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From the Department of Microbiology and Immunology,
Veterinary College of Norway, Oslo.

SEROLOGICAL, ENTEROTOXIN-PRODUCING AND BIOCHEMICAL PROPERTIES OF ESCHERICHIA COLI ISOLATED FROM PIGLETS WITH NEONATAL DIARRHEA IN NORWAY*

By
Arve Lund, Kåre Fossum and *Eivind Liven*

LUND, ARVE, KÅRE FOSSUM and EIVIND LIVEN: *Serological, enterotoxin-producing and biochemical properties of Escherichia coli isolated from piglets with neonatal diarrhea in Norway.* Acta vet. scand. 1982, *23*, 79—87. — Altogether 235 strains of Escherichia coli isolated from jejunal content of piglets with neonatal diarrhea were examined for serological, enterotoxin-producing and certain biochemical properties. Of 198 strains examined, 84 (42 %) belonged to O-group 149, while 14 (7 %) strains belonged to each of the O-groups 8 and 64. Seventy strains (35 %) could not be grouped with the sera used. The remaining strains were distributed among the following O-groups with only a few strains in each group: 2,6,9,32,45,98 and 141. Eighty out of 84 E. coli strains of O-group 149 possessed the K88 antigen and produced heat labile enterotoxin (LT). Besides, LT production was demonstrated in 3 out of 14 strains of E. coli O8. K88 antigen was demonstrated in only 1 strain not belonging to O-group 149. Among strains of E. coli O64 12 out of 14 were K99 positive. This antigen was not demonstrated in E. coli strains of other O-groups. A close relationship was demonstrated between strains of E. coli O149 possessing the K88 antigen and the ability to ferment both raffinose and adonitol. This ability was only detected in 2 other strains of E. coli not belonging to O-group 149.

Escherichia coli; O-groups; K88 and K99 antigens; enterotoxin; biochemical properties; piglets.

Escherichia coli (E. coli) is a common pathogen causing neonatal diarrhea in piglets in countries with modern pig production (*Svendsen et al.* 1975). Several reports demonstrate that strains belonging to certain O-groups of E. coli occur more

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frequently than others in connection with neonatal diarrhea in piglets (*Sojka* 1965, 1971, *Søderlind* 1971). The importance of adhesins and enterotoxin production for the pathogenicity of E. coli is well documented (*Smith & Halls* 1967, *Smith & Lingood* 1971, *Søderlind & Møllby* 1979). In Norway serological studies of E. coli isolated from piglets during the years 1961—1964 revealed that O8 and O147 strains occurred most frequently (*Sørum* 1966). In a recent study *Liven* (1979) examined 92 strains of E. coli 54 of which belonged to O-group 149 and 8 strains belonged to O-group 138. Enterotoxin production was demonstrated in 55.4% of the strains examined, and among these strains about 64 % produced both heat labile (LT) and heat stable (ST) enterotoxin.

In the present work the relationship between serological, enterotoxin-producing and certain biochemical properties of E. coli isolated from piglets with neonatal diarrhea, is reported.

MATERIALS AND METHODS

Strains of E. coli

Altogether 235 E. coli strains from 190 piglets originating from 81 herds in Southern Norway were tested. The E. coli strains examined were isolated from the content of the anterior part of jejunum of necropsied* piglets less than 10 days old. The piglets had been suffering from neonatal diarrhea suspected to be caused by E. coli. The intestinal content was inoculated on bovine blood agar and bromthymolblue lactose agar. From each pig 1 or 2 representative colonies suspected of being E. coli were chosen for further examination. The strains were stored in meat extract broth with 5 % horse serum at —20°C and they were subcultured not more than 2—3 times before being tested.

Biochemical examinations

The collected strains were examined for the following biochemical properties: gas from glucose fermentation, fermentation of raffinose and adonitol, urease production, acetylmethyl carbinol production, indole production and growth on Simmon's ammonium citrate medium.

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* Necropsies were performed at the National Veterinary Institute, Oslo, Veterinary College of Norway, Oslo, or at the Regional Veterinary Laboratory, Sandnes.

Serological examinations

The E. coli strains were primarily examined for the O-antigens 8, 64 and 149 and for the K-antigens 88 and 99. Diagnostic antisera against these antigens were prepared according to *Søderlind* (1971) using type strains obtained from Dr. Ørskov, the State Serum Institute, Copenhagen, Denmark. Strains which did not belong to any of the above mentioned O-groups were kindly tested by Dr. Søderlind, National Veterinary Institute, Uppsala, Sweden, with antisera against the following O-groups: 2,6,9,32, 45,98,115,138,139,141,147, and 157. The tube agglutination test was used for determination of O-groups (*Søderlind*). The demonstration of K88 and K99 antigens were performed by using slide agglutination test with colonies grown on blood agar and Minca-Isovitalex agar, respectively (*Guinée et al.* 1977 b).

Enterotoxin examination

Detection of LT was performed using enzyme linked immunosorbent assay described by *Olsvik et al.* (in press).

Table 1. Relationship between O-antigens, K-antigens 88 and 99 and production of heat labile enterotoxin (LT) in 198 strains of Escherichia coli isolated from jejunal content of piglets with neonatal diarrhea.

O-antigen*	Number of strains	Number of positive strains		
		K88	K99	LT
2	2	0	0	0
6	1	0	0	0
8	14	0	0	3
9	2	0	0	0
32	1	0	0	0
45	6	0	0	0
64	14	0	12	0
98	2	0	0	0
141	2	0	0	0
149	84	81	0	80
O-antigens not demonstrated with the sera used	70	1	0	

* No strains with O-antigens 115, 138, 139, 147 and 157 were diagnosed.

RESULTS

All the strains tested were classified as E. coli although 7.7 % of the isolated strains did not produce gas from glucose fermentation and 10.8 % were usease positive. The distribution of O-groups among the E. coli strains isolated in relation to LT production and presence of K88 and K99 antigens is shown in Table 1.

O-group 149 was the most prevalent O-group accounting for 84 of the 198 isolated E. coli strains (42 %). Fourteen strains (7 %) belonged to O-groups 8 and 64, respectively. A few strains in each group were distributed among 9 different O-groups. In 70 of the isolated strains (35 %) O-antigens were not demonstrated with the antisera used. K88 antigen was demonstrated in 81 of the 84 E. coli strains belonging to O-group 149, but only in 1 strain belonging to another O-group. K99 antigen was demonstrated exclusively in E. coli strains of O-group 64. Twelve of 14 strains in this O-group possessed this antigen.

LT production was demonstrated in E. coli strains belonging to O-groups 8 and 149 with the frequencies of 3 out of 14 strains and 80 out of 84 strains, respectively. All the LT-producing strains of O-group 149 possessed the K88 antigen. This antigen was not demonstrated in LT-producing strains belonging to O-group 8. Production of LT was not demonstrated in E. coli strains possessing the K99 antigen.

Table 2. Relationship between O-antigens, K-antigens 88 and 99 and ability to ferment raffinose and adonitol among 235 strains of Escherichia coli isolated from jejunal content af piglets with neonatal diarrhea.

O-antigen	Number of strains	Number of strains*					
		K88	K99	raf+/ad+	raf+/ad−	raf−/ad+	raf−/ad−
8	17	0	0	0	13	0	4
64	15	0	12	0	12	0	3
149	101	98	0	100	0	1	0
O-antigens not demonstrated with the sera used	102	2	0	2	38	14	48

* raf+, −: ability to ferment raffinose.
ad +, −: ability to ferment adonitol.

The relationship between O- and K-antigens and ability to ferment raffinose and adonitol among 235 strains of E. coli is presented in Table 2.

Among 101 strains of E. coli belonging to O-group 149, 100 strains fermented raffinose as well as adonitol. Ninetyeight of these strains also possessed the K88 antigen. Fermentation of both raffinose and adonitol was detected in only 2 out of 134 strains belonging to O-groups other than 149.

Hemolytic activity was not detected among strains of E. coli O149. Of the isolated strains only 2 were hemolytic (both O141).

DISCUSSION

In the present investigation 42 % of the isolated E. coli strains belonged to O-group 149. This result is in accordance with the frequency presented by *Liven* (1979). The importance of E. coli O149 in connection with neonatal diarrhea in piglets in Norway is similar to the situation in Denmark and Sweden (*Dam & Knox* 1974, *Søderlind & Møllby* 1978). In Ireland *Sweeney et al.* (1976) reported that 47.5 % of isolated E. coli strains from piglets with neonatal diarrhea belonged to O-group 149. Several reports during the 1960's demonstrated that E. coli strains of other O-groups than 149 were dominating in piglets with neonatal diarrhea (*Sojka* 1965, *Sørum* 1966). Thus, during the last 10—15 years E. coli strains possessing O-antigen 149 have spread through the pig population of many countries. This change in pattern of O-groups of E. coli causing neonatal diarrhea in piglets is of important epidemiological interest. Future studies should therefore also include O-groups of E. coli other than those generally accepted as enteropathogenic. *Guinée et al.* (1977 a) have listed several O-groups representing E. coli strains causing neonatal diarrhea in piglets without mentioning O64. The present investigation shows that strains belonging to this O-group most probably have caused neonatal diarrhea in piglets in some herds. E. coli O64 strains were isolated from piglets originating from 7 different herds in this study. By using 3 antisera (anti- O 8, 64 and 149) 56 % of the isolated strains could be grouped. Thus, the majority of enteropathogenic strains of E. coli belongs to a limited number of O-groups.

The close correlation between E. coli O149, K88 antigen and LT production in the present investigation is in accordance with the results of *Søderlind & Møllby* (1978). There is also a close

relationship between the presence of O-antigen 64 and K-antigen 99 in the strains examined. Porcine strains of E. coli was first shown to possess K99 antigen by *Guinée et al.* (1977 a) and *Moon et al.* (1977). These authors reported the presence of K99 antigen in porcine strains of E. coli belonging to O-groups 9, and 101. Further, *Smyth et al.* (1981) demonstrated K99 antigen in strains belonging to O-groups 8, 64 and 140.

Except for 3 strains of E. coli O8, LT was detected only among strains belonging to O-group 149. According to *Søderlind & Møllby* (1978) strains of different O-groups of E. coli including O6, O8, O9, O32, O64, O138, O139, O141, 0147 and O149 are able to produce LT. The strains in this investigation have not been examined for production of heat stable enterotoxin. Several reports demonstrate that E. coli O149 is able to produce both LT and ST (*Smith & Gyles* 1970, *Liven* 1979). Further, *Smyth et al.* (1981) demonstrated that all K99 positive strains examined produced ST. Therefore, it is likely that several of the strains in the present investigation are ST producers.

The use of vaccines in the prophylaxis of neonatal diarrhea in piglets should be based on knowledge about the characteristics of the E. coli strains occurring in the various herds. Four vaccines are currently used in Norway, 3 of which contain strains of killed E. coli 0149 and 2 containing strains of killed E. coli O64. The fourth vaccine recently available, contains LT-toxoid and the K88 ab and ac antigens (*Nagy et al.* 1978, *Næss* 1979, *Norberg* 1981).

In contrast to earlier reports concerning hemolytic activity of E. coli O149 (*Ørskov et al.* 1969, *Dam & Knox* 1974) none of the isolated strains belonging to this O-group were hemolytic in the present investigation.

There is a close correlation between the presence of O-antigen 149, K-antigen 88 and the ability to ferment raffinose and adonitol in E. coli strains. Besides, E. coli O149 possessing K88 antigen nearly always seem to produce LT. Thus, it seems as if the above mentioned properties in some way are linked together. Fermentation of raffinose and adonitol was demonstrated in only 2 strains belonging to other O-groups than 149. *Shipley et al.* (1978) demonstrated that the K88 and raffinose genes were located on a single nonconjugative plasmid in most of the porcine enteropathogenic E. coli strains. Results from other studies with adonitol fermenting E. coli strains indicate that genes encoding for

adonitol fermentation are not located on plasmids (*Franklin* 1981). In conclusion one can say that fermentation of both raffinose and adonitol among porcine E. coli strains is largely a characteristic of those belonging to O-group 149 possessing K88 antigen and being LT positive. Thus, fermentation of these 2 compounds may serve as a marker for the diagnosis of the most prevailing O-group of E. coli isolated in connection with neonatal diarrhea in piglets.

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SAMMENDRAG

Serologiske, enterotoksin-produserende og biokjemiske egenskaper hos Escherichia coli isolert fra spedgriser med diare i Norge.

Tilsammen 235 stammer av *Escherichia coli* isolert fra tynntarminnhold fra spedgriser med diare ble undersøkt for serologiske og visse biokjemiske egenskaper, samt for evne til å produsere termolabilt enterotoksin (LT). Blant 19 stammer tilhørte 84 (42 %) O-gruppe 149, mens 15 (7 %) stammer tilhørte O8, og det samme antall tilhørte O64. Det var ikke mulig å plassere 70 (ca. 35 %) av stammene i O-gruppe med de benyttede antiseraer. Få stammer ble funnet å tilhøre følgende O-grupper: 2,6,9,32,45,98 og 141. Åtti av 84 E. coli stammer tilhørende O-gruppe 149 hadde K88 antigen og produserte LT. Blant stammene

tilhørende O64 var 12 av 14 positive med hensyn til K99 antigenet. Dette adhesinet ble ikke påvist hos andre O-grupper av E. coli, mens K88 antigenet ble påvist hos 1 stamme tilhørende en ukjent O-gruppe foruten hos E. coli O149. Produksjon av LT ble i tillegg påvist hos 3 av 14 stammer tilhørende O-gruppe 8.

Av 101 stammer tilhørende O-gruppe 149 hadde 100 evne til å forgjære raffinose og adonitol samtidig. Nittiåtte av disse var K88 positive. Evnen til å omsette raffinose og adonitol samtidig ble forøvrig bare påvist hos 2 andre stammer tilhørende ukjente O-grupper.

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Reprints may be requested from: Kåre Fossum, the Department of Microbiology and Immunology, Veterinary College of Norway, P. O. Box 8146, Dep., Oslo 1, Norway.