umayam, Lowell. (2000). DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*. *Nature, 406*(6795), 477.

Hennekinne, Jacques-Antoine, Ostyn, Annick, Guillier, Florence, Herbin, Sabine, Prufer, Anne-Laure, & Dragacci, Sylviane. (2010). How should staphylococcal food poisoning outbreaks be characterized? *Toxins, 2*(8), 2106-2116.

Herrero, Mario, Grace, Delia, Njuki, Jemimah, Johnson, Nancy, Enahoro, Dolapo, Silvestri, Silvi, & Rufino, Mariana C. (2013). The roles of livestock in developing countries. *Animal, 7*(s1), 3-18.

Hilal, Sobiya. (2016). *PREVALENCE OF Listeria monocytogenes IN SHEEP AND GOAT FLOCKS OF NOMADS.* Division of Veterinary Public Health and Epidemiology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu.

Hirst, K Kris. (2008). The History of the domestication of goats. *About. com*.

Honda, T, Lertpocasombat, KANCHALEE, Hata, A, Miwatani, T, & Finkelstein, RA. (1989). Purification and characterization of a protease produced by Vibrio cholerae non-O1 and comparison with a protease of *V. cholerae* O1. *Infection and immunity, 57*(9), 2799-2803.

Howell, Deborah. (1972). Survey on mastitis caused by environmental bacteria. *Veterinary Record, 90*(23), 654-657.

Islama, F (2016). Black Bengal goat keeping at Mymensingh sadar upazila in Bangladesh. *Journal of Bioscience and Agriculture Research, 6*(02), 541-546.

Janda, JM, Powers, C, Bryant, RG, & Abbott, SL. (1988). Current perspectives on the epidemiology and pathogenesis of clinically significant *Vibrio* spp. *Clinical Microbiology Reviews, 1*(3), 245-267.

Järvinen, Anna-Kaarina, Laakso, Sanna, Piiparinen, Pasi, Aittakorpi, Anne, Lindfors, Merja, Huopaniemi, Laura, . Mäki, Minna. (2009). Rapid identification of

bacterial pathogens using a PCR-and microarray-based assay. *BMC microbiology*, *9*(1), 161.

Jiwa, SFH, Kazwala, RR, & Namahungu, E. (1994). Prevalence of Campylobacter spp. in clinically normal goats kept under various management systems in urban Tanzania. *Small Ruminant Research, 15*(1), 97-100.

Johnson, Gayle Christy, Maddox, Carol Wolfgang, Fales, William Harold, Wolff, William Allmond, Randle, Richard Fox, Ramos, Jose Antonio, . Wesley, Irene Varelas. (1996). Epidemiologic evaluation of encephalitic listeriosis in goats. *Journal of the American Veterinary Medical Association*, *208*(10), 1695-1699.

Julander, IG, Granström, M, Hedström, SÅ, & Möllby, R. (1983). The role of antibodies against alpha-toxin and teichoic acid in the diagnosis of staphylococcal infections. *Infection*, *11*(2), 77-83.

KALENDER, HAKAN. (2003). Detection of *Listeria monocytogenes* in faeces from chickens, sheep and cattle in Elazığ province. *Turkish Journal of Veterinary and Animal Sciences*, *27*(2), 449-451.

Kalogridou-Vassiliadou, D. (1991). Mastitis-related pathogens in goat milk. *Small Ruminant Research*, *4*(2), 203-212.

Kandolo, K, & Wauters, Georges. (1985). Pyrazinamidase activity in *Yersinia enterocolitica* and related organisms. *Journal of clinical microbiology, 21*(6), 980-982.

Kapatral, Vinayak, Olson, John W, Pepe, Jeffrey C, Miller, Virginia L, & Minnich, Scott A. (1996). Temperature-dependent regulation of *Yersinia enterocolitica* Class III flagellar genes. *Molecular microbiology, 19*(5), 1061-1071.

Kaper, JB, Lockman, H, Colwell, RR, & Joseph, SW. (1979). Ecology, serology, and enterotoxin production of Vibrio cholerae in Chesapeake Bay. *Applied and Environmental Microbiology, 37*(1), 91-103.

Kashem, MA, Hossain, MA, Ahmed, SS U, & Halim, MA. (2012). Prevalence of diseases, morbidity and mortality of Black Bengal Goats under different management systems in Bangladesh. *University Journal of Zoology, Rajshahi University, 30*, 01-04.

Kerremans, JJ, Verboom, Paul, Stijnen, Theo, Hakkaart-van Roijen, L, Goessens, Wil, Verbrugh, HA, & Vos, MC. (2007). Rapid identification and antimicrobial susceptibility testing reduce antibiotic use and accelerate pathogen-directed antibiotic use. *Journal of antimicrobial chemotherapy, 61*(2), 428-435.

Klimešová, Marcela, Manga, Ivan, Nejeschlebová, Ludmila, Horáček, Jiří, Ponížil, Antonín, & Vondrušková, Eva. (2017). Occurrence of *Staphylococcus aureus* in cattle, sheep, goat, and pig rearing in the Czech Republic. *Acta Veterinaria Brno, 86*(1), 3-10.

Kovacevic, Steven, Veal, LE, Hsiung, HM, & Miller, JR. (1985). Secretion of staphylococcal nuclease by *Bacillus subtilis*. *Journal of bacteriology, 162*(2), 521-528.

Kramer, JOHN M, & Gilbert, RICHARD J. (1989). Bacillus cereus and other Bacillus species. *Foodborne bacterial pathogens, 19*, 21-70.

Krogstad, O, Teige, J, & Lassen, J. (1972). Yersinia enterocolitica type 2 associated with disease in goat. *Acta veterinaria scandinavica*.

Lachica, R Victor F, Hoeprich, Paul D, & Riemann, Hans P. (1972). Tolerance of staphylococcal thermonuclease to stress. *Applied microbiology, 23*(5), 994-997.

Lachica, RVF, Genigeorgis, C, & Hoeprich, PD. (1971). Metachromatic agar-diffusion methods for detecting staphylococcal nuclease activity. *Applied microbiology, 21*(4), 585-587.

Lastovica, Albert J. (2006). Emerging Campylobacter spp.: the tip of the iceberg. *Clinical Microbiology Newsletter, 28*(7), 49-56.

Lastovica, Albert J, & Allos, Ban Mishu. (2008). Clinical significance of Campylobacter and related species other than *Campylobacter jejuni* and *Campylobacter coli Campylobacter, Third Edition* (pp. 123-149): American Society of Microbiology.

Law, Jodi Woan-Fei, Ab Mutalib, Nurul-Syakima, Chan, Kok-Gan, & Lee, Learn-Han. (2015). Rapid methods for the detection of foodborne bacterial pathogens: principles, applications, advantages and limitations. *Frontiers in microbiology, 5*, 770.

Le Monnier, Alban, Abachin, Eric, Beretti, Jean-Luc, Berche, Patrick, & Kayal, Samer. (2011). Diagnosis of Listeria monocytogenes meningoencephalitis by real-time PCR for the hly gene. *Journal of clinical microbiology, 49*(11), 3917-3923.

Lee, Nari, Kwon, Kyung Yoon, Oh, Su Kyung, Chang, Hyun-Joo, Chun, Hyang Sook, & Choi, Sung-Wook. (2014). A multiplex PCR assay for simultaneous detection of *Escherichia coli* O157: H7, *Bacillus cereus*, *Vibrio parahaemolyticus*, *Salmonella* spp., *Listeria monocytogenes*, and *Staphylococcus aureus* in Korean ready-to-eat food. *Foodborne pathogens and disease, 11*(7), 574-580.

Li, Ruichao, Wang, Yang, Shen, Jianzhong, & Wu, Congming. (2014). Development of a novel hexa-plex PCR method for identification and serotyping of *Salmonella* species. *Foodborne pathogens and disease, 11*(1), 75-77.

Liu, Dongyou. (2006). Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important foodborne pathogen. *Journal of medical microbiology, 55*(6), 645-659.

Low, JC, & Donachie, William. (1997). A review of *Listeria monocytogenes* and listeriosis. *The Veterinary Journal, 153*(1), 9-29.

Madison, BERENEICE M, & Baselski, VICKIE S. (1983). Rapid identification of *Staphylococcus aureus* in blood cultures by thermonuclease testing. *Journal of clinical microbiology, 18*(3), 722-724.

Mahmood, AK, Khan, MS, Khan, MA, Khan, MA, & Bilal, M. (2014). Prevalence of salmonella in diarrheic adult goats in field conditions. *The Journal of Animal and Plant Sciences, 24*(1), 98-102.

Mandal, PK, Biswas, AK, Choi, K, & Pal, UK. (2011). Methods for rapid detection of foodborne pathogens: an overview. *American Journal Of Food Technology, 6*(2), 87-102.

Markey, BK, Leonard, FC, & Archambault, M. (2013). Collection and submission of diagnostic specimens. *Clinical Veterinary Microbiology*, 3-7.

Masalha, Mahmud, Borovok, Ilya, Schreiber, Rachel, Aharonowitz, Yair, & Cohen, Gerald. (2001). Analysis of Transcription of the *Staphylococcus aureus* Aerobic Class Ib and Anaerobic Class III Ribonucleotide Reductase Genes in Response to Oxygen. *Journal of bacteriology, 183*(24), 7260-7272.

Mavangira, Vengai, Angelos, John A, Samitz, Eileen M, Rowe, Joan D, & Byrne, Barbara A. (2013). Gangrenous mastitis caused by Bacillus species in six goats. *Journal of the American Veterinary Medical Association, 242*(6), 836-843.

McClane, Bruce A, Uzal, Francisco A, Miyakawa, Mariano E Fernandez, Lyerly, David, & Wilkins, Tracy. (2006). The enterotoxic clostridia *The prokaryotes* (pp. 698-752): Springer.

McDonel, James L. (1986). Toxins of *Clostridium perfringens* types a, B, C, D and E. *Pharmacology of bacterial toxins*, 477-517.

McOrist, S, & Miller, GT. (1981). Salmonellosis in transported feral goats. *Australian veterinary journal, 57*(8), 389-390.

Mishra, AK, Sharma, Nitika, Singh, DD, & Gururaj, K. (2018). Prevalence and bacterial etiology of subclinical mastitis in goats reared in organized farms. *Veterinary world, 11*(1), 20.

Momin, MA, Islam, MA, Khatun, MM, & Rahman, MM. (2012). Characterization of bacteria associated with pneumonia in black bengal goats. *Bangladesh Journal of Veterinary Medicine, 9*(1), 67-71.

Nath, Tilak Chandra, Bhuiyan, Md Jamal Uddin, Mamun, MA, Datta, Real, Chowdhury, SK, Hossain, M, & Alam, Mohammad Shafiul. (2014). Common infectious diseases of goats in Chittagong district of Bangladesh. *Int. J. Sci. Res. Agric. Sci, 1*, 43-49.

Nathawat, Prerna, Bhati, Taruna, Sharma, Sandeep K, Mohammed, Nazeer, & Kataria, Anil K. (2013). Prevalence of *Staphylococcus aureus* in lactating goats with clinical mastitis and their antibiogram studies. *Animal Biology & Animal Husbandry, 5*(1).

Nazki, Salik, Wani, Shakil A, Parveen, Rafia, Ahangar, Showkat A, Kashoo, Zahid A, Hamid, Syed, . Dar, Pervaiz A. (2017). Isolation, molecular characterization and prevalence of *Clostridium perfringens* in sheep and goats of Kashmir Himalayas, India. *Veterinary world, 10*(12), 1501.

Niilo, L. (1980). Clostridium perfringens in animal disease: a review of current knowledge. *The Canadian Veterinary Journal, 21*(5), 141.

Nikolaou, K, Hensel, A, Bartling, C, Tomaso, H, Arnold, T, Rösler, U, . . . Neubauer, H. (2005). Prevalence of anti-Yersinia outer protein antibodies in goats in Lower Saxony. *Journal of Veterinary Medicine, Series B, 52*(1), 17-24.

Oakley, CL, & Warrack, G Harriet. (1953). Routine typing of *Clostridium welchii*. *Epidemiology & Infection, 51*(1), 102-107.

Olubunmi, PA, & Adeniran, MOA. (1986). Isolation of Campylobacter species from man and domestic animals in the western part of Nigeria. *Bulletin of animal health and production in Africa= Bulletin de la sante et de la production animales en Afrique*.

Osman, KM, & Elhariri, M. (2013). Antibiotic resistance of *Clostridium perfringens* isolates from broiler chickens in Egypt. *Rev Sci Tech, 32*(3), 841-850.

Pa, Wayne. (2006). Clinical and Laboratory Standard Institute C. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A7. Clinical and Laboratory Standard Institute*.

Pacini, Filippo. (1854). *Osservazioni microscopiche e deduzioni patologiche sul cholera asiatico*: tip. di F. Bencini.

Parvez, MA, Faruque, MR, Sutradhar, BC, Rahman, MM, Mannan, A, & Khatun, R. (2014). Clinical diseases and manifestations of goats and cattle recorded at teaching veterinary hospital in Chittagong Veterinary and Animal Sciences University. *Bangladesh Journal of Veterinary Medicine, 12*(1), 73-81.

Petersson-Wolfe, Christina S, Mullarky, Isis K, & Jones, Gerald Murray. (2010). *Staphylococcus aureus* mastitis: cause, detection, and control.

Porrero, M Concepción, Hasman, Henrik, Vela, Ana I, Fernández-Garayzábal, Jose F, Domínguez, Lucas, & Aarestrup, Frank M. (2012). Clonal diversity of *Staphylococcus aureus* originating from the small ruminants goats and sheep. *Veterinary microbiology, 156*(1-2), 157-161.

Postollec, Florence, Falentin, Hélène, Pavan, Sonia, Combrisson, Jérôme, & Sohier, Danièle. (2011). Recent advances in quantitative PCR (qPCR) applications in food microbiology. *Food microbiology, 28*(5), 848-861.

Prpic, J Kaya, Robins-Browne, Roy M, & Davey, R Brent. (1983). Differentiation between virulent and avirulent Yersinia enterocolitica isolates by using Congo red agar. *Journal of clinical microbiology, 18*(3), 486-490.

Radostits, OM, Gay, C, Hinchcliff, Kenneth W, & Constable, Peter D. (2007). A textbook of the diseases of cattle, sheep, goats, pigs and horses. *Veterinary Medicine 10th edition Bailliere, Tindall, London, UK*, 1576-1580.

Rahn, K, De Grandis, SA, Clarke, RC, McEwen, SA, Galan, JE, Ginocchio, C, . Gyles, CL. (1992). Amplification of an invA gene sequence of Salmonella typhimurium by polymerase chain reaction as a specific method of detection of Salmonella. *Molecular and cellular probes*, *6*(4), 271-279.

Raji, MA, Adekeye, JO, Kwaga, JKP, & Bale, JOO. (2000). Bioserogroups of Campylobacter species isolated from sheep in Kaduna State, Nigeria. *Small Ruminant Research, 37*(3), 215-221.

Richards, RB, Norris, RT, Dunlop, RH, & McQuade, NC. (1989). Causes of death in sheep exported live by sea. *Australian veterinary journal, 66*(2), 33-38.

Roberson, JR, Fox, LK, Hancock, DD, Gay, JM, & Besser, TE. (1994). Ecology of *Staphylococcus aureus* isolated from various sites on dairy farms1. *Journal of dairy science, 77*(11), 3354-3364.

Robinson-Dunn, Barbara. (2002). The microbiology laboratory's role in response to bioterrorism. *Archives of pathology & laboratory medicine*, *126*(3), 291-294.

Rood, Julian I. (2006). *Clostridium perfringens* and histotoxic disease *The Prokaryotes* (pp. 753-770): Springer.

Saha, Gobindha Kumar, Paul, Ashit Kumar, Abdus samad, M, & Khan, M Shahidur Rahman. (2013). Epidemiological Investigation and Antibiotic Sensitivity of Salmonellosis in Goats at the Selected Areas of Bangladesh. *Journal of Embryo Transfer, 28*.

Saha, Gobindha Kumar, Paul, Ashit Kumar, Samad, Mohamed Abdus, Islam, Mohammad Ariful, & Khan, Mohammad Shahidur Rahman. (2014). Prevalence of Salmonella associated with goats in Bangladesh. *Suranaree Journal of Science & Technology*, *21*(3).

Saleh, Nahed S, & Allam, Tamer S. (2014). Pneumonia in sheep: bacteriological and clinicopathological studies. *American J. of Research Communication, 2*(11), 70-88.

Sanyal, SC, Singh, SJ, Tiwari, IC, Sen, PC, Marwah, SM, Hazarika, UR, . Sakazaki, R. (1974). Role of household animals in maintenance of cholera infection in a community. *Journal of infectious Diseases, 130*(6), 575-579.

Sarangi, Laxmi Narayan, & Panda, HK. (2012). Isolation, characterization and antibiotic sensitivity test of pathogenic Listeria species in livestock, poultry and farm environment of Odisha. *Indian Journal of Animal Research, 46*(3).

Savini, Vincenzo. (2016). *Bacillus cereus* Disease in Animals *The Diverse Faces of Bacillus cereus* (pp. 107-115): Elsevier.

Schukken, Ynte H, González, Ruben N, Tikofsky, Linda L, Schulte, Hal F, Santisteban, Carlos G, Welcome, Frank L, . Zadoks, Ruth N. (2009). CNS mastitis: Nothing to worry about? *Veterinary microbiology, 134*(1-2), 9-14.

Senesi, Sonia, & Ghelardi, Emilia. (2010). Production, secretion and biological activity of Bacillus cereus enterotoxins. *Toxins, 2*(7), 1690-1703.

Sharma, Alok K, Tripathi, BN, Verma, JC, & Parihar, NS. (2001). Experimental Salmonella enterica subspecies enterica serovar Typhimurium infection in Indian goats: clinical, serological, bacteriological and pathological studies. *Small Ruminant Research, 42*(2), 125-134.

Shewan, JM. (1974). Genus I Vibrio Pacini 1854. *Bergey's manual of determinative bacteriology*.

Slee, KJ, & Button, C. (1990). Enteritis in sheep and goats due to *Yersinia enterocolitica* infection. *Australian veterinary journal, 67*(11), 396-398.

Songer, J Glenn. (1996). Clostridial enteric diseases of domestic animals. *Clinical microbiology reviews, 9*(2), 216.

Sterne, M, & Batty, I. (1975). Criteria for diagnosing clostridial infection, p79–122. In. Pathogenic clostridia: Butterworths, London, United Kingdom.

Swaminathan, B, Harmon, MC t, & Mehlman, IJ. (1982). A review: *Yersinia enterocolitica*. *Journal of Applied Bacteriology, 52*(2), 151-183.

Tenhagen, Bernd-Alois, Hansen, Inken, Reinecke, Annette, & Heuwieser, Wolfgang. (2009). Prevalence of pathogens in milk samples of dairy cows with clinical mastitis and in heifers at first parturition. *Journal of dairy research, 76*(2), 179-187.

Thoerner, P, Kingombe, CI Bin, Bögli-Stuber, K, Bissig-Choisat, B, Wassenaar, TM, Frey, J, & Jemmi, T. (2003). PCR detection of virulence genes in Yersinia enterocolitica and Yersinia pseudotuberculosis and investigation of virulence gene distribution. *Applied and environmental microbiology, 69*(3), 1810-1816.

Titball, Richard W, & Rood, Julian I. (2002). *Clostridium perfringens*: wound infections *Molecular medical microbiology* (pp. 1875-XII): Elsevier.

Trinidad, P, Nickerson, SC, & Alley, TK. (1990). Prevalence of Intramammary Infection and Teat Canal Colonization In Unbred and Primigravid Dairy Heifers1. *Journal of Dairy Science, 73*(1), 107-114.

Uzal, Francisco A, Giannitti, Federico, Finnie, John W, & García, Jorge P. (2016). Diseases produced by *Clostridium perfringens* type D. *Clostridial Diseases of Animals; Wiley Blackwell: Ames, IA, USA*, 157-172.

Vaidya, GR, Chaudhary, SP, Zade, NN, Khan, WA, Shinde, SV, Patil, A, & Kalambhe, DG. (2018). Prevalence, Virulence and Antibiotic Susceptibility of *Listeria monocytogenes* Recuperated from Slaughtered Goats and Pigs of Nagpur, Central India. *Int. J. Curr. Microbiol. App. Sci, 7*(4), 1566-1578.

Vanderhaeghen, Wannes, Hermans, Katleen, Haesebrouck, Freddy, & Butaye, Patrick. (2010). Methicillin-resistant *Staphylococcus aureus* (MRSA) in food production animals. *Epidemiology & Infection, 138*(5), 606-625.

Verbrugh, Henri A, Nelson, Robert D, Peterson, Phillip K, Wilkinson, Brian J, & Thompson, Rodney L. (1983). Serology of *Staphylococcus aureus* infections

using multiple antigens and serial serum samples. *The Journal of infectious diseases, 148*(3), 608-608.

Verbrugh, Henri A, Peters, Roel, Goessens, Wil HF, & Michel, Marc F. (1986). Distinguishing complicated from uncomplicated bacteremia caused by *Staphylococcus aureus*: the value of "new" and "old" serological tests. *Journal of Infectious Diseases, 153*(1), 109-115.

Visser, IJR, ter Laak, EA, van Dijk, NW, & Wouda, W. (1991). Toxigenic *Vibrio cholerae* non-O: 1 isolated from a goat in the Netherlands. *Veterinary Quarterly, 13*(2), 114-118.

Watts, Jeffrey L. (1988). Etiological agents of bovine mastitis. *Veterinary microbiology, 16*(1), 41-66.

Winblad, S. (1967). Studies on serological typing of *Yersinia enterocolitica*. *Acta Pathol. Microbiol. Scand. Suppl, 187*, 115.

Xayarath, Bobbi, Smart, Jennifer I, Mueller, Kimberly J, & Freitag, Nancy E. (2011). A novel C-terminal mutation resulting in constitutive activation of the *Listeria monocytogenes* central virulence regulatory factor PrfA. *Microbiology, 157*(11), 3138-3149.

Yadav, MM, & Roy, A. (2009). Prevalence of Listeria spp including *Listeria monocytogenes* from apparently healthy sheep of Gujarat State, India. *Zoonoses and public health, 56*(9-10), 515-524.

Zachary, James F, & McGavin, M Donald. (2013). *Pathologic Basis of Veterinary Disease-E-Book*: Elsevier Health Sciences.

Zaman, Sharmin, Ahad, Abdul, & Sarker, Md Samun. (2018). Isolation and identification of buccal and intestinal bacteria in goats in Chittagong, Bangladesh. *Int. J. Adv. Res. Biol. Sci, 5*(4), 64-71.

Zhang, Rong-Guang, Scott, David L, Westbrook, Mary L, Nance, Sharon, Spangler, Brenda D, Shipley, Graham G, & Westbrook, Edwin M. (1995). The three-

dimensional crystal structure of cholera toxin. *Journal of molecular biology*, *251*(4), 563-573.

Zhao, Xihong, Lin, Chii-Wann, Wang, Jun, & Oh, Deog Hwan. (2014). Advances in rapid detection methods for foodborne pathogens. *J. Microbiol. Biotechnol*, *24*(3), 297-312.

Zhou, Zuoyong, Zhang, Mengsi, Li, Hexian, Yang, Haoyue, Li, Xiaoxia, Song, Xinyue, & Wang, Zhiying. (2017). Prevalence and molecular characterization of *Staphylococcus aureus* isolated from goats in Chongqing, China. *BMC veterinary research*, *13*(1), 352.

Annex-I

Questionnaire for collecting relevant data from goat having fever and/or diarrhea

Case Reg No: **Date: ……/……/…….**

a. Species:

b. Age: ○ Neonate ○ Juvenile ○ Sub adult ○ Adult

c. Sex: ○ Male ○ Female ○ Castrate

d. Source of patient ○ Family livestock ○ Farm

e. Vaccination ○ Yes ○ No

f. Deworming ○ Yes ○ No

g. Is this animal suffered from any other disease(s) in last 6 months?

○ Yes ○ No

h. If yes, what kind of disease(s)?

○ Viral ○ Bacterial ○ Parasitic ○ Others

i. Is this animal treated with any antibiotic in last 6 months?

○ Yes ○ No

j. Duration of ongoing infection?

○

k. Clinical sign(s) of ongoing infection?

-
-
-
-
-

l. System affected due to ongoing infection?

- Digestive
- Respiratory
- Circulatory
- Uro-genital
- Integumentary
- Others

m. Tentative diagnosis of ongoing infection?

-

n. Antimicrobials Used for the treatment of ongoing infection?

-

Brief biography

Pranab Paul passed the Secondary School Certificate Examination in 2007 followed by Higher Secondary Certificate Examination in 2009. He obtained his Doctor of Veterinary Medicine Degree in 2014 (held in 2015) from Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh. Now, he is a candidate for the degree of MS in Medicine under the Department of Medicine and Surgery, Faculty of Veterinary Medicine, CVASU. He has immense interest to research in zoonotic and wildlife medicine.