



Isolation of Escherichia Coli from Clinical Mastitis Cases of Cows in Nangarhar Province

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Authors' contributions

This work was carried out in collaboration among all authors. Author NZ designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Authors ZAN, AMH, BK, RMSi and ZS helped in data analysis and revision of the manuscript. Author FA managed the literature searches. All authors read and approved the final manuscript

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ABSTRACT

Mastitis is a common inflammation of the mammary glands caused by a variety of microbial and non-microbial factors and one of the major agent is Escherichia coli (E. coli) bacteria. The disease affects lactating cow's production and bear economic consequences. The purpose of this study is to isolate E. coli bacteria from infected mammary glands and the prevalence of E. coli mastitis in lactating cows in Nangarhar province. In total 50 cow milk samples were collected from different districts of veterinary clinics, villages and dairy farms of Nangarhar province. The samples were examined with gram staining and catalase tests for diagnosis. The results revealed that 21 (42%) cows were infected with E. coli and 29 (58%) were infected by various susceptible microorganisms. In summary, considering the massive number of cultured E. coli bacteria, the research suggested that E. coli infection is highly ranked in Nangarhar different areas and could cause economic losses in cow bio productions.

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1. INTRODUCTION

Afghanistan is considered as an agricultural depended country and people profession to keep animals and cultivate lands for generating their household's income for their normal life support. Humans life have direct relations with animals for their benefits and these relations cause to transfer zoonotic disease from animals to humans. Therefore, veterinarians at the frontline for combating against zoonotic diseases and recommending preventive measures to the people in order to avoid its transmission.

Mastitis is considered as economic loses disease for farmers which is very common in many parts of the world which required continuous treatment to cure the infected animal [1,2]. said, mastitis is the infection of mammary glands in milking cows and caused by bacterial and non-bacterial factors, but mostly it caused through bacterial infections. Over 80% of the clinical and subclinical mastitis caused by *Streptococcus uberis*, *E. coli*, *Staphylococcus aureus*, *Streptococcus dysagalactacia*, *Streptococcus agalactacia*. In a review published in 2009 indicated that mastitis caused by *E. coli* exposed 3 types of pathological changes which are; changes in milk, changes in milk/ mammary glands and toxicity of mammary glands of cow.

In general, the severe mastitis caused by coliform bacteria family [3]. Mastitis is the most effective economic affective disease in livestock sector [4]. In a survey conducted in Pakistan in livestock indicated mastitis as first tire disease in cattle's which affect farmer's economic status. Similarly, previous studies reported that mastitis affected both the production of milk and mammary gland in cows [1-5].

Researches done in Pakistan also indicated that, the economical loses caused by mastitis are countless but particularly in Panjab the economical loses were more than 240 million Pakistani rupees within the year [6]. The economical loses happened in Pakistan probably higher compared to developed countries and its due to the fact that farmers not considering the preventative measures. For example, farmers not applying antiseptic prior to milking and no adequate hygienic practices inside the cowshed [6,7]. The environmental type of mastitis caused by *E. coli* and coliform bacteria's can transfer from infected cow to the healthy cows during

milking time and the predisposing factors are manure, organic materials, infected water, infected cowshed, moisture and etc. [8] reported in 2006 that, environmental causes of mastitis are the major bacteria such as *E. coli* (should be italic), *Klebsiella*, *Streptococcus uberis* (should be italic), *Streptococcus dysagalactacia* (*dysagalactiae*) (should be italic), which can be transmitted during milking time from surroundings to the healthy cow mammary glands. In 2007 [9] declared that *E. coli* (should be italic), *Enterobacter aerogens* (*aerogenes*) (should be italic), *Kelbsiella pneumonia* (should be italic) and *Serratia marcesans* (*marcescens*) (should be italic) are the etiologic causes of the mastitis and classified under the Coliform mastitis and over 50% of the infected cow can survive which decreased 25% of the milk production and the survived cows are the carrier of the disease and having clinical symptoms such as anorexia, weight loss and calcium deficiency. Therefore, the aim of the study was to isolate *E. coli* (should be italic) bacteria from farm and house animals caused by clinical mastitis and its prevalence in Nangarhar province through culture and selective media testes.

2. MATERIALS AND METHODS

2.1 Materials

The media which were applied during the study are Agar Agar, MacConkeys agar, Nutrient agar, Nutrient broth, EMB (Eosin Methylene Blue) and the chemicals which were used in this research were Grams Crystal violet, Grams Iodine, Grams Decolorizers, Grams Safranin and Hydrogen per oxide.

2.2 Experiment Location

Milk was collected from different veterinary clinics in the districts, dairy farms and houses which reported clinical mastitis cases in Nangarhar province.

2.3 Animals

The animals which were studied during the research were only cows.

2.4 Sample and its Size

The total collected milk samples were 50 and used 10ml for laboratory examinations.

2.5 Sample Collection Method

The samples were collected in 10 ml sterile tubes from different veterinary clinics, dairy collection points in the villages and houses which reported suspected clinical mastitis cases and stored in Nangarhar veterinary science faculty clinic for diagnosis.

2.6 Isolation of *E. coli*

In this technique, upon arrival of the samples to the laboratory, the samples were cultured in liquid media of nutrient broth for growing the microorganisms and incubate them for 12 hours in 37C. consequently, the incubated microorganisms are cultured in nutrient agar and MacConkey medias and keep them in incubator for 24 hours under 37C. The diagnosis of colony is dependent on morphological status of the grown bacteria like shape, color and size to identify the respective isolated bacteria. Those bacteria which are suspicious need to be culture again in Eosin Methylene Blue for verification.

2.7 Identification of *E. coli* Bacteria

For the identification and diagnosis of *E. coli* bacteria, we applied the methods used by [10] and [11]. For example, Gram Staining, Biochemical test and Catalase test.

2.8 Statistical Analysis

The data obtained are analyzed statistically using 5 Digo, Ca., USA (Graphpad. Prism) and Microsoft excel. All available data are recorded in tables and graphs based on the average deviation standard. Based on statistical experience and meaning findings are (Significant) data.

3. RESULTS

3.1 Prevalence of Clinical Mastitis

The total isolated microorganisms were 65 (100%) obtained from 50 heads of cows infected with clinical mastitis. 30 of the isolated microorganisms were gram negative and 35 were gram positive. However, the research target was gram negative bacteria. Therefore, 30 (46.1%) of these bacteria were grown in a pure media of culture as shown in Table 1 and Fig. 1.

3.2 Gram Staining

For the identification of gram negative (-) and gram positive (+) bacteria, it required to prepare slide from nutrient agar, MacConkey agar and selective medias and then stain with gram positive. The result indicated he *E. coli* bacteria which is gram negative shown in Fig. 3.

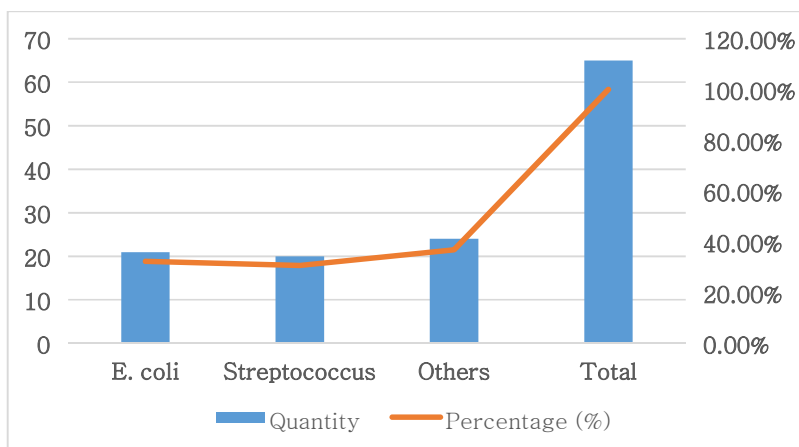


Fig. 1. Indicate the quantity and percentage of isolated bacteria in culture media

Table 1. Indicate the quantity and percentage of isolated bacteria during experiment

S/N	Bacteria	Quantity	Percentage (%)
1	<i>E. coli</i>	21	32.30 %
2	Streptococcus	20	30.76 %
3	Others	24	36.92 %
Total		65	100 %



Fig 2. The picture left indicates the growth, shape and color of bacteria in EMB media and the picture right indicate the growth, shape and color of bacteria in MacConkey agar media (Left picture shows the colony morphology of *E. coli* on EMB agar)



Fig 3. The image shows *E. coli* gram negative bacteria after gram staining

3.3 Catalase Test

Following to the gram staining, the researcher added 30% of H₂O₂ (Hydrogen Peroxide) one drop on slide and then pure culture microorganism over on it. In the result of reaction to this test the foaming's (bubbles) were produced on the surface of slide which represent gram negative bacteria presence and those slides which not produce the bubbles were considered as gram positive bacteria for this test and the total 21 samples were counted consisting gram negative bacteria for the said examination.

4. DISCUSSION

In year 2013 Katsande and his colleagues incidentally collected samples from 32 heads of cow in the city of Aylam and then cultured in the media [12]. Following to the culture, he performed gram staining and selective media and other biochemical examinations in laboratory to identify different genus and species of bacteria

involved in mastitis. Out of the 32 heads cows which went under diagnosis, it has been found that 21 of the total number (81.25%) were infected with *Klabcilla* (*Klabcilla*) pneumonia (should be italic) and *E. coli* (should be italic) bacteria and another dairy farm entirely was infected with *streptococcus* (should be started with capital letter and should be italic) *agalactacia* (*agalactiae*) which cause sever mastitis in cows. In 2013 [13] and his colleagues collected 51 milk samples from those cows which had clinical and sub clinical mastitis and isolated the suspicious bacteria in different culture media such as nutrient agar, blood agar and Mackconkey agar. The high percentage of isolated bacteria was *E. coli* (italic) which cover 37.50% of the entire microorganisms. *Staphylocococcus aureus* was calculated 31.94%, *streptococcus* (should be started with capital letter) spp 12.5% *proteus* (should be started with capital letter) spp 2.77% and *pseudomonas* (should be started with capital letter) spp was calculated 1.38% of the whole cultured media.



Fig. 4. Shows positive status of catalase test (Confirmation of gram negative bacteria)

Tanja in 2004 isolated 237 *E. coli* bacteria from clinical mastitis in Finland and Israel [14]. Vioral in 2010 has investigated that the major causes of mastitis caused by *E. coli*, *Mannheimia haemolytica* and *Klebsiella pneumoniae* bacteria. Resulting from his research, he isolated 216 species of bacteria which 17% were gram negative, 7.9% were environmental coliform bacteria [15].

[16], and Sisay in 2011 [17] carried similar investigation to isolate and identify the causative bacteria of mastitis and used the same media as we selected in above study. In the result, the isolated high percent was covered by *E. coli* bacteria and subsequently followed by other gram negative bacteria and confirmed the diagnosis of mastitis which caused by *E. coli* and other species of gram negative family [18-21].

5. CONCLUSION

Mastitis is defined as inflammation of mammary gland which has various causative agents including infections and non-infectious, resulting spoilage of milk with and considered as economic affecting disease for farmers. *E. coli* is one the most important bacteria causing mastitis and survive in various parts of the environment such as cowshed, manure, moisture and other organic products. The study was conducted in Nangarhar province to determine how many cows are infected with mastitis. 50 sample were collected from different veterinary clinics, dairy farms and village cows and then transferred to Nangarhar university/ veterinary science faculty clinic for diagnosis and laboratory examinations. We cultured the samples in different media such as nutrient agar, Macconkey agar, nutrient broth and also done gram staining for identification of causative bacteria of mastitis. The results

indicated that 42% of the mastitis infection was caused by *E. coli* bacteria and 58% were considered other microorganisms. To conclude, mastitis is mostly caused by gram negative (*E. coli*) and streptococcus bacteria family in Nangarhar province different districts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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