

**Brief Communication**

**O-ANTIGENS, K-ANTIGENS AND PRODUCTION OF ENTEROTOXIN IN ESCHERICHIA COLI-STRAINS ISOLATED FROM LITTER MATE PIGLETS WITH NEONATAL DIARRHEA**

The importance of virulence factors such as adhesins and enterotoxins of *Escherichia coli* in causing diarrhea in piglets is well documented. It is generally accepted that the intestinal tract of piglets suffering from neonatal diarrhea is thoroughly colonized by strains of enteropathogenic *E. coli* (EPEC) which carry identical O-antigens and virulence factors (*Søderlind & Møllby 1979*). In several countries, EPEC belonging to O-group 149 carrying the K88 antigen have been most commonly found in piglets with neonatal diarrhea. This paper reports on the isolation of EPEC, with different O- and K-antigens as well as different patterns of enterotoxin production, from 2 piglets originating from one litter in which 7 out of 13 piglets suffered from diarrhea during the first week of life.

The herd had for some time experienced diarrhea and elevated mortality among piglets. Necropsy of the 2 piglets submitted for laboratory examination revealed dilated small intestines with loose to watery content. The livers were slightly congested.

Bacteriological examination of the livers and of the content of the small intestine was carried out according to standard bacteriological techniques on blood agar and bromthymolblue lactose agar. Incubation was performed aerobically at 37°C for approx. 20 h. *E. coli* strains were primarily examined for the O-antigens 2, 6, 8, 9, 32, 45, 64, 98, 101, 115, 124, 125ab, 138, 139, 141, 145, 147, 149 and 157 and for the K-antigens 88 and 99. Demonstration of O- and K-antigens were performed using tube agglutination test (*Søderlind 1971*) and slide agglutination test (*Guinée et al. 1977*), respectively. Strains of *E. coli* not belonging to any of the O-groups mentioned were kindly tested by Dr. Ørskov at the State Serum Institute, Copenhagen, Denmark. Examination for heat labile toxin (LT) and heat stable toxin (ST) was performed by means of enzyme linked immunosorbent

assay\*) (*Olsvik et al.* 1982), and the suckling mouse test (*Dean et al.* 1972), respectively.

Bacteriological examination of intestinal content from both piglets showed abundant growth of non-haemolytic colonies which were identified as *E. coli*. From piglet No. 1 a single colony from the primary plate was subcultured for serological characterization. This strain proved to belong to O-group 64 and to possess the K99 antigen.

From piglet No. 2 *E. coli*-strains of 19 separate colonies from the primary plate were selected and tested for the presence of K88 and K99 antigen. Of these, strains from 10 colonies were examined for their O-antigens and production of LT and ST. The results of this examination are represented in Table 1.

Table 1. K-antigens, O-antigens, heat labile toxin (LT) and heat stable toxin (ST) in *Escherichia coli*-strains from 19 colonies isolated from 1 piglet suffering from neonatal diarrhea.

Colony No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
K88 antigen	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—
K99 antigen	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	—
O-antigen	149	149	149	149	149	149	NT <sup>a</sup>	84	84	84	84	NT	NT	NT	NT	NT	NT	NT	NT
LT	+	+	+	+	+	+	NT	—	—	—	—	NT	NT	NT	NT	NT	NT	NT	NT
ST	+	+	+	+	+	+	NT	+	+	+	+	NT	NT	NT	NT	NT	NT	NT	NT

<sup>a</sup> Not tested.

The EPEC demonstrated from the 2 piglets belonged to different O-groups (64, 84, 149) and separately possessed the K88 antigen or the K99 antigen. EPEC of O-group 149 all harboured the K88 antigen and produced both LT and ST. EPEC of O-group 84 all possessed the K99 antigen and produced only ST. EPEC of O-group 64 with the K99 antigen was not tested for LT or ST production. The present paper is the first report on the isolation of EPEC of O-group 84 from diseased piglets in Norway.

This report demonstrates that EPEC isolated in connection with a single outbreak of neonatal diarrhea in piglets may belong to different O-groups and carry various virulence factors. Consequently, in some cases the commonly employed procedure

\*) The test was kindly performed at the Norwegian Defence Microbiological Laboratory, National Institute of Public Health, Oslo.

of characterizing 1 or 2 colonies from the primary plates may be insufficient to elucidate important properties of the EPEC involved.

The findings further emphasize the necessity of performing a thorough bacteriological examination of diseased piglets from herds where vaccination programmes are planned.

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#### REFERENCES

- Dean, A. G., Yi-Chuan Ching, R. G. Williams & L. B. Harden:* Test for *Escherichia coli* enterotoxin using infant mice: Application in a study of diarrhea in children in Honolulu. *J. infect. Dis.* 1972, *125*, 407—411.
- Guinée, P. A. M., J. Veldkamp & W. H. Jansen:* Improved Minca Medium for the detection of K99 antigen in calf enterotoxigenic strains of *Escherichia coli*. *Infect. Immun.* 1977, *15*, 676—678.
- Olsvik, Ø., O. T. Hushovd, B. P. Berdal, T. Bergan & M. Mathiesen:* Production of enterotoxin by *Escherichia coli* at four, twenty-two and thirty-seven degrees centigrade. *Eur. J. clin. Microbiol.* 1982, *1*, 12—16.
- Søderlind, O.:* Studies on *Escherichia coli* in pigs. II. Serological investigations. *Zbl. Vet.-Med. B.* 1971, *18*, 569—590.
- Søderlind, O. & R. Møllby:* Enterotoxins, O-groups, and K88 antigen in *Escherichia coli* from neonatal piglets with and without diarrhea. *Infect. Immun.* 1979, *24*, 611—616.

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