

Congo red dye agar test as an indicator test for detection of invasive bovine *Escherichia coli* - short communication

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ABSTRACT

Congo red dye agar test (CR test) has been used to differentiate invasive and non invasive *E. coli* in poultry. This simple test was used to detect enteroinvasive *E. coli* of bovine origin, isolated from cases of neonatal calf diarrhoea. Out of 97 isolates tested 46 showed CR-positive reaction, while 51 were CR negative. Upon confirmation with the Sereny test, a standard test for detection of invasiveness, CR test was found 100% specific and 58.89% sensitive. This test can be used for primary screening of non invasive *E. coli* from potentially invasive *E. coli*.

Key words: Congo red dye agar, *Escherichia coli*, invasiveness, Sereny test, bovine

Introduction

NATARO and KAPER (1998) have classified pathogenic *Escherichia coli* (*E. coli*) into four major groups: (I) Enterotoxigenic *E. coli*; (II) Enteroinvasive *E. coli*; (III) Enteropathogenic *E. coli*, and (IV) Enterohaemorrhagic *E. coli*.

Enteroinvasive *E. coli* (EIEC) is a type of *E. coli* responsible for bacterial septicaemia. Their virulence armoury includes production of hydroxymate siderophore, resistance to bactericidal effect of serum, a colonization factor for mouse intestine and hydrophobic properties that makes bacteria self-agglutinable (SAID et al., 1988), and plasmid encoded surface antigen known as 31-A or CS-31A (CONTREPOIS et al., 1993). Enteroinvasive *E. coli* has been studied with the in vivo method of production of kerato conjunctivitis in guinea pig (SERENY, 1955), or in vitro tests such as Gentamicin assay after MDCK cell (KORTH

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et al., 1994). However, these tests have certain limitations, viz., use of live animals, and technically demanding tissue culture methods, respectively. In poultry, a simple method of Congo Red Dye binding test (CR test) has been used to differentiate between invasive and non-invasive *E. coli* (BERKHOFF and VINAL, 1986; PANIGARHY and YUSHEN, 1990) but its use for detection of mammalian EIEC has not been reported. We therefore designed the present study to use CR test to detect bovine EIEC. Further, the results were confirmed with the Sereny test, an indicator test for detection of EIEC.

Materials and methods

E. coli were isolated from 68 diarrhoeic calves by standard protocols (COWAN and STEEL 1975; EDWARD and EWING, 1986). The isolates were serotyped from the National *Salmonella* and *Escherichia* Centre, Kasauli, Himachal Pradesh, India. While 29 isolates recovered from the same condition, serovars already maintained at the Departmental Laboratory of Veterinary College, Bikaner, were also used. A total of 97 *E. coli* with different serovars were used for the study.

Congo red dye agar test (CR Test). The test was carried out as per the technique of BERKHOFF and VINAL (1986). The colonies were streaked on Congo red agar (BERKHOFF and VINAL, 1986) and incubated for 72 hours at 25 °C.

Reaction was recorded at 18, 24, 48 and 72 hours. Appearance of red colonies within 72 hours was recorded as a positive reaction. Negative colonies did not bind the dye and remained white or grey even after 72 hours and were declared negative.

Sereny test. Sereny test was carried out as per the technique of SERENY (1955). Bacterial cultures were grown overnight in nutrient broth. The growth was centrifuged at 5000 rpm for 15 min at 4 °C and the bacterial pellet was collected for each isolate. The concentration was adjusted with 0.9% normal saline solution to 5×10^9 bacteria per mL. A volume of approximately 50-microlitre suspension was inoculated in the conjunctival sac of guinea pig. Reaction was observed for 96 hours and development of keratoconjunctivitis was recorded as a positive reaction.

Results and discussion

Congo red dye agar test was first used by SURGALLA and BEASLY (1969) for differentiation of virulent and avirulent *Pasteurella* (now *Yersinia*) *pestis*. Subsequently, it was used as phenotypic marker of colisepticaemic (invasive) and non-colisepticaemic *E. coli* in poultry by BERKHOFF and VINAL (1986), GJESSING and BERKHOFF (1989) and PANIGARHY and YUSHEN (1990). In the present study, of 97 isolates tested 46 (47.42%) isolates gave positive reaction for CR test, while 51 (52.57%) did not bind the dye even after 72 hours and were thereafter declared negative. Such findings are in concurrence

with BERKHOFF and VINAL (1986) who found that about half of the *E. coli* was CR positive, which were obtained from environmental and cloacal origin. Likewise, PANIGARHY and YUSHEN (1990) also found 13/ 21 (61.9 %) *E. coli* were CR positive. However, such findings have not been confirmed for cattle or any other mammalian species. Although KALOREY et al. (2002) reported CR binding test with 50% and 89.09% CR binding at 25 and 37 °C, respectively, their relationship to invasiveness was not discussed. As this test was carried out for detection of invasiveness among *E. coli* of bovine origin, confirmation was obtained with the Sereny test, a standard test to detect enteroinvasive *E. coli*. Of 46 CR positive isolates, 27 *E. coli* were proved Sereny test-positive, while 16 were false positive for invasiveness. The sensitivity of the test was proved 58.69%. It was revealed that all 51 isolates, which were CR negative, were also negative with the Sereny test. The test proved 100% specific without showing any false negative. Therefore, this test can be used as a primary screening test to screen non-invasive *E. coli* from the potentially invasive *E. coli*. Binding of CR dye was also found different according to their serovars. It was observed that not all strains of same serovars were negative or positive. The same result was obtained with the Sereny test.

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SAŽETAK

Agar test s kongo-crvenilom rabljen je za razlikovanje enteroinvazivnih od enteroneinvazivnih izolata bakterije *Escherichia coli* izdvojenih iz novorođene teladi s proljevom. Od 97 pretraženih izolata, 46 ih je bilo pozitivno, a 51 je bio negativan. Za provjeru dobivenih rezultata rabljen je Serenijev test, koji je standardni test za dokazivanje invazivnosti *E. coli*. Agar test s kongo-crvenilom pokazao se 100% specifičnim i 58,89% osjetljivim. Test se može upotrijebiti za početno razlikovanje neinvazivnih od invazivnih izolata *E. coli*.

Ključne riječi: agar s kongo-crvenilom, *Escherichia coli*, Serenijev test, govedo
