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Isolation and identification of Escherichia coli causing diarrhea in calves

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Abstract:

Neonatal calf diarrhea remains one of the most important problems in young calves causing great economic losses. In the current study, a total number of 150 fecal samples collected from diarrheic and in-contact cattle and buffalo calves under 3 months old were examined bacteriologically for isolation of E.coli. All isolates were confirmed by PCR using 16SrRNA gene and screened for their pathogenicity using Congo red assay.

Key words: Diarrhea, calves, E. coli, 16SrRNA and Congo red assay.

Introduction:

Neonatal calf diarrhea (NCD) defined as diarrhea of calves from one week up to 12 weeks old. The disease considered as one of the major health problem among newly born calves **(Bazeley, 2003)**,causing economic losses include high morbidity and mortality rates, reduced growth rate, treatment costs and time spent caring for the affected calves **(Anderson et al., 2003** and **Ok et al., 2009)**. Escherichia coli (E. coli) has been incriminated as a major cause of diarrhea, which characterized by progressive dehydration and death may occur depends on the age of the calf when scour started and on the particular pathotypes of E. coli **(Tan et al., 2011)**. Pathogenic E. coli strains are distinguished from other E. coli by their ability to cause serious illness as a result of their genetic elements for toxin production, adhesion and invasion of host cells, interference with cell metabolism and tissue destruction **(Borgattaa et al., 2012)**. Polymerase chain reaction (PCR) is used for the diagnosis of E. coli with high accuracy, and considered as an easy tool for amplifying genes of interest specifically present in a target pathotype or serogroup **(Begum et al., 1993)**. Congo red test (CR test) has been used to detect pathogenic E. coli of bovine origin which isolated from cases of neonatal calf diarrhea **(Sharma et al., 2006)**.

This present study was aimed to investigate the presence of E. coli causing diarrhea in calves and to detect their pathogenicity using Congo red assay.

examined diarrheic and in-contact cattle and buffalo calves using sterile cotton swabs and transferred to the laboratory on ice box.

Materials and methods:

Collection of samples:

A total number of 150 fecal samples were directly collected from the rectum of the

Bacteriological Identification of E. coli according to Quinn et al. (2002):

Fecal samples were inoculated into trypticase soya broth and incubated at 37ºC for 24hrs for propagation of E. coli. Subcultures from trypticase soya broth were streaked on MacConkey agar and EMB agar and incubated at 37º C for 24hrs.

Identification of the isolated bacteria was done on the basis of colonial morphology, staining characters and biochemical reaction **(Quinn et al., 2002)**. After complete identification, the bacterial isolates were stored at -20°C in brain heart infusion broth containing 16% glycerol for long term preservation.

Identification of E. coli isolates by Polymerase chain reaction according to Sambrook and Russell (2001):

DNA was extracted from the bacterial colonies by boiling method **(Wani et al., 2003)**. The samples were tested using primer set (16SrRNA) for identification of E. coli **(Wang et al., 2002)** as illustrated in Table (1). The PCR reaction carried out in a 25µl volume. Each reaction consisted of 5µl of template DNA, 5µl of 5X master mix, 10pmol of 1µl of forward primer and1µl of reverse primer and 13µl of Nuclease Free Water (NFW).The PCR condition was adjusted at 1cycle at 95°C for 3 min followed by 30 cycle at 95°C for 20s, 58°C for 40s & 72°C for 30s and ended by 1cycle at 72°C for 8 min.

Amplified PCR products were analyzed by electrophoresis in 1.5% (wt/vol) agarose gel containing ethidium bromide (0.5µg/ml). The products were visualized under UV illumination and documented with Gel pro analyzer® version 4.

Study the pathogenicity of E. coli isolates by culturing onto congo red medium (Berkhoff and Vinal, 1986):

Each E. coli isolate was tested for its growth on Congo red medium. The reaction was best seen after 24 hrs. of aerobic incubation at 37°C and then left at room temperature for additional 2 days (not to exceed 4 days). Congo red positive (CR+) colonies gave red colour while Congo red negative (CR-) colonies didn't bind the dye.

Table 1: Oligonucleotide primers used in PCR.

Primer name (Target gene)	Oligonucleotide sequence (5–3`)	Product size (bp)
E16S (16SrRNA)	F:CCCCCTGGACGAAGACTGAC R:ACCGCTGGCAACAAAGGATA	401

Results

A total number of 150 fecal isolates collected from diarrheic and in-contact cattle and buffalo calves were examined for detection of E. coli at a rate of 100%. E. coli colonies were bright pink and green metallic sheen on MacConkey and EMB agar media respectively and appeared as gram negative coccobacilli arranged singly or in pairs. On TSI agar E. coli produce yellow color and gas, whereas on Simmon's citrate agar E. coli unable to utilize citrate so no change in greenish colour of the media. The result of the amplification of 16SrRNA gene using PCR (Figure 1) revealed that, all E. coli strains were positive for 16SrRNA gene (100%). Congo red assay showed that all E. coli strains were positive for congo red reaction (100%).

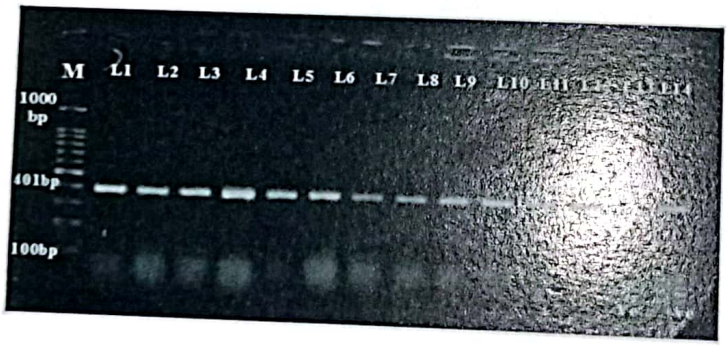

Fig. (1): Agarose gel electrophoresis for detection of 16SrRNA gene among E. coli strains. Lane M: Molecular weight marker, 100-1000 bp. Lanes 1-12: positive samples with band of amplicon size at 401bp. Lane 13: Negative control and Lane14: Positive control of 16SrRNA gene with band of amplicon size at 401bp.

Discussion

Neonatal calf diarrhea remains one of the most important health problems in livestock, causing great economic losses. Several agents contribute to the etiology of NCD in calves such as bacteria, viruses and parasites. But this study deals with only E. coli which is the most important cause of bacterial diarrhea in calves (**Kolenda et al., 2015**). The present study was conducted to detect E. coli isolates obtained from diarrheic and in-contact cattle and buffalo calves under 3 months old and their pathogenicity.

The presence of E. coli infection in all isolated samples (100%) is regarded to the fact that E. coli is normally inhabitant in the intestine of warm blooded animals.

The rate of E. coli infection was similar to that obtained by **Ibrahim (1995)** 100% and nearly similar to that obtained by **Perez et al. (1998)** 94%; **Mazhaheri Nejad Fard et al. (2005)** 88.7%; **Pourtaghi et al. (2013)** 86.7%; **Mohamed (2015)** 81% and **El-Seedy et al.**

(2016) 75.6%. On the other hand the result is not agreed with **Mosaad et al. (2008)** 48.47%; **Moussa et al. (2010)** 39.29%; **El-Shehedi et al. (2013)** 35.83%; **Gebregiorgis and Tessema (2015)** 36.8% and **Islam et al. (2015)** 57 %. The differences between this findings may be attributed to geographical locations, variations in age groups or may be due to managemental factors including inadequate nutrition, lack of hygiene, overcrowding, exposure to severe environment, insufficient attention to the newborn calf with insufficient intake of colostrum and lack of effective preventive measures as any stress factors allowing the opportunistic E. coli to flourish and express virulence genes causing pathogenic effect on calves.

All E. coli isolates were Congo red positive (100%). The obtained result is similar to that described by **Galal et al. (2013)** and **Osman et al. (2013)**100%. On contrast this result is disagreed with **Sharma et al. (2006)** 47.42% who also detected the sensitivity and specificity of Congo red assay was 58.69% and 100% respectively and **EL-Alfy et al. (2013)** 60%.

Conclusion:

Neonatal calf diarrhea remains one of the most important problems faced by livestock, causing great economic losses especially those caused by E. coli. The PCR using 16SrRNA gene is a sensitive, specific, and rapid method for the confirmation of the E. coli isolates and the application of Congo red assay can be used for detection of pathogenic E .coli.

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