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Summary

Adhesion mediated by fimbriae (pili) is responsible for attachment occurred between bacteria and host cells, is the first step in the pathogenesis leading to disease condition. So detection of fimbria was found to be a good predictor of enteropathogenicity.

A comparative study on 7 different namely: Minca IsoVitalex, Minca, minimal, basal with glucose, basal with casamino acid, Simmon`s adonitol citrate agar and MacConkey agar media was carried out using 39 faecal samples from newly-born diarrhoeic cow-calves to detect the highest recovery factor of K99 fimbrial antigen. Examination of the 39 faecal samples revealed that 89.7%, 76.9%, 71.8%, 56.4%, 43.6%, 12.8% and 5.1% were recovered from the previously mentioned media with a recovery factor of 4.9, 4.0, 2.9, 2.0, 1.4, 1.2 and 1.0 respectively.

Two diagnostic sera were locally prepared in rabbits against 2 standard type strains, B41 (O101 : K99) and B85 (O9: K99). Prior to antigen preparation and rabbit immunization the culture was tested by slide agglutination test using ready made trading K99 pilus antisreum (Denka, Seiken Co., LTD. Japan) to ensure the presence of K99 pilus antigen. Serial I/V inoculation of pilus antigen with doses of 0.25, 0.5, 1.0, 1.5, 2.0, 2.5 and 3 ml at 5 days intervals were used. Following the last injection by 5-7 days, bleeding was carried out by drawing 15-20 ml blood from the ear vein of the rabbit The prepared sera that gave positive reaction at a titer of 1/2560 or more was accepted. A working serum dilution 1/10 (after adding merthiolate to reach.

A final concentration of 0.01%) was used for K99 pilus detection in isolation of *E. coli* strains.

A total of 566 and 113 faecal samples from diarhhoeic and apparently healthy of different studied animals, were collected and cultured on Minca-Is. for pilus detection. From each culture plate 5 suspected colonies were selected, subcultured on Minca-Is and a portion was cultured on MacConkey agar medium to detect lactose fermentation. This resulted in isolation of 2830 and 565 colonies from diarrhoeic and apparently healthy animals respectively. These isolates were considered K99 positive, if gave positive agglutination (by microagglutination technique) with both the two prepared (B41 and B85) diagnostic sere.

The isolates which gave positive microagglutination test were subjected for morphological characterization and biochemical confirmation.

Comparison between the traditional culture method and qualitative enzyme immunoassay "Pathasure bovine enteritis kit" was employed using 217 faecal samples from diarrhoeic cow-calves. Results revealed that K99 pilus antigen was detected in an incidence of 27.2% and 29.5% respectively. The qualitative enzyme immunoassay was found more accurate in detection and preferably to be used in case of plenty of samples.

Correlation between piliated (K99+ *E. coli)* strains and calf age revealed an incidence of 43.2%, 40.6%, and 34.5% at the intervals of 1-2, 3-4 and 5-6 day of age respectively. At 7-8, 9-10 and 11-12 day of age intervals, it was 31.6%, 25.0% and 20.0% respectively. The

incidence continued to decrease in the third week and in calves aging more than 3 weeks (19 up to 30 days) only 4.0% was recovered. The highest incidence was recorded during the 1st two days of calf s age and still high up to the end of the first week and then decreased regularly and scadualy with life age up to almost three weeks of age.

Some virulence factors of piliated strains (K99+ *E. coli* isolates) were carried out. Heat stable enterotoxin (Sta) was detected in 66.1%, 65.1%, 47.4% and 36.4% positive strains in case of cow-calves, buffalo-calves, lambs, kids respectively. Only 1 out of 2 strains recovered from suckling-camels was found to be enterotoxigenic. The K99 pilus antigen and heat stable (Sta) enterotoxin are major *E. coli* virulence attributes.

Correlation between enterotoxin production of piliated strains (K99+ strains) that recovered from 59 diarrhoeic cow-calves and their age revealed an incidence of 81.2%, 76.9% and 70.0% at 1-2, 3-4 and 5-6 day of age respectively. Enterotoxigenic K99+strains were mostly detected during the first days after birth and then decreased in older age.

Among the potential virulence factors produced by piliated strains, was the haemagglutination activity. Examination of piliated strains (K99+strains), recovered from all studied animal species, showed higher activity with their homologous red cell type. The incidence was amounted 79.7%, 55.8%, 63.2% and 54.5% in case of cow-calves, buffalo-calves, lambs and kids (each with its homologous RBCs) respectively. This highest haemagglutination activity was followed by high rate with erythrocytes obtained from another animals but of the same species i.e. cow-calves strains highly agglutinated with buffalo erythrocytes---

kids strains highly agglutinated with lamb erythrocytes. In case of using human erythrocytes, a high agglutination rates were obtained with strains recovered from cow-calves, buffalo-calves, while strains recovered from kids and lambs did not record high rate of this activity. It was found that 64.6% of haemagglutination (HA) positive strains were D-mannose resistant type which pointed out to its virulent activity. Multiple pattern of haemagglutination reaction, using RBCs from different animal species were detected in $K99^{+}$strains recovered from different investigated animal species.

Dealing with more virulence markers of pathogenic *E. coli*, serum resistance and haemolytic activity were carried out. Results revealed that 25.4%, 27.9%, 21.1% and 18.2% of piliated *E. coli* strains recovered from cow-calves, buffalo-calves, lambs and kids respectively showed serum resistant activity. In case of haemolytic activity, 8.5%, 7%, 5.3% and 9.1% isolates recovered from the same previously mentioned animal species respectively, were positive for this activity. The two isolates recovered from suckling-camels were negative for serum resistant and haemolytic activity.

It was found interesting to document the presence of pili in piliated *E. coli* isolates. An electron microscopy was conducted for confirmation and visual (electronical) way for characterization of pilus. The scanning electron microscope (SEM) was used, which provided images of external morphology similar in appearance to those formed by the light (ordinary) microscope, but greater in magnification as it reaches up to 100,000 times. The specimen was suspended in distilled water and a drop of the suspension was placed on formvar coated grids, then transferred on fluorescent (200-mesh)

screen and examined with electron microscope operated at 80 K.V., then photography was carried out. Fimbriae were seen as numerous rods like structures which were fine and irregularly bent originating almost from the surface of the bacterial cells.

Conclusion:

1- Minca IsoVitalex (Minca-Is) is the most preferable media used for K99 pilus antigen detectability. It is recommended to be used in central labs. where plenty of cases and samples to be tested.

2- Minca medium (without IsoVitalex) can also be used for K99 pilus antigen detection as it has no adverse effect on its production.

3- The micromtechnique method has several advantages in that smaller amounts of antisera and antigens, less space in the laboratory and less time are required.

4- Qualitative enzyme immunoassay technique "Pathasure Bovine Enteritis Kit" was considered a high, sensitive, accurate and fast test which is used in detection of K99 pilus antigen directly from the faeces.

5- During the first two days of calves age *E. coli* K99 pilus antigen showed the highest incidence which was continued to be still high up to the end of the 1st week. This incidence was then decreased with calves age.

6- Heat stable enterotoxin (Sta) in parallel with pilus antigen was detected in more than 60% of the piliated isolates. Both pili and enterotoxins are major *E. coli* virulence attributes which express in the pathogenicity.

7- Enterotoxigenic *E. coli* (ETEC) strains with pilus antigen were highly detectable in new born calves during the first few days after birth.

8- Fimbriated *E. coli* strains showed haemagglutination activity (with and /or without D-mannose) specially with erythrocytes of the same animal species which added a more virulence factor.

9- Scanning electron microscope (SEM) which provided an images that clarified and magnified the external morphology of the bacterial cell was employed as a confirmatory tools for the documentation of the presence of pili.