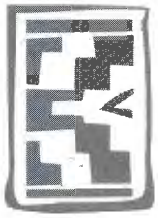

Escherichia coli serotypes in pigs in South Africa

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ABSTRACT

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This retrospective study was based on 674 cases of colibacillosis in pigs submitted to the diagnostic bacteriology laboratory of the Onderstepoort Veterinary Institute (OVI) over the 20-year period ranging from 1971–1991. During this time, 28 840 cases from various livestock species were received, of which 4 162 (14,4%) were from pigs. The 674 porcine cases selected for this study were included if an *E. coli* infection had been suspected by the referring veterinarian, and typable *E. coli* strains were then isolated by this laboratory.

Enteritis (45,5%) and septicaemia (46,9%) were the most common syndromes, with agalactiae (1,4%) and abortion (1,1%) representing a far lower prevalence. Oedema-disease signs were described by the submitting veterinarian in only 12 cases. Samples were received from weaners and sucklers in relatively equal numbers until 1981, but subsequently samples from sucklers declined, while those from weaners remained high.

There were 69 different somatic and capsulated (OK) antigen groups associated with *E. coli* infections in pigs. *Escherichia coli* O149 was the most common isolate (45,8%), while *E. coli* O141 was the next most common isolate (18,3%). This was followed by O9 (8,9%), O20 (5,2%) and O8 (3,1%). All other serotypes together accounted for less than 20 % of the total number of cases, and were isolated fewer than 20 times each. The fimbrial attachment factor, F4 (K88) was found associated with 46,9% of isolates.

Keywords: *Escherichia coli*, pigs, serotypes

INTRODUCTION

Escherichia coli is the predominant bacterium of the facultatively anaerobic flora of the intestine of many animal species. While most strains are non-pathogenic, certain of them may cause disease. The main syndromes produced by *E. coli* are enteritis (enteric colibacillosis) and septicaemia (colisepticaemia) in piglets, and oedema disease in older pigs. *Escherichia coli* occasionally also causes pyogenic infections in a variety of tissues and is responsible for some forms of mastitis and uterine infection. Enteric colibacillosis occurs most commonly in new-born animals in intensive farming systems, but only rarely in animals raised under extensive conditions.

Escherichia coli infections occur worldwide (Sojka 1965; Cooke 1985; Hinton 1985; Wray & Morris 1985), and can be of major economic significance. In southern Africa, enteric colibacillosis occurs most commonly in piglets, and less frequently in calves, lambs and kids (Wray & Morris 1985; Henton & Hunter 1994). At the start of the study, it was not known which serotypes were important in pigs in South Africa. Knowledge of the common types is important for vaccine selection and disease monitoring.

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Different strains of *E. coli* are typable according to the characteristics of capsular (K), somatic (O) and flagellar (H) antigens. Many strains, especially those isolated from extra-intestinal sites, possess polysaccharide capsules that are used for typing K antigens. The cell wall of the bacterium is used for O-antigen typing and is partially composed of lipopolysaccharide, of which the lipid-A portion is responsible for the toxic activities of what is referred to as endotoxin. If a strain possesses neither a capsule nor flagellae, it is characterized by the O antigen alone.

The classification of *E. coli* is based on both biochemical tests and serotyping of the O, K and H antigens, the latter being one of the most useful ways of identifying pathogenic types. At present, 170 O antigens, 80 K antigens, and 56 H antigens are recognized. The number of possible serovars or serotypes is estimated to be 10^3 (Germani 1995) as there is considerable variation in the combinations of the three antigens.

Virulence factors coded for by plasmids are toxin production, possession of pili (also referred to as fimbriae), and antibiotic resistance. The virulence of enteropathogenic *E. coli* is attributed mainly to the presence of pili and the production of toxins (Morris, Thorns, Wells, Schott & Sojka 1983). Certain serotypes are usually associated with specific virulence factors. Although most of the virulence factors are coded for by transmissible plasmids, and while in theory any serotype could harbour them, in practice there is usually no environmental pressure for most of them to be transmitted. The only exception is for the plasmids coding for antibiotic resistance, which is usually complex and involves multiple antibiotic resistance (Sojka 1973; Wray & Morris 1985).

In addition to flagellae, many strains possess antigenic proteinaceous pili, some of which function as adherence factors and are therefore responsible for the attachment of *E. coli* to mucosal epithelial cells of the intestinal tract (Bijlsma, De Nijs, Van der Meer & Frik 1982; Sellwood 1984). When these pili antigens were first described, their chemical nature was unknown. The first two discovered were thought to be capsular antigens and were named K88 and K99 (Orskov 1984). These are now known, respectively, as F4 and F5 (F for fimbrial antigen). Adherence factors are produced in the greatest quantity in the small intestine but are elaborated only under certain circumstances in culture (Dean 1990).

Colibacillosis in pigs occurs in two distinct age groups: neonates and those in the immediate post-weaning period. The percentage of pathogenic strains of *E. coli* relative to non-pathogenic strains that are carried by the sow, increases markedly at farrowing. This facilitates their transmission to piglets early in life (Glantz 1971). Whether or not neonatal colibacillosis develops in piglets which are exposed to pathogenic strains of *E. coli,* depends to a large extent on the presence or absence of colostral and lacteal antibodies; other factors play a lesser role (Armstrong & Cline 1977; Bertschinger, Eggenberger, Jucker & Pfirter 1979). On the other hand, stress plays an important predisposing role in the development of the disease in weaned piglets. Piglets are exposed to many stress factors at weaning, including loss of maternal contact, movement to new pens, new pen-mates, overcrowding, and erratic environmental temperature caused in part by lack of maternal warmth. In addition, declining levels of lacteal IgA may play a role. Weaning stress also results in a temporarily increased gastric pH which results in decreased gastric bactericidal activity (Schulman 1973). Previously, changes in dietary composition at weaning or after transport to a different farm were believed to predispose to colibacillosis in piglets. It is now believed that if piglets are fed a creep-feed while still suckling their dams, hypersensitivity to dietary antigens may develop. This could predispose to enteric colibacillosis after weaning if they are fed a weaning diet containing the ingredients to which they developed sensitivity in the creep-feed (Miller, Newby, Stokes, Hampson & Bourne 1983; Miller, Newby, Stokes & Bourne 1984).

Escherichia coli, particularly non-pathogenic strains, form part of the normal intestinal flora of warm-blooded animals. Pathogenic strains form only a small part of the total *E. coli* population of adult animals (Sojka 1973). They are usually transmitted by the faecal-oral route, and are among the first organisms to establish themselves in the large intestine soon after the piglets are born (Sojka 1973), with the small intestine being subsequently colonized. Once established, and irrespective of the age of the animal, few *E. coli* are present in the cranial part of the small intestine, relatively large numbers are present in the caudal part, while the largest numbers occur in the large intestine (Sojka 1973).

Pathogenic strains of *E. coli* produce several toxins categorized either as enterotoxins (Germani 1995) or cytotoxins. The enterotoxigenic strains produce two main toxins, designated heat-labile toxin, which is chemically closely related to cholera toxin, and heat- stable toxin. Each toxin may be produced alone or toxins may be produced in combination. The toxins are usually associated with specific serotypes, and toxin production is coded for by specific plasmids. The heat-labile toxin is antigenic, while the heat-stable toxin is not. Only human and porcine *E. coli* strains, particularly those of the K88 serotypes, have been shown to produce heat-labile toxin (Levine 1987; Germani 1995). The heat-labile toxins produced by different animal-derived strains of *E. coli* are antigenically similar. Both toxins induce biochemical changes in the epithelial cells of the mucosa (the cells of the mucosa remain intact and show no

morphological changes) that result in the hypersecretion of fluids and electrolytes into the intestinal lumen (Levine 1987).

Enteropathogenic *E. coli* produce Vero cytotoxins (VT), also known as Shiga-like toxins (SLT), which are similar to the toxins of *Shigella dysenteriae* type 1. These toxins are cytotoxic for HeLa and Vero cells, enterotoxic in ligated rabbit ileal segments, and lethal for mice (O'Brien & Holmes 1987). Three types of toxin are known: VT1, which can be neutralized by anti-Shiga toxin; VT2; and VTe, the latter being a variant which was previously known as 'edema disease principle (EDP)' or 'oedema disease toxin'.

Colisepticaemia may develop terminally in animals suffering from enteric colibacillosis or it may occur without any evidence of enteric involvement. Infection may be contracted *per os* (tissue invasion taking place in the nasopharynx or intestinal tract) or via the umbilicus (Sojka 1965; Glantz 1971). Very young animals, and particularly those that are hypogammaglobulinaemic owing to insufficient absorption of colostral antibodies, are prone to contract colisepticaemia.

This retrospective study was based on the results obtained on bacteriological examination of organ specimens submitted to the Onderstepoort Veterinary Institute (OVI) from unselected cases of porcine disease diagnosed as colibacillosis by the attendant veterinarians over a 20-year period. Its purpose was to establish which serotypes of *E. coli* are important in diseases of pigs in South Africa, and their relationship to the syndromes mentioned above.

MATERIALS AND METHODS

Antisera

Antisera were prepared from type strains obtained from the Statens Serum Institute, Copenhagen, Denmark, by courtesy of Dr F. Orskov, in accordance with standard methods (Sojka 1965; Orskov & Orskov 1984; Ewing 1986; Germani 1995). These methods are briefly as follows:

Production of O antisera

Antisera were prepared from 151 O strains of *E. coli*, from O1–O157, with the exception of O31, O47, O67, O72, O94 and O122, as these have been removed from the register, because they either do not exist or were subsequently found to be *Citrobacter* (Orskov & Orskov 1984).

Each serotype was cultured overnight on blood tryptose agar (Oxoid). Growth was harvested with a curved glass rod, suspended in 10 mℓ of normal saline and autoclaved at 121 °C for 1 h to destroy the K antigens. The density of the suspension was adjusted to correspond to the turbidity of Browns 3 which corresponds to 10^9 bacteria per mℓ. This suspension was used to vaccinate adult rabbits from the OVI colony, with ascending doses of 0,5, 1,0, 1,5 and 2,0 mℓ intravenously at 4-d intervals. Each animal was bled 7 d after the final injection. The sera were harvested and preserved with 0,5% phenol and stored in a refrigerator at 4 °C.

Each serum was diluted with normal saline to a working strength at which it showed good agglutination with its homologous serotype and no cross reactions with any of the other O serotypes in a plate agglutination test. Serum was considered satisfactory if there was rapid clear agglutination at a dilution of 1/100 or more. Antisera were absorbed if significant cross reactions occurred.

Production of OK antisera

Antisera were produced from 95 OK strains, from K1–K103, with the exception of K21, K64, K65, K77, K92, K100, K101 and K102, as these strains were not available.

Each serotype was cultured and made into a suspension as described above but was not autoclaved. Vaccination of rabbits was carried out at 4-d intervals with ascending doses of 0,5, 0,5, 1,0 and 1,5 mℓ. As these live suspensions were usually toxic to rabbits, formalin (40% m/v) to reach a final concentration of 0,3% (m/v) was added to a portion of the suspension 18 h before the first injection, and 4 h before the second. The third and fourth injections were of the suspension only. Rabbits were bled 7 d after the final injection and their sera were harvested and preserved with 0,5% phenol.

Sera were considered satisfactory when the K titre was 1/80 and higher in the tube agglutination test, and the O titre was not more than one dilution higher than the K titre.

Absorption of K antisera

Antisera prepared as above from live cultures, contained both O and K precipitating antibodies. Sera were absorbed either with the homologous culture, or a culture was used in which the O antigen was similar to, but the K antigen differed from, the capsular type against which the serum was raised.

Sera prepared from strains that had the K(B) capsular type, were used as OK sera and not absorbed, but diluted to a working strength as above.

Slide agglutination test

This was performed in the same manner as described by Glantz (1971). Sera were dispensed in cartridges. Polyvalent pools were prepared, each containing ten sera, except the last pool in each

group, which contained fewer than ten sera. There were eight sera in the final O pool and five in the final K pool. A drop of antiserum was added to a drop of bacterial suspension on a 20-circled glass slide. These were mixed for 1 min and then read.

Isolation of *E. coli*

All the organ specimens from the cases submitted by veterinarians, and in which *E. coli* infections were suspected, were cultured at 37 °C for 24 h on blood tryptose agar which was prepared with 10% bovine blood and McConkey agar (Difco and Biolab). Colonies suspected of being *E. coli* were identified according to standard methods (Sojka 1965; Orskov 1984; Orskov & Orskov 1984). Smooth colonies were selected visually.

At least three colonies were serotyped from each organ specimen from which *E. coli* was cultured. The organs from which specimens were taken and submitted from each animal, varied to some extent, but generally small and large intestine, mesenteric lymph node, liver, spleen and lung were represented. In about 30% cases, initial bacteriological isolations were performed at laboratories other than the OVI, and only specific cultures of *E. coli* were received for serotyping. In these cases, no selection of colony types was made. Each colony tested was inoculated into duplicate test tubes containing nutrient broth, and cultured overnight. The following day one of the tubes was heated to 121 °C under pressure in an autoclave for 15 min, and the sediment typed with the O antisera, the other was centrifuged and the deposit used for typing with the K and OK antisera.

Test for heat-stable enterotoxin (ST) in suckling mice

The method initially described by Dean, Ching, William & Harden in 1972, with modifications suggested by B.D. Schoeb, SAIMR, Johannesburg (personal communication 1976), was used.

Not every strain that was isolated was tested for ST, but those that were, were cultured overnight in nutrient broth at 37 °C. The next day the broth was centrifuged at 2 000 rpm for 30 min. The clear supernatant was drawn off and 50 µℓ of Evans Blue solution (2%) was added as a tracer dye. Suckling mice from several litters were randomly assigned to groups containing at least three animals. A stomach tube, prepared from a pediatric intravenous catheter, was passed into each infant mouse, and 0,1 mℓ of the supernatant was deposited in the stomach. The successful performance of this prodedure was assured if the white stomach contents, as seen through the transparent abdominal wall, changed to blue. At least three suckling mice per strain were used. Each group of mice was placed in a gauze bag, separated from the mother, and kept at 35 °C for 4 h. The mice were then sacrificed and the entire gastrointestinal tracts and their contents of the three mice in each group were weighed together. The combined mass of the remainder of the carcasses of the three mice was also determined. If heat-stable enterotoxin was present, fluid accumulated in the gastrointestinal tract, which was then relatively heavier than the intestinal tract of a suckling mouse dosed with a non-toxigenic strain. If no Evans Blue dye was visible in the gastrointestinal contents, the mouse was rejected, as this indicated that the supernatant had not been correctly deposited. The ratio of the mass of the gastrointestinal tracts divided by the mass of the rest of the bodies, was calculated. If the ratio was greater than 0,09, the strain was considered to be positive for ST production, but if it was less than 0,06, the strain was considered negative. If the ratio fell between 0,06 and 0,09, the test was repeated, and this usually resulted in a clear-cut result.

The 2 x 2 chi-squared test was used for all statistical determinations.

RESULTS

The total number of specimens received from cases of *E. coli* infections in pigs was 674 over a span of 20 years from July 1971 to July 1991, averaging 34 cases per year (Table 1).

The three years during which most positive cases were diagnosed, were 1973, 1978 and 1981, when specimens from 55, 58 and 60 cases, respectively, were received. Cases were relatively evenly spread throughout the year, the total of the highest numbers being in September and November (67 and 66, respectively) and the lowest in December and January (38 and 49, respectively) (Table 1). These differences were not significant (P = 0,3006). In some cases, more than one serotype of *E. coli* was present in an outbreak (Table 1).

Samples were received from all over southern Africa, with the greatest numbers from the province previously known as Transvaal (70%), which is the principal area served by the OVI (Table 2).

As more than one serotype may be isolated from a case of colibacillosis, the total number of serotypes isolated from the 674 cases was 782 (Table 3).

There were 69 different serotypes associated with disease in pigs (Table 3). Two serotypes were isolated more than 100 times each. These were *E. coli* O149 which was isolated 358 times and *E. coli* O141 which was isolated 144 times.

Nine serotypes were isolated fewer than 100 times but more than ten times. They were *E. coli* O20:K- (isolated 28 times); *E. coli* O139 (19 times); *E. coli* O9:K28 and *E. coli* O9:K30 (each 18 times); *E. coli*

TABLE 1 The total number of cases of *E. coli* infections in pigs, received over a 20-year period, for each month, and the total number of serotypes isolated

Year	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Total cases	Total serotypes for year
1971	NT[a]	NT	NT	NT	NT	NT	2	–[b]	–	–	2	2	6	7
1972	1	5	–	1	–	2	4	5	3	3	4	2	30	36
1973	9	3	–	2	2	–	2	9	9	9	7	3	55	71
1974	3	3	6	2	3	1	2	5	3	8	3	3	42	48
1975	–	1	5	7	5	2	4	1	2	–	3	5	35	36
1976	–	5	3	1	–	2	–	–	2	3	3	2	21	24
1977	1	4	–	3	1	–	1	2	3	5	4	2	26	28
1978	5	8	7	2	4	9	3	3	13	1	3	–	58	65
1979	4	3	–	4	3	3	3	3	3	2	4	1	33	39
1980	3	–	4	3	2	4	5	5	4	4	3	2	39	41
1981	7	4	6	3	6	6	12	4	5	4	3	–	60	67
1982	1	3	4	2	2	2	2	3	1	2	4	1	27	34
1983	5	2	1	–	2	1	2	2	2	1	5	–	23	24
1984	4	3	5	–	2	2	–	5	1	1	4	1	28	30
1985	–	2	–	11	1	4	1	1	–	–	3	2	25	27
1986	–	4	2	2	3	3	2	1	2	–	2	2	23	26
1987	–	2	–	1	1	3	3	4	–	2	–	4	20	30
1988	2	1	2	3	1	2	1	2	7	4	3	2	30	39
1989	2	1	2	5	5	2	2	2	4	3	2	1	31	35
1990	–	1	5	5	8	1	5	3	3	3	4	3	41	50
1991	2	5	6	5	2	1	NT	NT	NT	NT	NT	NT	21	25
Total cases	49	60	58	62	53	50	56	60	67	55	66	38	674	782

[a] Not included
[b] No cases.

TABLE 2 Distribution of *E. coli* samples received over a 20-year period, according to previous geographical provinces

Area	Total (%)	Prevalence of the two most common types	
		O149 (%)	O141 (%)
Transvaal	472 (70,0)	261 (72,9)	114 (79,2)
Cape	126 (18,7)	48 (13,4)	15 (10,4)
Natal	42 (6,2)	31 (8,6)	6 (4,2)
Free State	28 (4,2)	14 (3,9)	9 (6,3)
Other countries	6 (0,9)	4 (1,1)	– (–)
Total	674	358	144

O78 (15 times); *E. coli* O138 (14 times); *E. coli* O9:K35 (12 times) and *E. coli* O20:K83 and *E. coli* O157 (each ten times).

Thirty-one serotypes were isolated two to nine times each and 27 serotypes were isolated only once during the 20-year period (Table 3).

The most common serotype was *E. coli* O149 (O149:K91), previously known as Abbotstown A1 (Sojka 1965). It was isolated 358 times (45,8%). The next most common isolate was O141 (O141:K85), previously known as E68 (Sojka 1965). Together, these two serotypes were responsible for 64,2% of the *E. coli* infections in pigs diagnosed by the OVI.

All the other serotypes lagged far behind these two, with the next most common, *E. coli* O20:K- being isolated only 28 times, which represents 3,6% of the total (Table 3). If the isolates as listed by O serogroup are considered, the third most common group was O9, with 70 isolations (8,9% of the total isolations).

There were 11 different capsular groups associated with *E. coli* O9, the three most frequently isolated being O9:K28, O9:K30 and O9:K35.

Escherichia coli O20 was the fourth most common group, with 41 isolations (Table 3) or 5,2% of the total number of isolations. There were only two capsular types associated with *E. coli* O20; these were *E. coli* O20:K83 and *E. coli* O20:K84. The majority of *E. coli* O20 strains did not possess a detectable capsule.

Escherichia coli O8 group was in fifth place with 24 isolations. There were five capsular types associated with it, the two most frequent isolates either being *E. coli* O8:K87 or possessing no detectable capsule.

The two groups associated with oedema disease, O139 and O138, followed in fourth and eighth places, with 19 and 14 isolations, respectively (Table 3). Fifteen isolations were made of *E. coli* O78, which is a serotype commonly found in ruminants and poultry as well as pigs in South Africa. *Escherichia coli* O157 was the ninth most commonly isolated, with ten

TABLE 3 Total number of *E. coli* serotypes and the syndromes associated with them

Serotype	F4 (K88)[a]	Syndromes					Infant mouse test[b]	Total
		Enteritis	Septicaemia	Agalactiae	Abortion	Other		
O149	308	182	167	–	2	9	1/1[c]	358
O141	46	81	56	–	2	5	–	144
O20:K-	–	9	17	1	–	1	5/7	28
O139	2	9	9	–	–	1	–	19
O9:K28	–	5	13	–	–	–	1/1	18
O9:K30	–	8	10	–	–	–	1/1	18
O78	1	3	11	1	–	–	0/1	15
O138	–	4	9	–	–	1	–	14
O9:K35	–	2	8	1	–	1	–	12
O20:K83	–	4	5	1	–	–	–	10
O157	1	7	2	–	–	1	–	10
O8:K-	–	5	4	–	–	–	–	9
O8:K87	6	4	3	–	1	–	–	8
O9:K34	–	2	4	–	–	–	–	6
O101:K103	–	2	3	–	1	–	–	6
O154:K-	–	2	4	–	–	–	3/3	6
O2:K-	1	3	2	–	–	–	–	5
O142	–	3	2	–	–	–	–	5
O5:K4	–	3	–	–	–	1	–	4
O9:K9	–	3	1	–	–	–	–	4
O55	–	–	2	–	1	1	–	4
O114	–	–	3	1	–	–	–	4
O5:K-	–	2	1	–	–	–	0/1	3
O8:K8	–	2	1	–	–	–	–	3
09:K26	–	2	1	–	–	–	1/1	3
O9:K32	–	–	2	–	1	–	–	3
O20:K84	–	1	2	–	–	–	–	3
O26	–	2	–	–	–	1	–	3
O86	–	1	2	–	–	–	–	3
O1:K-	–	–	1	–	–	1	–	2
O2:K1	–	2	–	–	–	–	–	2
O8:K48	–	2	–	–	–	–	–	2
O9:K36	–	–	–	2	–	–	–	2
O9:K39	–	1	–	1	–	–	–	2
O12K-	–	2	–	–	–	–	–	2
O64:K-	–	1	1	–	–	–	–	2
O101:K-	–	2	–	–	–	–	–	2
O112	–	1	1	–	–	–	–	2
O119	–	–	2	–	–	–	–	2
O126	–	1	1	–	–	–	–	2
O137	1	–	1	–	1	–	–	2
O147	1	2	–	–	–	–	–	2
O1:K1	–	–	1	–	–	–	–	1
O2:K54	–	–	1	–	–	–	–	1
O4:K12	–	–	1	–	–	–	–	1
O8:K25	–	–	1	–	–	–	–	1
O8:K40	–	–	1	–	–	–	–	1
O9:K31	–	1	–	–	–	–	–	1
O9:K38	–	–	–	1	–	–	–	1
O12:K12	–	1	–	–	–	–	–	1
O21:K-	–	1	–	–	–	–	–	1
O27:K-	–	–	1	–	–	–	–	1
O50:K-	–	1	–	–	–	–	–	1
O54:K	–	1	–	–	–	–	–	1
O68:K-	–	–	1	–	–	–	–	1
O92:K-	–	–	1	–	–	–	–	1
O95:K-	–	–	1	–	–	–	–	1
O95:K1	–	–	1	–	–	–	–	1
O98:K-	–	–	1	–	–	–	–	1
O108:K-	–	–	1	–	–	–	–	1
O121:K-	–	–	1	–	–	–	–	1
O121:K87	–	–	–	1	–	–	–	1
O124	–	–	1	–	–	–	–	1
O125	–	1	–	–	–	–	–	1
O132:K-	–	–	–	1	–	–	–	1
O133:K3	–	–	1	–	–	–	–	1
O145:K-	–	–	1	–	–	–	0/1	1
O152:K-	–	–	1	–	–	–	0/1	1
O153:K-	–	–	–	–	–	1	–	1
Total	367	371	367	11	9	24		782

[a] Number of strains also carrying the F4 (K88) plus antigen
[b] Infant-mouse test to detect heat-stable enterotoxin
[c] Numbers positive/numbers tested

TABLE 4 Prevalence of selected *E. coli* serotypes isolated over a 20-year period

Year	Serotype									
	O149	O141	O20	0139	0157	O138	09:35	O101	Other	Total
1971	–	3	–	–	–	2	–	–	2	7
1972	13	9	1	–	–	3	4	–	7	37
1973	9	13	4	3	–	5	4	–	34	72
1974	23	5	1	–	1	1	2	–	15	48
1975	17	3	3	–	1	–	1	–	11	36
1976	14	5	–	2	–	–	–	–	3	24
1977	12	5	2	1	–	–	–	–	8	28
1978	32	5	5	5	1	–	–	1	16	65
1979	19	7	2	2	1	–	–	–	8	39
1980	25	6	2	2	–	–	–	–	6	41
1981	31	7	2	2	1	3	1	–	20	67
1982	18	8	2	1	–	–	–	–	5	34
1983	14	5	2	1	–	–	–	–	2	24
1984	20	4	1	–	–	–	–	–	5	30
1985	16	6	2	–	–	–	–	1	2	27
1986	21	4	1	–	–	–	–	–	–	26
1987	14	4	3	–	1	–	–	1	7	30
1988	14	9	1	–	1	–	–	1	13	39
1989	11	10	5	–	3	–	–	1	5	35
1990	23	18	1	–	–	–	–	2	6	50
1991	12	8	1	–	–	–	–	1	3	25
Total	358	144	41	19	10	14	12	8	178	784
Average %	45,8	18,3	5,2	2,4	1,3	1,8	1,5	1,0		

isolations during the 20 years. All the other types were isolated fewer than six times each during the period under review, from pigs manifesting clinical signs of *E. coli* infection.

Some serotypes showed a relatively even distribution of isolations per year over the 20-year period, and the number of isolations as well as the percentage of the particular strain over the number of isolations per year remained relatively constant for *E. coli* O141 and *E. coli* O20, as is seen in Table 4. *Escherichia coli* O149 was isolated in somewhat small numbers, both in absolute numbers and as a percentage of total numbers, from 1971 until 1974, but subsequently an average of about half the isolations were *E. coli* O149. *Escherichia coli* O139, one of the types causing oedema disease, was identified for the first time in 1973, regularly isolated from 1976 to 1983, but has not been isolated since. *Escherichia coli* O138, which also causes oedema disease, showed a similar pattern. It was regularly isolated from the start of the period until 1974, then again in 1981 but not subsequently.

The fimbrial antigen F4 (K88) was found in association with 367 isolates (46,9%) belonging to nine strains (Table 5). It was carried most frequently in association with O149 (86,0%). The next most common carrier of F4 was *E. coli* O8:K87, carried by six of the eight isolates (75,0%). This is, however, a very small sample and may therefore be unreliable. The third most common strain carrying F4 was *E. coli* O141, which showed a carriage rate of 31,9%. The other six strains listed in Table 5 had only single occurrences of F4 association, with the exception of O139, where two out of 19 strains (10,5%) carried the antigen. The two most frequent porcine pathogenic strains isolated in this study, namely O149 and O141, which together were responsible for 64,2% of *E. coli* infections in the pigs, together carried F4 (K88) in 354 of 502 isolations (70,5%).

The ability of certain strains to produce ST was tested in infant mice only 18 times. Strains were tested only if there was some doubt about the association of the isolate with the disease. Of the 18 strains tested, 12 were found to produce ST (Table 6). *E. coli* O20:K- produced ST in five out of seven cases. The few strains of *E. coli* O154, O149, O9:K28, O9:K30 and O8:K8 that were tested, all produced ST, but the single strains of *E. coli* O78, O5:K-, O145:K- and O152:K- tested did not.

In this study, the *E. coli* infections in pigs most commonly manifested as syndromes associated with either enteritis or septicaemia (Table 3), with the prevalence of the enteritis syndrome (47,5%) being almost equal to that of the septicaemia (46,9%). Agalactiae at 1,4% and abortion at 1,1%, represented a far lower prevalence. Other infections represented conditions such as metritis, cystitis, arthritis and abscessation in which *E. coli* was isolated as

TABLE 5 Serotypes of *E. coli* found to be associated with the F4 (K88) fimbrial antigen

Serotype	No. of isolations with F4	Total no. of isolations	(%)
O149	308	358	(86,0)
O141	46	144	(31,9)
O139	2	19	(10,5)
O78	1	15	(6,7)
O157	1	10	(10,0)
O8:K87	6	8	(75,0)
O2:K-	1	5	(20,0)
O137	1	2	(50,0)
O147	1	22	(50,0)
Other	0	415	(0,0)
Total	367	782	(46,9)

TABLE 6 Detection of heat-stable toxin by maens of the infant mouse test

Serotype	Positive	Negative	Total
O20	5	2	7
O154	3	0	3
O149	1	0	1
O9:K28	1	0	1
O9:K30	1	0	1
O78	0	1	1
O5:K-	0	1	1
O8:K8	1	0	1
O145:K-	0	1	1
O152:K-	0	1	1
Total	12	6	18

either an opportunist or as a sequel to chronic infection.

In 447 cases, the age of piglets that had suffered from colibacillosis was known. There were 311 cases (69,5%) in weaners as opposed to 136 cases (30,4%) in sucklers. Fig. 1 illustrates the change seen over the years in the relative percentage of samples received from cases of *E. coli* infections in young animals. Until 1981, samples from weaners and sucklers were received in relatively equal proportions, but after 1981, the number of samples received from sucklers declined, whereas that from weaner samples remained relatively high.

In weaners, more specimens were received from piglets with enteritis (187 or 60,1%) than from those with septicaemia (122 or 39,2%), but the converse was found in sucklers where septicaemia (81 or 59,5%) was more common than enteritis (51 or 37,5%). According to the 2 x 2 chi-squared test, this difference was significant at $P < 0,0001$. More weaner problems were due to *E. coli* O149 (59,8%) than to

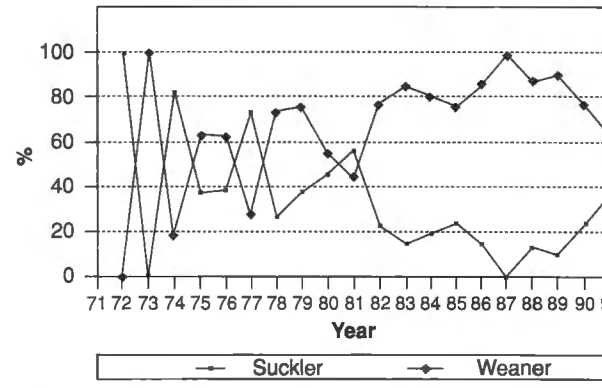

FIG. 1 The change in the number of cases of colibacillosis in suckler and weaned piglets expressed as a percentage of the total received each year

E. coli O141 (23,5%) (Table 7), and this phenomenon also appeared to hold for the sucklers, but in the case of the latter the difference was not significant ($P = 0,79$). The fimbrial antigen F4 (K88) was more common in isolates from weaners (63,3%) than from those of the sucklers (49,3%).

As there were noteworthy differences between the various serotypes of *E. coli* infecting the pigs, the results of the investigation presented below are given in more detail according to the serotype of *E. coli* involved.

The two types, O149 and O141, which together comprised 64,2% of all isolations, mirrored the general results and have been included in detail in Tables 3, 4, 5, 6 and 7. In 83 cases, mixed infections of bacteria, which included *E. coli* O149 as well as other serotypes of *E. coli* and species of *Salmonella*, *Streptococcus*, *Serpulina*, *Clostridium perfringens* type A, *Klebsiella pneumoniae* capsule type 31, *Pasteurella multocida*, *Pasteurella pneumotropica*, *Haemophilus parasuis* and *Chlamydia*, were isolated from pigs or their litter mates. These infections had induced a clinical disease similar to that of colibacillosis. The most common of these species that was found together with *E. coli* O149, was *E. coli* O141. These two together comprised 37 of the 83 cases (0,05% of the total number). Sixteen of these were piglets suffering from septicaemia, and 20 piglets suffering from enteritis. One was an aborted foetus. Other relatively common *E. coli* strains found in conjunction with O149 were *E. coli* O20 (five cases), O138 (three cases) and O139 and O8:K87 (two cases each). *Salmonella* Typhimurium was found together with *E. coli* O149 on four occasions. *Streptococcus equisimilis* was the most common *Streptococcus* species identified together with both *E. coli* O149 and O141.

Escherichia coli O141 was found together with other bacterial infections in 72 cases. Most of the latter were other serotypes of *E. coli* (excluding *E. coli*

TABLE 7 Cases of *E. coli* infections from sucklers and weaners by clinical sign, year and whether O141 of O149 or K88 was involved

Year	Suckler												Weaner											
	O149				O141				F4 (K88)				O149				O141				F4 (K88)			
	Total	E[a]	S[b]	O[c]	Total	E	S	O	Total	E	S	O	Total	E	S	O	Total	E	S	O	Total	E	S	O
1971	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1972	3	2	1	–	–	–	–	–	3	2	1	–	–	–	–	–	–	–	–	–	–	–	–	–
1973	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1	–	–	–	–
1974	7	4	3	–	1	1	–	–	6	3	3	–	1	1	–	–	1	–	1	–	1	1	–	–
1975	5	1	4	–	–	–	–	–	3	1	2	–	8	1	7	–	3	–	3	–	9	1	8	–
1976	5	2	3	–	–	–	–	–	4	2	2	–	10	4	6	–	7	2	5	–	3	3	–	–
1977	7	2	5	–	1	–	1	–	8	2	6	–	1	1	–	–	3	2	1	–	3	2	1	–
1978	4	1	3	–	2	1	–	1	6	2	3	1	24	17	7	–	1	1	–	–	22	15	7	–
1979	5	2	3	–	1	1	–	–	5	2	3	–	8	5	3	–	4	2	2	–	11	7	4	–
1980	6	2	4	–	–	–	–	–	7	2	5	–	11	7	4	–	3	2	1	–	10	6	4	–
1981	5	1	4	–	2	2	–	–	5	1	4	–	9	6	3	–	3	2	1	–	10	6	4	–
1982	2	2	–	–	1	1	–	–	3	3	–	–	10	3	7	–	4	1	3	–	10	4	6	–
1983	1	–	1	–	–	–	–	–	1	–	1	–	9	8	1	–	1	1	–	–	8	7	1	–
1984	3	1	1	1	–	–	–	–	2	–	1	1	15	5	10	–	4	2	2	–	16	6	10	–
1985	2	–	2	–	2	1	1	–	3	–	3	–	13	5	8	–	3	–	2	1	12	5	7	–
1986	2	1	1	–	1	–	1	–	2	1	1	–	15	8	7	–	3	1	2	–	13	6	7	–
1987	–	–	–	–	–	–	–	–	–	–	–	–	11	6	5	–	3	2	1	–	13	7	6	–
1988	2	–	2	–	1	–	1	–	1	–	2	–	11	6	5	–	8	6	2	–	13	8	5	–
1989	–	–	–	–	1	–	1	–	–	–	–	–	11	7	4	–	7	7	–	–	10	7	3	–
1990	3	1	2	–	4	–	4	–	4	1	3	–	17	13	4	–	13	11	12	–	19	15	4	–
1991	2	2	–	–	1	1	–	–	3	3	–	–	5	4	1	–	5	3	2	–	7	5	2	–
Total	64	24	39	1	18	8	9	1	67	25	40	2	186	105	81	–	73	46	25	2	197	112	85	–

[a] E = Enteritis
[b] S = Septicaemia
[c] O = Other infections

O149). From three of these cases *E. coli* O138 was isolated and from another three, *E. coli* O78. *Salmonella* Typhimurium was detected in four of the cases. Other isolates were *Serpulina*, *Streptococcus*, *Actinomyces pyogenes* and *Erysipelothrix rhusiopathiae*.

The *E. coli* O20 group was isolated from 14 sucklers, 13 weaners and four adults. The suckler samples were from nine cases of septicaemia, three of enteritis and one of arthritis, while the nature of the clinical disease in one was unknown. In the weaners, the *E. coli* O20 group was isolated from equal numbers of animals that had suffered from septicaemia or enteritis (6:7). Four of the cases were adults—two of the isolates were from mastitic milk and two from septicaemic animals. In only five of the cases was *E. coli* O20 found in conjunction with other bacterial pathogens; these were all other strains of *E. coli*.

Escherichia coli O139 was isolated 19 times, but oedema disease was described in only five of the animals. Other syndromes associated with this organism were congestion (four cases), diarrhoea (two cases) and septicaemia (one case). Nine of the cases were weaners and two were 3–10-d-old sucklers with congestion. From three of the cases, *E. coli* O139 was isolated together with other pathogenic *E. coli* strains, while in one, *Serpulina hyodysenteriae* was detected.

Escherichia coli O78 was isolated from four sucklers, four weaners, and two adult pigs, one with agalactia and one with septicaemia. Ten of these cases of *E. coli* O78 showed signs of septicaemia and four of enteritis.

The ages of only three animals of the 14 cases in which *E. coli* O138 was detected were known—two were sucklers and one was a weaner. Septicaemia occurred in eight cases, enteritis in four and oedema disease in one.

Escherichia coli O157 was isolated from five weaners, one suckler and one adult in the seven cases of which the ages were known. The clinical syndromes associated with this organsim were enteritis (seven cases of which three were described as being haemorrhagic), septicaemia (two cases) and metritis (one case).

Escherichia coli O8:K87 was isolated from seven weaners and one foetus. The weaners had shown enteritis (four cases), septicaemia (two cases) or oedema disease (one case).

Lesions of oedema disease were described by the submitting veterinarian in only 12 of the cases from which specimens were submitted. *E. coli* O149:K88 was isolated five times, *E. coli* O139 three times, *E. coli* O141 twice and *E. coli* O138, O20, O8:K87 and O9:K32, once each. Where the ages of the piglets were known, most piglets (seven) were about 2 weeks post weaning. One piglet was 3 weeks old and one 3 d old. The attachment factor F4 (K88) was present in seven of the 14 isolates. Seventy-five per cent of the oedema-disease cases were submitted during the first half of the study period.

DISCUSSION

This retrospective study was based on samples from pigs submitted to the OVI diagnostic bacteriology laboratory from cases suspected by the referring veterinarians to be colibacillosis. *Escherichia coli* was found to be the cause or the contributing cause of the disease process in 674 cases, which amounts to 16,2 % of all cases received from pigs.

The distribution of cases of colibacillosis over the years and seasons remained relatively constant. The few peaks that were obtained are considered to be due to increased numbers of samples being submitted by inexperienced veterinarians who required confirmation of their diagnoses more often than their experienced colleagues did. Although specimens were received from a smaller number of cases of colibacillosis during December and January, this may not reflect a real drop in the number of cases of colibacillosis, but only mirror a trend generally experienced in diagnostic laboratories during holiday periods. If the total number of cases of colibaccilosis that were diagnosed is considered on an annual basis (Table 1), it would appear that it remained more or less constant throughout the entire 20-year period of the study. This could give the impression that the control of the disease (*E. coli* infection) remained a problem during this period. What must be taken into consideration, however, is that the number of pigs slaughtered in the South Africa doubled during the period, from 1 222 756 in 1971 to 2 052 804 in 1991, according to the Meat Board of South Africa, and that the number of veterinarians who are members of the Pig Veterinary Society and have a particular interest in pig diseases, have increased from three to the current number of about 50. In addition, other diagnostic laboratories are now also being used by many of these veterinarians. If these facts are taken into account it would seem, therefore, that colibacillosis can now be considered to be less of a problem than at the start of the study. This decrease may be attributed to an improved understanding of the pathogenesis of the disease, improved management (Schulman 1973) including improved feeding (Armstrong & Cline 1977; Bertschinger *et al.* 1979; Miller *et al.* 1983; Miller *et al.* 1984), availability of superior genetic material and the use of vaccines.

Pathogenic factors of *E. coli*, such as verocytotoxins (or Shiga-like toxins) (Gonzales & Blanco 1985; Levine 1987; O'Brien & Holmes 1987; Brown, Echeverria & Lindberg 1991), cytotoxic necrotizing factor

(CNF) and F6 pili (Morris *et al.* 1983; Nataro, Scaletsky, Kaper, Levine & Trabulsi 1985; Dean 1990), were not recognized at the commencement of the study and have therefore not been discussed here. The adherence factor K88 (F4) (Bijlsma *et al.* 1982; Sellwood 1984) was recognized and was found in almost half of the isolates.

As the heat-labile toxin gene is carried on the same plasmid that codes for the F4 adhesion factor, it was not actively searched for in this study. The ST (Urban, Pipper, Dreyfus & Whipp 1990) of *E. coli* was investigated in the small number of cases (Table 6) where there was doubt about the significance of a particular isolate. No attempt was made to distinguish between STa and STb. Although only 18 strains were examined for this factor, the high percentage of positive cases (66,7%) indicates that ST of *E. coli* probably plays a significant role in neonatal and weanling diarrhoea in South Africa.

Two serotypes, *E. coli* O149 and O141, were the most common over the entire 20-year period, and although annual variations in incidence did occur, these were confined to particular years and no real upward or downward trend was observed. The other 67 different OK groups (or 42 other serogroups if only the O antigen is taken into account) that were detected, were considered to be to a large extent unusual and isolated types. The common serotypes in South Africa are a small group that can be relatively easily controlled by vaccination. It is difficult to compare these results with those of other reports, as other time spans are covered (Sojka 1965; Glantz 1971; Sojka 1973; Awad-Masalmeh, Quakyi & Willinger 1988) or only a limited number of typing sera were used (Garabal, Gonzalez, Vazquez, Blanco, Blanco & Blanco 1996).

The two main clinical syndromes described in this report on pigs, are enteric colibacillosis and septicaemia. Septicaemia is often a sequel to enteritis, particularly in young, immunodeficient piglets. A similar relative decline in the number of *E. coli* infections in sucklers compared to the number of cases in weaners found in this study, was noted by Awad-Masalmeh *et al.* in 1988 in Austria. They attributed this decline to the lowering of weaning age from 6–8 weeks to 2–3 weeks, and to the use of vaccines to control the disease. Both these reasons may be valid in South Africa. Few farmers wean earlier than 3 weeks in this country, because sophisticated management is necessary for earlier weaning and vaccines to control colibacillosis in pigs have been available since 1972, when the first whole-cell bacterin vaccine was produced at the then OVI. It is now produced at Onderstepoort Biological Products (OBP). This vaccine originally contained the five most common serotypes in South Africa at the time, two of them carrying the K88 antigen. The vaccine produced by the OBP has changed in composition (the number of serotypes included was increased to eight strains and later decreased to the present six strains) in response to the fluctuation in isolation of serotypes over the years. The initial alum-based vaccine was changed to an oil-emulsion vaccine in 1982. Strains that have always been included in the vaccine are O149, O141 and O138, and F4 (K88) has also always been included. *E. coli* O139 has been incorporated into the OBP vaccine since 1979. Other vaccines in use in South Africa at present, were developed during the 1980s and are based on pili (F4, F5, F6 and F41) and toxins (LT) and are available in various combinations.

There has been no real change in the incidence of problem cases due to *E. coli* O149, O141 or strains possessing F4 (K88), even though these antigens are included in the various vaccines in use in South Africa. *Escherichia coli* O138 and *E. coli* O139 show a different pattern. *E. coli* O138 was included in the vaccine in 1972. Several cases due to it occurred up to 1974, then none until 1981 when three cases were diagnosed. It has subsequently not been detected again (Table 4). *Escherichia coli* O139, commonly associated with oedema disease, was not initially included in the Onderstepoort vaccine, because at the time it was believed by some investigators that oedema disease was a hypersensitivity reaction (Sojka 1965), and inclusion in the vaccine would presumably have exacerbated the problem. This strain was included in the vaccine in 1979, after it had been shown by Clugston, Nielsen & Smith (1974) that oedema disease was caused by a specific toxin. This was subsequently confirmed by Dobrescu (1983). Two cases of *E. coli* O139 were isolated each year from 1979–1981, and one case in 1982 and one in 1983. No cases due to *E. coli* O139 have been recorded since. These findings seem to indicate that some strains, such as *E. coli* O138 and O139, are better controlled by vaccination than others, such as O149 and O141.

The predominant oedema disease serotypes of *E. coli* O138, O139 and O141 (Sojka 1965; Clugston *et al.* 1974; Nielsen 1986; Bertschinger & Nielsen 1992; Imberechts, De Greve & Lintermans 1992) were isolated from fewer than half the number of the cases submitted by veterinarians from pigs with oedema disease. As the genes coding for oedema disease toxin (VTe or SLT-IIv) are likely to be chromosomal (Johnson, Pollard, Lior, Tyler & Rozee 1990) only a small number of strains are known to be capable of producing oedema disease. Johnson *et al.* (1990) claim to have identified VTe in strains such as O2, O8, O65, O120, O121 and O157, which are not known as oedema-disease strains. In this study, five isolates of *E. coli* O149 were made from cases of oedema disease, but this type is not, according to several authors (Sojka 1965; Johnson *et al.* 1990; Imberechts *et al.* 1992), associated with clinical

oedema disease nor does it produce the toxin. Further detailed studies of not only these isolates but also of enterohaemorrhagic strains that produce large amounts of verocytotoxin are warranted. These latter strains are best represented by *E. coli* O157 (Tzipori, Karch, Wachsmuth, Robins-Browne, O'Brien, Lior, Cohen, Smithers & Levine 1987; Whittam & Wilson 1988). In this study, specimens were received from ten cases from which O157 was isolated. These were poorly documented by the referring veterinarians, the enteritis being described as haemorrhagic in only three of them.

Septicaemia-associated strains, such as *E. coli* O78, and some of the serotypes of the O8, O9 and O101 groups, formed only a small part of the whole survey (Tables 3 and 4). As it was difficult to assign a specific serotype to the categories of primary or secondary (terminal) septicaemia because of the limited amount of information supplied in each case, it was not possible to calculate exact numbers. Vaccination aimed at preventing colibacillosis is therefore much more likely to be successful if it is aimed at the prevention of enteric colibacillosis rather than of colisepticaemia.

Until this project was initiated, little was known about the serotypes of *E. coli* present in pigs in southern Africa and no *E. coli* vaccines were in use at that time. This study has indicated which strains are commonly found in pigs in South Africa, and what disease syndromes are associated with them. Most of the virulence factors of *E. coli* were unknown at the start of the study, but have been the focus of attention of many researchers world-wide over the past 20 years. The host-pathogen relationship has been found to be intricate with many factors playing a role, and several still have to be elucidated. Most methods of determining virulence are very complicated and expensive, with many variables playing a role. Serotyping, however, remains a simple, relatively inexpensive alternative for diagnosing pathogenic *E. coli* and for determining their epidemiological distribution. It also serves to pinpoint which strains should be used for further virulence determinations. Future diagnostic tests should include tests for virulence determinants, as and when they become available and economical to use.

Continuous monitoring of serotypes isolated from herds in which *E. coli* infections are a problem in southern Africa is important from the perspective of their being selected for inclusion in vaccines. The choice of strains and virulence determinants to be included in vaccines should have as broad a spectrum as possible. This was not possible at the commencement of this study, and the first vaccine produced at Onderstepoort was based on empirically chosen strains.

Changes in the methods of pig farming, such as early weaning, have had an effect on the prevalence of colibacillosis. Further changes are probable in the future, and constant monitoring of colibacillosis would enable veterinarians to select correct disease-prevention regimens.

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