PATHOGENIC PROPERTIES AND THE OCCURRENCE OF CAPSULAR ANTIGENS, BIOCHEMICAL CHARACTERISTICS AND SUSCEPTIBILITY TO COLICINES IN *ESCHERICHIA COLI* STRAINS OF GROUP O149. II. PATHOGENIC PROPERTIES AND THE OCCURRENCE OF CAPSULAR ANTIGENS *

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In the first part of the work (1) the kind of K antigen occurring in *E. coli* strains of the O149 group was determined and then biochemical properties and susceptibility to colicines of Fredericq's set were studied. The aim of the present work was to determine to eventual effect of K antigens, especially K88(L), on their pathogenic properties for mice and chick embryos and to investigate enteropathogenic properties of individual strains of this group in ligated segments of pig intestine.

Material and Methods

Bacterial strains. *E. coli* strains of the O149 group with determined previously (1) K antigen, isolated from diseased pigs (4, 5), were used. Out of 225 strains of this group, 12 strains with the single K antigen (K88ab and K85ab) and 5 strains with undetermined K antigen (K?) were examined and 21 strains containing simultaneously K antigen of B and L types (K91, K88ac) were randomly selected. The particular strains were selected basing on their titres in the O agglutination test and every 7 strains with the lowest (400), average (1600) and highest (3200) titres were used. All the strains were stored in a lyophilized state until used.

Biological tests. White mice and chick embryos were used as experimental animals. Mice weighing 18—20 g and originating from a colony bred by the Veterinary Institute were injected intraperitoneally with different dilutions of strains tested. The dose was 0.2 ml and 3 mice were used for each dilution. Mice were observed for 6 days after vaccination. Ten-day-old chick embryos, originating from Leghorn hens, were injected intraallantoically with 0.1 ml of every bacterial culture, undiluted or diluted 10^{-10}. Five chick embryos were used for every bacterial dilution. The number of dead and live chick embryos was determined after 16-hour incubation period. LD_{50} of live bacterial culture for mice and chick embryos was calculated by the method of Reed and Muench (3).

Two piglets, aged 8—9 week, weighing about 20 kg each. were used to test the enteropathogenic properties of *E. coli* strains belonging to the O149 group. Fifteen ml of the 24-hour bacterial culture of every tested strain were injected into a ligated segment of pig intestine. Injections were made according to the method of De et al. (2) modified by Truszczyński et al. (7). Intensity of dilatation of the ligated segment was evaluated using criteria described previously (7).

Results

LD_{50} values for mice and chick embryos of living bacterial cultures and the degree of enteropathogenicity in the ligated segment of strains containing simultaneously K91 and K88ac were presented in Table 1.

It is evident from the Table that the strains tested differed greatly in their pathogenic properties for mice. LD_{50} ranged from the dilution

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Table 1

Virulence of *E. coli* strains, group O149, with the double K antigen, for mice and chick embryos and their enteropathogenicity in ligated segment of pig intestine

Item	Strain No.	Kind of K antigen	O agglutination titre	LD_{50} for		Enteropathogenicity
				mice	chick embryos	
1	76	K91 (B), K88ac (L)	400	1:67.2	$10^{-7.6}$	++++***
2	133	„	400	1:5	10^{-9}	0****
3	166	„	400	1:27.4	10^{-6}	—***
4	172	„	400	1:20	$10^{-7.4}$	++++
5	477	„	400	1:16.8	$10^{-7.6}$	0
6	1125	„	400	1:2.9	$10^{-2.2}$	++++
7	1153	„	400	1:3.5	$10^{-7.7}$	++++
8	89	„	1600	1:3.9	10^{-4}	++++
9	108	„	1600	1:5.9	$10^{-2.5}$	+++
10	184	„	1600	1:23.5	$10^{-7.7}$	++++
11	420	„	1600	1:10	10^{-8}	++++
12	805	„	1600	1:10	$10^{-8.2}$	0
13	403	„	100*—1600**	1:16.8	10^{-7}	++++
14	813	„	200—1600	1:13.7	10^{-8}	0
15	205	„	100—3200	1:3.5	$10^{-6.8}$	+
16	358	„	100—3200	1:1.7	10^{-10}	++
17	1186	„	100—3200	1:3.5	10^{-8}	++++
18	799	„	200—3200	1:27	$10^{-8.3}$	+++
19	812	„	200—3200	1:23.8	10^{-7}	++++
20	181	„	3200	1:16.8	$10^{-8.3}$	++++
21	34	„	3200	1:2.9	$10^{-1.8}$	0

* — agglutination titre after inactivation of antigen at 100°C
** — agglutination titre after inactivation of antigen at 121°C
*** — negative results or a degree of dilatation of ligated intestinal segments according to criteria described previously (7)
**** — not tested

of 1:1.7 (less virulent strains) to 1:67.2 (very virulent strains). Similar results were obtained in determining the pathogenicity of these strains for chick embryos. LD_{50} ranged from the dilution of $10^{-1.8}$ to 10^{-10}. Comparison of virulence of every tested strain for mice and chick embryos revealed that, in most cases, the strains weakly pathogenic for mice killed chick embryos even at low dilutions while strains highly virulent for mice caused death of chick embryos after administering high bacterial dilutions.

Table 1 presents also the results of experiments on the enteropathogenic properties of 16 strains with double K antigen. All the strains tested, except one, showed enteropathogenic properties in the ligated segment of pig intestine.

Table 2 presents LD_{50} values of five bacterial strains, with single or undetermined K antigens, for mice and chick embryos and their entero-

pathogenic properties in ligated segments of pig intestine. It is evident from this table that the variety in degree of virulence for mice was smaller in this group of strains than in the previous one. Except one strain, no. 492, nonpathogenic for mice, no strain had LD_{50} lower than the 1:10 dilution, while in the previous group this was found in 50 per cent of strains. Similarly, LD_{50} of live bacterial cultures for chick embryos remained almost on the same level (10^{-7}—10^{-8}).

Table 2

Virulence of *E. coli* strains, group O149, with the single or undetermined K antigens, for mice and chick embryos and their enteropathogenicity in ligated segments of pig intestine

Item	Strain No.	Kind of K antigen	O agglutination titre	LD_{50} for		Enteropathogenicity
				mice	chick embryos	
1	295	K88ac (L)	800	1:10	$10^{-6.7}$	++*
2	541	K85ab (B)	400	1:32.8	$10^{-7.7}$	++++
3	1138	,,	400	1:16.8	$10^{-8.3}$	—*
4	1139	,,	400	1:16.8	$10^{-8.1}$	0**
5	577	,,	800	1:10	$10^{-5.9}$	—
6	1114	,,	800	1:54.8	$10^{-7.6}$	++
7	1128	,,	800	1:16.8	$10^{-8.3}$	0
8	1135	,,	800	1:16.8	$10^{-7.6}$	++++
9	1137	,,	800	1:16.8	$10^{-8.4}$	—
10	1145	,,	800	1:16.8	$10^{-7.5}$	++++
11	1096	,,	1600	1:109	$10^{-6.6}$	—
12	1060	,,	6400	1:23.5	$10^{-8.2}$	++++
13	534	K ?	800	1.28.3	10^{-8}	—
14	492	,,	1600	nonpathogenic	$10^{-4.5}$	—
15	980	,,	1600	1:16.8	10^{-6}	0
16	1119	,,	1600	1:16.8	10^{-8}	++
17	775	,,	3200	1.25.9	$10^{-7.8}$	++

* — negative results or a degree of dilatation of ligated intestinal segments according to criteria described previously (7)
** — not tested

Table 2 presents also the results of testing enteropathogenic properties of 14 strains with the single or undetermined K antigens. It has been found that most of the strains dilated ligated segments of pig intensine. Similarly as in the group of strains with the double K antigen no relationship was found between the degree of virulence for mice and chick embryos and the ability to dilate ligated segments of pig intestine.

Discussion

Investigations on the virulence for mice and chick embryos of *E. coli* strains, with O149 antigen and the single or double capsular antigen, confirmed the results of the previous own investigations (6) showing that

mutants of different virulence occur in a given serotype. The range of LD_{50} for strains of the O149 group approached to that found, on mice and chick embryos, for serogroups O138, O139, O141 and O8. Similary as in the previous investigations (6) a correlation was found in LD_{50} for mice and chick embryos.

A great percentage of strains with the O149 antigen showed enteropathogenic properties in ligated segments of pig intestine. However, more strains showed these properties if they contained simultaneously K antigens of B and L types than if they had only single K antigen of B type. This may suggest that K88(L) antigen occurs more often than other K antigens in strains with enteropathogenic properties. However, this does not mean that this antigen is the same as a factor conditioning the enteropathogenicity of *E. coli* strains. The comparison of results concerning the O149 group with their virulence for mice and chick embryos showed no relationship between these two properties. Thus, the results of the previous own investigations (7) were confirmed.

Conclusions

1. Little, mean and very virulent strains were found among strains with the double K antigen; strains with the single K antigen had more uniform pathogenic properties.

2. Strains belonging to the O149 serogroup were often enteropathogenic; strains with the double K antigen were enteropathogenic more often than those with the single K antigen.

3. Correlation has been found between pathogenicity of strains for mice and chick embryos and no correlation was demonstrated between pathogenicity of strains for these animals and their enteropathogenicity for pigs.

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