Research Article

Bacteriological analysis of blood samples collected from goats in altered behavior

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Abstract

Present investigation was based on isolation and identification of bacterial isolates occurs in blood samples taken from 50 healthy and 50 unhealthy goats during the period from February 2017 to January 2018. A total of 100 blood samples were taken from goats to find out their bacterial burden. Out of these samples, 45 samples of unhealthy goats were found to be infected by several harmful bacteria, which reveals their prevalence as, *Enterobacter aerogenes* (6.6%), *Escherichia coli* (8.8%), *Streptococcus bovis* (8.8%), *Klebsiella pneumoniae* (11.1%), *Corynebacterium pseudotuberculosis* (11.1%), *Streptococcus agalactiae* (11.1%), *Listeria monocytogenes* (13.3%), *Staphylococcus epidermidis* (13.3%), *Staphylococcus aureus* (15.5%). These bacterial pathogens may particularly cause harmful infections, which could be responsible for economic losses in goat farms, reduction of milk and meat productions enterprises. Therefore, our present work would be helpful in prevention and control of certain diseases that could be transmitted to human by milk and meat products. Moreover, as goat is also an indispensable source of income, therefore our present information may also enable the farmers to become more attractive in goat breeding and enable them in rising yield levels. Furthermore, in many countries, goat blood samples are more readily accessible than the other ruminants, thence have also been used an alternative source of blood for the usage in bacteriological culture media. Our present work is one of very few studies that had investigated the bacteriological analysis of blood samples of goats, and would be helpful for assessing their effects on goat health as well as its consumer's health.

Keywords: Blood samples; Goat diseases; Pathogenic bacteria

Introduction

The term "behavior" can be defined as the whole activities, which the organism shows in response to certain external and internal stimuli received from its environment. In fact, it is the response of any individual or group or species according to its environmental conditions. It's also helps the

organism in finding food, water, shelter, mates, as well as in avoiding certain unfavorable environmental conditions i.e., protection from enemies, food scarcity, floods, fires etc. Therefore, now behavior can be classified into three major categories e.g., learned, inherit and social behaviors. Hence, behavior is a very essential criterion in

assessing the animal health or well-being [1]. Though animal behavior also varies greatly among different domesticated species of livestock; however, for the goat, temperature, daylight, land use, type of vegetation and season, breeds, stage of production, herd size, place for water, types of supplemental feedstuffs, and properties of diets provide in farms have been found the main factors that are influencing mostly on feeding and reproductive behaviors of goats in management practices [2-6]. Therefore, Knowledge about these behaviors is quite essential for raising the productivity of domesticated animals that are cultured as well as for enhancing the animal welfare of animals. As these domesticated animals are selected for the sake of profitable production of milk, meat and various other food products usually exhibited different behavior than their ancestors. Nowadays, most farmed animals are placed in captivity under more comfortable environments controlled by humans [7-11]. As goat is one of the widespread species of domesticated animals that can be raised in many altered environments in almost every parts of the world, because of its ability to adapt them in highly varied ecological regions and climate conditions. Therefore, all information related to raising goats and their behavior is essential to ensure their better health care, diets and environment conditions. Hence, knowledge and understanding of goat behavior would also be helpful to raise yield levels, which would provide more income to farmers, milk and meats production for consumers; thus, decreasing the stress on animal and its producer [12-14]. The common signs and symptoms regarding to goat health status is essential to be familiar for evaluating that whether goat is healthy or unhealthy, and could also be helpful to recognize clinical signs of certain diseases that are most common in goats. Therefore, farmers should have ability to assess the goat health

on a regular basis including, e.g., normal body condition, coat, and vital signs and symptoms of diseases caused by certain pathogens. In healthy status, a normal body temperature of goats is ranged from 101.5°F and 103.5°F, and respiration rate is about 12 to 15 breaths per minute depends upon on environmental temperature, heart rate is 70 and 80 beats/minute, show healthy hair coat, eating and drinking normally, bright eyes with pink eye membrane are good indications of overall health. Whereas, the unhealthy goat shows signs of abnormal eating habits, loss of appetite, depression, diarrhea, abnormal feces and discolored urine, swellings of certain body parts, dull eyes, running nose, abnormal feeding and reproductive behaviors [15].

Bacteria are the smallest creatures of the earth belonging to the prokaryotic family with different morphologies ranging from few micrometers in length [16]. Mostly they are found in the normal flora of gut and skin. Though majority of them are beneficial, but very few have shown harmful in their impacts and produce certain pathogenic diseases [17]. The bacteria can be identified by gram staining as, gram positive and gram negative bacteria [18]. The gram positive bacteria contain thick peptidoglycan layer, and purple when stain, while the gram negative bacteria contain the thin layer of peptidoglycan, and pink when stain [19]. Some pathogenic bacteria that are found in goats includes i.e., *Brucella* is small sized coccobacilli, gram negative bacteria belongs to the phylum Proteobacteria [20]. *Corynebacterium* is a genus belonging to the phylum Actinobacteria, which are gram positive non-spore forming rod shaped bacteria, which in some phases of life appears as club shaped [21]. *Enterococcus* is a gram positive large genus of lactic acid bacteria belonging to phylum Firmicutes, which appearing in pairs (diplococcus) or small chains [22].

Escherichia is a genus of gram negative, facultative anaerobic rod shaped bacteria belongs to the family Enterobacteriaceae [23]. In animals, especially in goats, *Escherichia coli* serotype O157:H7 caused the serious illness that may cause death [24]. *Listeria* belongs to phylum Firmicutes is a gram positive, non-spore producing, and facultative anaerobic bacteria [25]. *Listeria ivanovii* and *Listeria monocytogenes* are specifically affecting goats and can cause non-bloody diarrhea, dehydration, vomiting, and low grade fever [26]. *Streptococcus* is a genus of gram positive bacteria belongs to Phylum Firmicutes, which is differing from *Staphylococcus* because of its appearance in chains [22]. Further investigation on bacteriological analysis of goats and other ruminants have been done by [27-32].

Bacterial diseases that are commonly occurs in goats including e.g., *Anthrax* is caused by gram positive *Bacillus anthracis* [33]. Johne's disease (Bovine paratuberculosis) is a chronic, infectious bacterial disease of adult goats and is caused by *Mycobacterium paratuberculosis* [34], and shows symptoms included thickening of intestinal wall, weight loss gradually and chronic diarrhoea dehydration and severe cachexia [35]. *Mastitis* occur in cattle is caused by gram positive bacteria, such as, *Streptococcus agalactiae* [36], but it is also caused by different species of *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, and *Corynebacterium bovis* [37]. Symptoms include reduction in milk production, eyes narrowed to slits [38]. *Brucellosis* is caused by its species includes i.e., *Brucella melitensis*, *Brucella ovis*, *Brucella abortus*, *Brucella suis*, *Brucella neotomae*, *Brucella ovis* and *Brucella canis* [39], and shows the symptoms that are fever, night sweats, weakness, and hygromas on the knee joint [40]. *Listeriosis* is caused by *Listeria monocytogenes* and *Listeria ivanovii* belongs to genus *Listeria*, which is a gram

positive, anaerobic rod shaped bacteria that cause the severe infections in domestic animals [41, 42]. *Caseous lymphadenitis* is caused by the *Corynebacterium pseudotuberculosis* is very difficult to control due its chronic features [43], and forms the abscesses in lymph nodes internally and externally [44]. *Tetanus* is also a chronic disease, which is caused by the toxins of *Clostridium tetani* [45], and its symptoms includes, e.g., lock jaw, stiffness, head high and lameness [46]. *Enterotoxaemia* (Pulpy kidney) is a fatal disease in goats, which is caused by *Clostridium perfringens* and diagnosis by diarrhoea, involuntary contraction of body muscles, paralysis and sudden death. *Malignant edema* is a bacterial disease of goats is caused by *Clostridium septicum* and is manifested by deep wound infection with trauma, which provides ideal condition for its growth [27]. Moreover, tuberculosis, listeriosis, salmonellosis, toxoplasmosis, brucellosis, as well as streptococcal, staphylococcal and campylobacter infections are examples of some bacterial diseases that have been reported in raw milk and can be transmitted to humans. Prevalence of mastitis agents found in goats includes, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli* and *Pseudomonas* species have also been isolated from goat milk. Therefore, all such bacterial diseases are now wide-spread throughout the world and consumers that used raw milk are still in facing the risk of food poisoning [47-50].

More recently, new technologies have been developed for the analysis of the complex bacterial diseases found in various body parts of host. Further, due to the profound analysis and comparison of all available information, the whole mechanisms that are contributing in harmful impacts on host health can be well-understood, and new strategies could be made to make sure that all these harms caused

by bacterial pathogen's is almost negligible [29, 51].

As both goat milk and meat are popular amongst the health aware consumers, as well as in certain ethnic groups, therefore, in order to support and increase small-scale meat and dairy products enterprises, various schemes are required to developed for increasing the investments in goat raise farms and also promote small-scale dairy product industries. Likewise, In order to determine the qualities of raw milk and meat produced by smallholder goat farms, a farm-based research studies are essential for extensive production system that would be adequate for the production of safe raw goat milk and meat [52]. As overcrowding, poor housing conditions, transportation and environmental changes stress, improper management, chronic infections, and many other stressful conditions may increase more susceptibility of goats to attain several bacterial diseases. Therefore by keeping in view about the significance of goat as an animated source of both milk and meat products for human consumption, the present study was conducted with based on identification of the different bacteria found in blood samples collected from healthy and unhealthy goats

that could be responsible for producing the several harmful impacts on health of goats.

Materials and methods

Samples of blood were collected from the jugular vein of 100 goats in which 50 goats were healthy and 50 goats were unhealthy. Two tubes were used for the collection, vented and non-vented for the aerobic and anaerobic organisms respectively. About 5 ml of blood were collected in each with anticoagulant to prevent the samples from clotting. EDTA (ethylenediamine tetra acetic acid) was used as an anticoagulant (Fig. 1 & 2). The samples were cultured on their selective and differential media including i.e., MacConkey agar, Blood agar and Mannitol salt agar for isolation of microorganisms. The samples were incubated at 37°C. Gram staining was performed for the detection of gram positive and gram negative bacteria. Gram negative bacteria were introduced to TSI (Triple Sugar Iron Agar) and Citrate test for the confirmation. Culture were inserted in the TSI and Citrate tubes through the needle or wire loop and then placed it for incubation at 37°C for 24 hours by following the methods of [32, 53-55].

Figure 1. Blood samples of goats

Figure 2. Isolation of blood sample by loopful method

Results and discussion

Out of 100 blood samples, 45 samples of unhealthy goats were found to be affected by the bacteria. Several pathogenic bacteria were isolated and identified from blood samples of goats of this study that includes, *Enterobacter aerogenes* (6.6%), *Escherichia coli* (8.8%), *Streptococcus bovis* (8.8%), *Klebsiella pneumoniae* (11.1%), *Corynebacterium pseudotuberculosis* (11.1%), *Streptococcus agalactiae* (11.1%), *Listeria monocytogenes* (13.3%), *Staphylococcus epidermidis* (13.3%), and *Staphylococcus aureus* (15.5%). The overall results revealed that the prevalence of

Enterobacter aerogenes were low, while *Staphylococcus aureus* were showing their highest rate of contamination in blood. It means *Staphylococcus aureus* was the main caprine mastitis agent of producing clinical mastitis in goats as reported by [56]. Furthermore, *Staphylococcus aureus* is the worldwide predominant food poisoning agent of goat milk that may cause the staphylococcal food poisoning in consumers, because many traditional milk products are mostly not exposed for pasteurization in accordance with [57], as shown in (Table 1 and Fig. 3 & 4).

Table 1. Shows number of goats effected by bacteria, and their percentage in blood samples of goats

Pathogenic bacteria found from blood samples	Number of Goats effected by bacteria	Percent (%)
<i>Enterobacter aerogenes</i>	3	*6.6%
<i>Klebsiella pneumoniae</i>	5	11.1%
<i>Escherichia coli</i>	4	8.8%
<i>Streptococcus bovis</i>	4	8.8%
<i>Staphylococcus aureus</i>	7	**15.5%
<i>Staphylococcus epidermidis</i>	6	13.3%
<i>Listeria monocytogenes</i>	6	13.3%
<i>Streptococcus agalactiae</i>	5	11.1%
<i>Corynebacterium pseudotuberculosis</i>	5	11.1%
Note: * shows lowest percentage; ** shows highest percentage		

Figure 3. Number of goats effected by bacteria

Figure 4. Percentage of bacteria that effects the goats

Streptococcus agalactiae is major cause of intramammary infection that causes the serious infection due to which both meat and milk production decreases. Modern techniques, such as, selective media method can be used for the detection of *Streptococcus agalactiae* from milk and blood samples in accordance to [36]. The present study shows the presence of *Listeria monocytogenes* isolated from the blood samples of the goat by detecting method, which can cause the serious illness known as listeriosis that may results into death, and also responsible for the

transmission to other animals by their fecal materials, as it is excreted out with feces and that contaminated the soil in accordance with [58]. In the present study, there is no finding of Enterotoxaemia, which another disease is caused by the *Clostridium perfringes* that is responsible for the severe illness in goats, and is commonly found in goats and detected by the PCR analysis and streaking on agar plates. Diarrhea is a common sign of this disease, which is mostly prevalence in North America as reported by [59]. Brucellosis is another main disease occurs in goats caused

by the *Brucella melintensis* and *Brucella abortus*, which also shows the different strains, and highly pathogenic that causes serious illness like abortion in goats in accordance with [60]. The present study shows the presence of causative agent of mastitis i.e., *Staphylococcus aureus* is also isolated from the blood sample of goat which the main source of economic losses in Pakistan due to the reduction in goat milk. The rate of neutrophils become high in mastitis as compare to the healthy goats and increases death risk if not diagnosis earlier as reported by [61]. Tetanus is commonly found in Pakistan and is considered as a contagious disease caused by the *Clostridium tetani*,

which show different signs, such as, reduction in urination that is predominantly occur in goats. It is a severe disease, which may leads to death of animal if not cured. It is also common in calves by transmitting of the bacteria from mother to young ones during pregnancy. It may be control by the diagnostic programs in accordance with [45]. Johne's disease is also occurs in high rate can cause serious illness of goats, which is caused by the *Mycobacterium avium* was not reported in the present study. It's detection from serum is more effective than the fecal materials in accordance with [34], as shown in (Tables 2-5, Fig. 5-8) respectively.

Table 2. Bacteria that identified from the blood samples, their origin and diseases caused by them

Pathogenic bacteria found from blood sample	Part of the body of goat in which bacteria effects	Diseases caused by bacteria
<i>Enterobacter aerogenes</i>	Urinary tract, intestinal tract	Urinary tract infection
<i>Klebsiella pneumoniae</i>	Lungs	Pneumonia
<i>Escherichia coli</i>	Intestinal tract	Diarrhea, Infection of the urinary tract, Pneumonia, Respiratory illness
<i>Streptococcus bovis</i>	Alimentary tract	Ruminal acidosis
<i>Staphylococcus aureus</i>	Skin, gastrointestinal tract	Bacteremia, Respiratory infections, Intramammary infections.
<i>Staphylococcus epidermidis</i>	Mammary gland	Mastitis
<i>Listeria monocytogenes</i>	Gastrointestinal tract	Listeriosis, Encephalitis
<i>Streptococcus agalactiae</i>	Mammary gland, udder	Mastitis
<i>Corynebacterium pseudotuberculosis</i>	Lymph nodes	Caseous lymphadenitis

Table 3. Morphology of pathogenic bacteria

Pathogenic bacteria	Gram reaction	Shape	Spore formation	Aerobes
<i>Enterobacter aerogenes</i>	Negative	Rods in filaments	Non spore forming	Facultative
<i>Klebsiella pneumoniae</i>	Negative	Rods in filaments	Non spore forming	Facultative
<i>Escherichia coli</i>	Negative	Rods in filaments	Non spore forming	Facultative
<i>Streptococcus bovis</i>	Positive	Cocci in chains	Non spore forming	Facultative
<i>Staphylococcus aureus</i>	Positive	Cocci in clusters	Non spore forming	Facultative
<i>Staphylococcus epidermidis</i>	Positive	Cocci in clusters	Non spore forming	Facultative
<i>Listeria monocytogenes</i>	Positive	Rods	Non spore forming	Facultative
<i>Streptococcus agalactiae</i>	Positive	Cocci in chains	Non spore forming	Facultative
<i>Corynebacterium pseudotuberculosis</i>	Positive	Rods	spore forming	aerobes

Table 4. Growth characteristics of bacteria on MacConkey agar, Blood agar and MSA

Pathogenic bacteria	MacConkey agar	Blood agar	Mannitol salt agar (MSA)
<i>Enterobacter aerogenes</i>	Lactose ferment	Gamma hemolysis	No growth
<i>Klebsiella pneumoniae</i>	Lactose ferment	Beta hemolysis	No growth
<i>Escherichia coli</i>	Lactose ferment	Alpha hemolysis	No growth
<i>Streptococcus bovis</i>	Lactose non-ferment	Gamma hemolysis	No growth
<i>Staphylococcus aureus</i>	Lactose ferment	Beta hemolysis	No growth
<i>Staphylococcus epidermidis</i>	Non ferment	Gamma hemolysis	No growth
<i>Listeria monocytogenes</i>	Lactose non-ferment	Beta hemolysis	No growth
<i>Streptococcus agalctiae</i>	Lactose ferment	Beta hemolysis	No growth
<i>Cornyebacterium pseudotuberculosis</i>	Lactose non-ferment	Beta hemolysis	No growth

Table 5. Biochemical tests of gram negative bacteria

Gram negative bacteria	TSI tube	Citrate tube	Gas production
<i>Enterobacter aerogenes</i>	Lactose fermenter with acetic acid	Positive	Negative
<i>Klebsiella pneumoniae</i>	Lactose fermenter with acetic acid	Positive	Positive
<i>Escherichia coli</i>	Lactose fermenter with acetic acid	Negative	Negative

Figure 5. Bactrial growth on MacConkey agar (Pinkcolour indicate the lactose fermenters and Yellow colour indicates the Lactose non-fermenters)

Figure 6. Bacterial growth on Blood agar plates (a) greenish discoloration shows Alpha hemolysis, (b) Colorless shows Beta hemolysis, (c) no change in agar plate shows no hemolysis)

Figure 7. TSI (Orange) and citrate tubes (Green)

Figure 8. Gram negative bacteria introduce to TSI and Citrate tubes for the confirmation

Conclusions

From the present results, it was concluded that goats in Pakistan are suffering from certain pathogenic bacteria that effects on their health and also reduced their meat and milk production, which validating the great economic losses to farmers and industries. They are serving as transmitting vectors that transfers bacterial diseases through their fecal material that contaminated food, soil and water and also to its consumers that used dairy products. Therefore, for the prevention and control of various bacterial diseases, there must be diagnostic programmed should arranged for the diagnosis or examination of goats. Further studies on etiology of these pathogenic bacteria are essential to categorize the main causative agent of goat diseases. Moreover, In many developing countries, due to the high prevalence of certain blood-borne hepatitis pathogens, and lack of satisfactory safety precautions in clinical microbiology laboratory, the goat blood have been most commonly used as bacterial media for the isolation of microorganisms from human tissue and body fluids, as reported by [62-65].

Authors' contributions

Conceived and designed the experiments: N Ahmad & Z Masood, Performed the experiments: M Jawaid & N Afshan, Analyzed the data: M Jawaid, N Ahmad & Z Masood, Contributed reagents/ materials/ analysis tools: N Afshan, N Ahmad & Z Masood, Wrote the paper: M Jawaid & Z Masood.

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