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The sub-specific differentiation of *Escherichia coli* with particular reference to ecological studies in young animals including man

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SUMMARY

It is possible to differentiate isolates of *Escherichia coli* using a number of techniques including the determination of the serotype, biotype and phage type and the profiles for resistance to antibacterial agents and toxic chemicals, intracellular enzymes, outer membrane proteins and plasmids and the production of enterotoxin and colicines. These methods have been used principally for the study of pathogenic *E. coli* and plasmid-mediated drug resistance. However they can also be used successfully for ecological purposes and the application of several of these techniques for the study of the ecology of *E. coli* in healthy young animals including man is described.

INTRODUCTION

The year 1985 marks both the centenary of Theodor Escherich's original description of the bacterial species now known as *Escherichia coli* and the twentieth anniversary of the publication of Walter Sojka's impressive monograph '*Escherichia coli* in domestic animals and poultry'.

Sojka (1965) described in detail the general characteristics and biochemical behaviour of *E. coli* and also the role of the species as a pathogen since it causes a number of economically important diseases of farm animals including neonatal diarrhoea and mastitis in cattle, post-weaning diarrhoea and oedema disease in pigs and coli septicaemia in chickens.

Since the 1950s antibacterial agents have been administered to farm animals for the purpose of therapy, prophylaxis and growth promotion but when Sojka prepared his review there was relatively little information then available on the phenomenon of drug resistance in *E. coli*, particularly the genetical aspects. Since that time, however, many papers on this subject have been published together with an increasing literature concerning the ecology of *E. coli* in animals including man.

E. coli is essentially a commensal organism in the animal host although certain strains are pathogenic due to the presence of virulence factors, the causative genes of which are frequently transmissible *in vitro*. In order to study the ecology of both commensal and pathogenic isolates it is essential to differentiate between strains and a number of schemes have been proposed to achieve this end. However, the aims of the clinical bacteriologist and the bacterial ecologist are rather different.

In the study of pathogens the detailed fingerprinting of isolates may be necessary before the epidemiology or pathogenesis of the disease can be understood and consequently the use of exacting or sophisticated techniques such as comprehensive serological differentiation of isolates or plasmid profile analysis may be justifiable. On the other hand the ecologist will wish to examine relatively large numbers of isolates and consequently will be attracted to methods which are simple, reproducible and economical.

Methods employed for the sub-specific differentiation of *E. coli* will be reviewed in the first part of this paper, while the second part will consider how some of these techniques have assisted in the study of the ecology of *E. coli* in young animals. Some hitherto unpublished data will be presented together with additional analyses of data already published. The majority of these studies involved the examination of faecal swabs collected from cattle, chickens and pigs. Up to 10 colonies of *E. coli* were examined from each sample. The isolates were O-serogrouped using antisera prepared against at least 150 internationally-recognized O-group antigens (Hartley *et al.* 1975; Howe & Linton, 1976). In some of the surveys the *E. coli* were also biotyped (Hinton, Allen & Linton, 1982) and their resistance to antibacterial agents (usually eight) determined. Two methods were employed which gave comparable results, namely a disk diffusion test (Linton, Howe & Osborne, 1975) and a technique in which the agents were incorporated separately into the sensitivity test agar (Hinton *et al.* 1984).

SUB-SPECIFIC DIFFERENTIATION OF *ESCHERICHI COLI*

A number of different methods have been developed for differentiating *E. coli* isolates on the basis of their phenotype. The most widely used is serotyping with additional differentiation being provided by such techniques as biochemical biotyping, phage typing, plasmid profile analysis and the determination of resistance to antibacterial agents and toxic chemicals. The use of several methods in parallel has been recommended by Old *et al.* (1980), Crichton & Old (1980) and Wilson, Crichton & Old (1981) and obviously this is essential for the study of the distribution of specific clones (Achtman *et al.* 1983; Stenderup & Ørskov, 1983).

Serotyping

The serotyping of isolates using a full set of either O or O and H antisera is necessarily time-consuming and consequently a limited range of antisera, raised against specific pathogenic *E. coli* serotypes, are frequently used by clinical bacteriologists. Two groups which have employed the more comprehensive serotyping technique for ecological studies during the last 15 years have been those of Shooter and Bettelheim and their colleagues working in London and New Zealand and Richmond and Linton, and their co-workers in the University of Bristol.

One of the problems associated with serotyping is that some isolates will be either non-typable or autoagglutinable (rough) and if the proportion happens to be large the value of the method is necessarily limited. Shooter *et al.* (1974) serotyped 798 *E. coli* isolates from abattoirs with 150 O-antisera and found 509 (64 %) non-typable. This compared to only 2 (0·1 %) of 1580 human isolates. However, Bettelheim *et al.* (1974*a*, *b*), Hartley *et al.* (1975) and Hartley, Clements & Linton (1977) found

Table 1. *The proportion of non-O-typable* Escherichia coli *isolated from cattle, chickens and pigs*

Survey index no.	Animal species	No. of animals*	Age (weeks)	Source of isolates	No. samples per animal	No. *E. coli* isolates	No. non-O-groupable	%	Reference
1	Cattle	NS	Calves	Faeces	1	174	20	11·5	Hartley *et al.* (1975)
2	Cattle	6	Adult	Faeces	6	94	3	3·2	Howe & Linton (1976)
		8	3–8	Faeces	8	380	63	16·6	
3	Cattle	400	0–8	Faeces	1	946	118	12·5	Howe, Linton & Osborne (1976*a*)
4	Chickens	12	0–7	Faeces		1498	37	2·5	Howe, Linton & Osborne (1976*b*)
5	Chickens	175	7	Faeces	1	4713	595	12·6	Linton *et al.* (1977*b*)
		196		Carcases	1				
6	Pigs	2	10–30	Faeces	21	4312	845	19·6	Linton, Handley & Osborne (1978)
7	Cattle	10	1–6	Faeces	11	1055	287	27·2	Linton, Timoney & Hinton (1981)
8	Cattle	–	< 6	Slurry	8	752	233	31·0	Hinton & Linton (1982)
9	Chickens	NS	1–14	Faeces	NS	1131	201	17·8	Hinton *et al.* (1982)
10	Cattle	219	Adult	Milk	1	290	53	18·3	Linton & Robinson (1984)
11	Cattle	3	1–28	Faeces	28	814	187	23·0	Hinton *et al.* (1984)
12	Cattle	18	1–4	Faeces	17	2930	612	20·9	Hinton, Hedges & Linton (1985)
13	Cattle	10	Adult	Faeces	1	102	20	19·6	Hinton, Linton & Hedges (1985)
		16	< 26	Faeces	5–28	2445	435	17·8	
14	Pigs	39	3	Faeces	1	387	91	23·5	Hinton *et al.* (1985)
15	Pigs	1	Adult	Faeces	16	116	47	40·5	Hinton & Linton (unpublished data)
		5	< 4	Faeces	18	870	87	10·0	
16	Cattle	6	Adult	Faeces	1–15	323	111	34·4	Hinton & Linton (unpublished data)
		6	< 2	Faeces	14	811	212	26·1	

* NS, not stated.

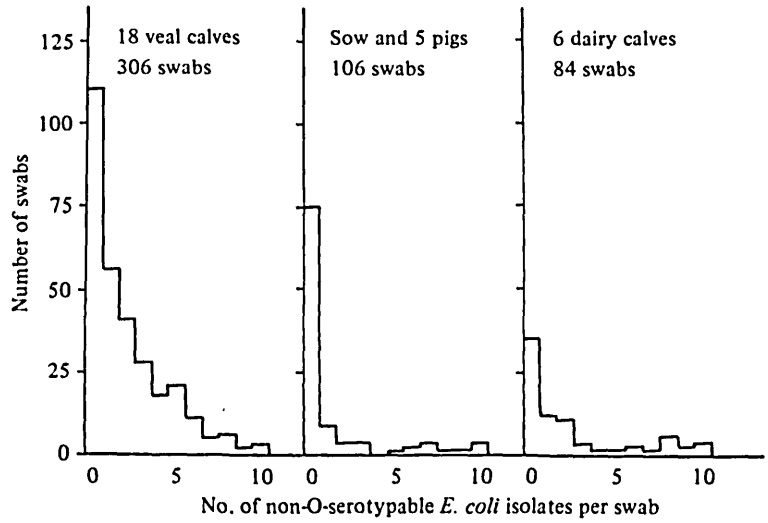

Fig. 1. The distribution of swabs collected from 18 veal calves, a sow and 5 piglets and 6 dairy calves according to the number of non-O-typable *Escherichia coli* colonies per swab.

between 11 % and 33 % of human isolates non-typable and this suggests that the proportion of non-typable *E. coli* isolates from human sources may vary widely. An analysis of data from cattle, chicks and pigs confirms that this is the case in animals (Table 1). Overall 4257 (17·6 %) of the 24143 isolates were non-typable with a range of 2·5 % for isolates from a group of chickens to 40 % for isolates from an individual sow.

The distributions of the number of non-typables per swab (10 colonies) obtained in three surveys (Surveys 12, 15 and 16 in Table 1) are illustrated in Fig. 1. Between 29 % and 63 % of the swabs examined yielded at least one non-typable isolate and in all three surveys there were a few swabs in which all isolates were non-typable.

Shooter *et al.* (1974) concluded that non-typables probably comprise O-serogroups as yet undefined, since 13 different H antigens were found among 509 isolates untypable by O-grouping sera. Non-typable and rough strains have been differentiated using phages (Budde, Nimmich & Naumann, 1978) while biotype and resistance pattern has been used to define non-typable *E. coli* (NTEC) strains (Hinton, Linton & Hedges, 1985). Up to 4 NTEC strains being identified from 10 colony samples obtained from dairy calves with an average of 0·6 per swab.

The examination of *E. coli* isolates from a large number of individuals reveals that the O-serogroups are unequally distributed and this implies that the more frequently isolated are probably better colonizers of the intestinal tract (Hartley *et al.* 1975; Howe & Linton, 1976). A similar situation also obtains when groups of animals are examined on several occasions (Fig. 2). In each of three surveys (Nos. 12, 13 and 16 in Table 1) the distribution was skewed with a few O-serotypes being isolated from all or most animals and an increasing number being identified from fewer and fewer animals.

E. coli O-serogroups are not necessarily equally distributed within the same faecal specimen (Bettelheim, Faiers & Shooter, 1972). The problems associated with sampling of *E. coli* from intestinal sources for O-serogrouping have been

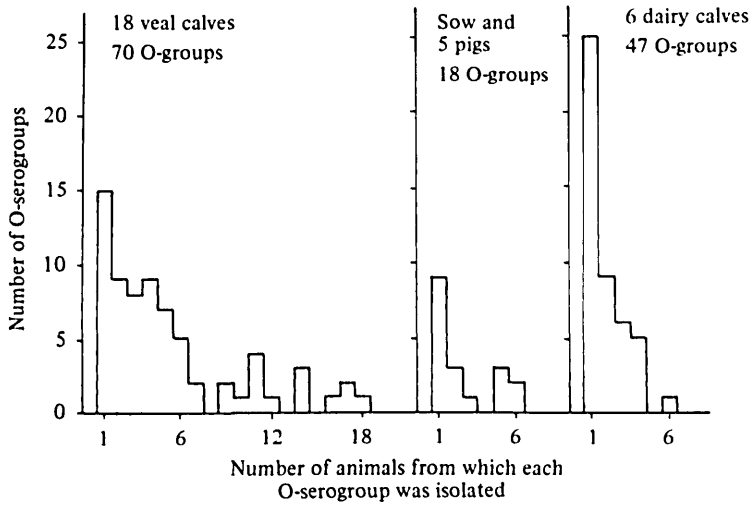

Fig. 2. The distribution of O-serogroups isolated from faecal samples of 18 veal calves, a sow and 5 piglets and 6 dairy calves according to the number of animals within the group from which each O-serogroup was isolated.

considered by Hedges, Howe & Linton (1977). The more complex the *E. coli* flora the greater the number of colonies that have to be examined from each specimen to obtain a reasonable estimate of the 'majority' O-serogroups present. Nevertheless a comparison of the results obtained by examining 100 colonies and 10 colonies per sample revealed that the smaller sample gave better results than expected because colony selection by the investigator was not random as there was preferential selection for different colonial forms. Further evidence in favour of small samples is provided by an analysis of the number of O-serogroups identified from each of 1023 swabs collected from cattle and pigs of various ages. The number of O-serogroups per 10 colony sample was relatively low at 2·6 (Table 2) while more than 4 O-serogroups were found in only 126 (13%) of the 979 samples from which O-typable *E. coli* were isolated (Fig. 3).

Post-mortem investigations are required to establish whether the majority O-serogroups present in the faeces are representative of those colonizing other parts of the intestine and there is evidence that this is not necessarily the case in young chickens and pigs (Hinton *et al.* 1982, 1985). In 51 pigs up to 12 O-serogroups were identified from the rectum and caecum (Table 3). In 23 (39%) of the animals no O-serogroups were common to both sites. In all but 1 of the remainder the total was only 1 with the flora at both sites being dominated by fewer than 5 O-serogroups in 19 (70%) of the 27 pigs.

Biochemical biotyping

The ability of *E. coli* to metabolize certain substrates varies considerably and this phenomenon has been exploited in the development of biotyping schemes and several, of varying complexity, have been described (Bettelheim & Taylor, 1969; van der Waaij *et al.* 1975; Buckwold *et al.* 1979; Crichton & Old, 1979; Gargen, Brumfitt & Hamilton-Miller, 1982; Hinton, Allen & Linton, 1982).

Table 2. *The average number of O-serogroups identified from 10* Escherichia coli *colonies isolated from faecal swabs from cattle and pigs*

Survey index no.*	Animals sampled	No. of animals	Age (weeks)	Swabs per animal	Total swabs	O-serogroups per swab	
						Range	Average
7	Cattle	10	1–6	11	110	0–8	3·15
11	Cattle	3	1–28	28	84	0–5	2·39
12	Cattle	18	1–4	17	306	0–8	3·04
13	Cattle	16	< 26	5–28	259	0–7	2·69
14	Pigs	39	3	1	39	1–6	2·64
15	Pigs	1	Adult	16	16	0–5	1·75
		5	< 4	18	90	0–5	2·07
16	Cattle	6	Adult	1–15	35	0–5	1·83
		6	< 2	14	84	0–7	2·25
	All samples	104	—	1–28	1023	0–8	2·68

* See Table 1.

The advantages of biotyping are (1) it is possible to obtain a profile for each isolate and (2) the biotype remains stable after storage of the isolate for months or years (Crichton & Old, 1979).

There are some differences of opinion about the suitability of certain of the tests used because they were insufficiently discriminatory, poorly reproducible or plasmid-mediated and the case for and against a number of these has been discussed by Crichton & Old (1982), Gargen, Brumfitt & Hamilton-Miller (1982) and Hinton, Allen & Linton (1982). In any event it is important to standardize the test protocol and, in the author's opinion, to read the tests after a relatively short period so as to obtain a qualitative assay of constitutive enzymes and to minimize the chance of a mutation occurring.

The API 20E system has also been employed for biotyping purposes (Davies, 1977; Mee & Nikolette, 1983) but this approach was considered unsatisfactory by Crichton & Old (1979) since the majority of tests are insufficiently discriminatory although this shortcoming has been overcome by Gargen, Brumfitt & Hamilton-Miller (1982) who employed the system in conjunction with six additional tests.

Antibacterial drug resistance pattern

The resistance of *E. coli* to a range of antibacterial agents is usually determined as a matter of routine in both clinical and ecological investigations and the information obtained used to assist in the differentiation of isolates.

Resistogram typing

The resistance of *E. coli* isolates to potentially toxic chemicals was investigated by Elek & Higney (1970). They evaluated a variety of inorganic and organic compounds, including dyes, and selected eight for a resistogram typing scheme. They concluded that the resistogram profile of strains should remain stable since there would be no selection pressure for resistance as *E. coli* would not encounter these compounds under normal circumstances. However, this may not always be

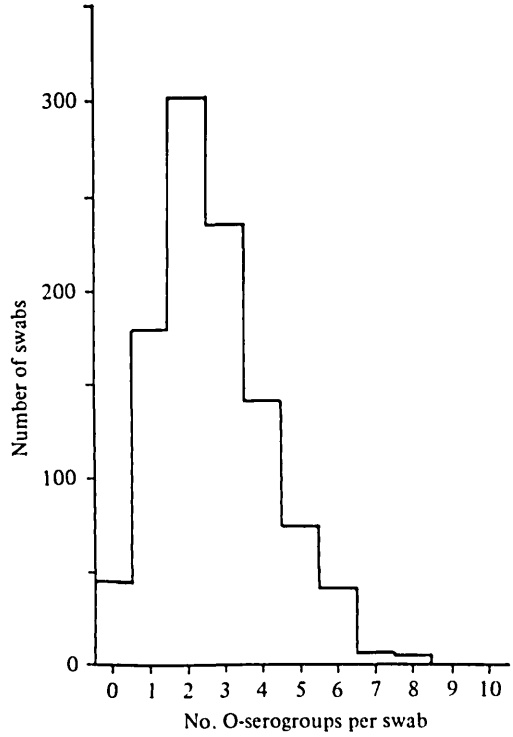

Fig. 3. The distribution of 1023 swabs of faeces according to the number of O-serogroups identified among 10 *Escherichia coli* colonies examined from each.

the case since copper sulphate, one of the compounds in their test protocol, is frequently included in the diet of fattening pigs as a growth promoter.

It is possible to obtain a resistogram profile for all isolates and the method has been used to good effect, in conjunction with other tests, for differentiating *E. coli* isolates from the urinary tract of human patients (Old *et al.* 1980; Wilson, Crichton & Old, 1981; Crichton & Old, 1982).

Enzyme profile analysis

Selander & Levin (1980) investigated the electrophoretic variation of 20 enzymes among isolates of *E. coli* and demonstrated extensive variability for several of them although they concluded that the total number of distinctive genotypes was probably limited since identical clones were obtained from unassociated hosts. A further study, involving 12 enzymes, provided evidence of extensive genetic structure in natural *E. coli* populations (Ochman *et al.* 1983). These authors concluded that recombination of genes probably occurs infrequently and that the natural population of *E. coli* consists of a mixture of more or less independently evolving lines (clones) (Ochman *et al.* 1983), some of which have a very widespread distribution (perhaps world-wide) while others have more restricted ranges (Caugant, Levin & Selander, 1984). Recently Ochman & Selander (1984*b*) have proposed a set of 72 reference strains of *E. coli* which represent the range of genotypic variation in the species and which can be used to study genetic structure of natural populations.

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Table 3. *The distribution of 51 pigs 3–4 weeks old according to the number of* Escherichia coli *O-serogroups identified in the rectum and caecum and the number of O-serogroups common to both sites*

No. of O-serogroups common to both sites	No. of pigs	Total no. of *E. coli* O-serogroups identified									Median
		1	2	3	4	5	6	7	8	9–12	
0	23	—	3	3	3	4	3	1	3	3	5
1	27	3	10	3	3	–	2	2	1	3	3
2	1	—	—	—	—	1	—	—	—	—	—

Outer membrane protein profile analysis

The outer membrane proteins (OMP) of *E. coli* are heterogeneous in respect to both number and electrophoretic mobility (Overbeeke & Lugtenberg, 1980). The analysis of OMP has been employed to demonstrate the relationship of clones of pathogenic *E. coli* (Achtman *et al.* 1983; Stenderup & Ørskov, 1983). Achtman *et al.* (1983) concluded that each membrane pattern was not necessarily associated with one O:K serotype although there was unexpected uniformity within the same serotype group while members of two clones with the same OMP pattern, but of differing serotype have been shown to be very similar to each other (Mercer *et al.* 1984). Ochman & Selander (1984*a*) determined the enzyme profiles of the *E. coli* K1 isolates examined by Achtman *et al.* (1983) and their findings support the hypothesis that the genetic structure of natural populations of *E. coli* is basically clonal with very limited recombination of chromosomal genes.

Plasmid profile analysis

There are several techniques available for the extraction and separation of plasmid DNA and there is a considerable literature on the association of plasmids with resistance (R) determinants. However, sensitive *E. coli* may carry plasmids (Platt *et al.* 1984) and the distribution of plasmids among *E. coli* isolates, based on molecular weights, have been found to be non-random (Caugant, Levin & Selander, 1981).

Within any particular O-serogroup a variety of plasmid profiles have been demonstrated (Baumgartner & Nicolet, 1983). Plasmid profile analysis has been employed in the elucidation of a number of ecological problems including the demonstration of (1) similarities and differences between clones or strains (e.g. Achtman *et al.* 1983; Baumgartner & Nicolet, 1983; Linton & Hinton, 1984; Mercer *et al.* 1984), (2) the relatedness of plasmids carried by apparently epidemiologically unrelated isolates, e.g. pathogens of infants and pigs (Jørgensen, 1983) and (3) the transmission of *E. coli* from animals to man (Levy, Fitzgerald & Macone, 1976; Linton *et al.* 1977*a*).

Phage typing

Phages have been employed to differentiate additional strains within O-serogroups (Marsik & Parisi, 1971; Gershman, Merrill & Hunter, 1981; Gersham, Markowsky & Hunter, 1984), biotypes (Ansari *et al.* 1980) and also *E. coli* isolates which are autoagglutinable (rough) (Budde, Nimmich & Naumann, 1978)

although the technique has the same limitation as O-serotyping in that a proportion of isolates will be non-typable.

Phage typing has been employed to study the ecology of *E. coli* in cows and calves (Smith & Crabb, 1956) and also *E. coli* isolates from diseased chickens (Bhatia, 1977) and cows with coliform mastitis (Gershman, Merrill & Hunter, 1981; Gershman, Markowsky & Hunter, 1984).

Colicine typing

Colicine production is plasmid encoded and one (Col V) has been identified as a virulence marker (Smith, 1978). O-serogroups of *E. coli* can be subdivided into several colicine types although colicine typing is of limited value for epidemiological purposes because not all isolates produce colicines (Craven & Barnum, 1972; Hettiaratchy, Cooke & Shooter, 1973). However, on occasions, colicine typing has been helpful in assessing the significance of isolating the same O-serotype from an individual patient (Hettiaratchy, Cooke & Shooter, 1973) while it has been employed together with other tests for differentiating urinary isolates (Wilson, Crichton & Old, 1981) and clones of pathogenic *E. coli* (Achtman *et al.* 1983).

Virulence factors

It is not the purpose of this review to consider the pathogenesis of diseases caused by *E. coli*. Suffice it to say that certain surface antigens, enterotoxin production and haemagglutinating activity have been employed to differentiate pathogenic and non-pathogenic strains. Biochemical heterogeneity has been demonstrated among pathogenic serotypes (Braaten & Myers, 1977; Scotland, Gross & Rowe, 1977; Isaacson, Moon & Schneider, 1978) and consequently it is unlikely that a search for a correlation between virulence factors and specific biochemical properties will prove rewarding. Nevertheless, in porcine isolates haemolysin production is frequently associated with the ability to elaborate enterotoxin while some investigators have indicated an association between pathogenicity and some other phenotypic properties. For instance, Silva, Toledo & Trabulsi (1980) and Toledo & Trabulsi (1983) concluded that *E. coli* isolated from human patients with diarrhoea are likely to be invasive if they are non-motile and unable to decarboxylase lysine while Harry & Chubb (1964) and Kulshrestha & Kumar (1977) recorded that the majority of pathogenic isolates from poultry fermented dulcitol and decarboxylated ornithine.

THE INSTABILITY OF THE *ESCHERICHIA COLI* FLORA

The population of *E. coli* in the faecal flora of individuals is not static and a turnover in strains has been demonstrated in man and other animal species by the use of O-serogrouping (e.g. Sears, Brownlee & Uchiyama, 1950; Cooke, Ewins & Shooter, 1969; Craven & Barnum, 1971; Hartley, Clements & Linton, 1977; Linton, Handley & Osborne, 1978), a combination of O-serogrouping, biotyping and antibacterial resistogram typing (Hinton, Hedges & Linton, 1985; Hinton, Linton & Hedges, 1985), phagetyping (Smith & Crabb, 1956) and enzyme profile analysis (Caugant, Levin & Selander, 1981).

Sears, Brownlee & Uchiyama (1950), who O-serogrouped isolates, proposed that

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Table 4. *Isolation rate of* Escherichia coli *strains from young cattle and pigs*

	Diet	No. of animals	No. of swabs examined per animal	Sampling period (days)	No. of *Esch. coli* strains isolated per animal	Reference
Veal calves	Milk substitute	18	17	21	32–88	Hinton, Hedges & Linton (1985)
Dairy calves	Cow's milk for days then milk substitute	9	9–10	10	7–28	Hinton, Linton & Hedges (1985)
Piglets	Sow's milk and creep feed	5	13	20	5–9	Hinton & Linton (unpublished data)

E. coli strains could be considered as either 'residents' or 'transients'. Subsequently Guinee *et al.* (1972) defined a 'resident' strain as one which constituted 5 or more of 20 colonies isolated from each faecal specimen during a period of 6 weeks or more. Later investigations by Hartley, Clements & Linton (1977), however, indicated that the distinction between these two categories was not clear cut and they recommended that their use be abandoned. They suggested that because the demonstration of O-serogroups was necessarily limited by the number of colonies examined, the term 'majority' strain should be used for strains identified from a 10-colony sample while any O-serogroup detected only when more colonies were examined should be regarded as a 'minority' strain.

Individuals acquire new strains from either the food, the environment or other individuals and evidence that this is the case is provided indirectly by Caugant, Levin & Selander (1981) who concluded that the genetic diversity among isolates, as measured by enzyme profiles, was too great to be accounted for by either the recombination or the mutation of genes within the individuals intestinal tract.

THE ECOLOGY OF *ESCHERICHIA COLI* IN YOUNG ANIMALS

Bettelheim *et al.* (1974*a–d*) differentiated *E. coli* strains by serotype and biotype and found that strains isolated from babies born in a London hospital could also be present in their mothers faeces, a fact confirmed by Ørskov & Sørensen (1975) and Gothefors *et al.* (1976). They also reported that a proportion of strains were isolated from several of the babies studied, but not their mothers, and an environmental source of these isolates was suggested. Subsequent studies in a maternity ward in New Zealand revealed little evidence of spread of strains among babies and this was ascribed to the fact that the mothers predominantly handled their own babies in the New Zealand survey whereas in London they were frequently handled by nursing staff (Bettelheim, Peddie & Chereshsky, 1983). Gothefors *et al.* (1976) reported that on occasions no common O-serogroups were isolated from mothers and their infants while in other cases the transfer of strains occurred from the babies to their mothers, presumably following contact with nappies contaminated with faeces.

Similar investigations in cattle have revealed that a proportion of strains isolated from young calves may also be identified in the faeces of their mothers (Smith & Crabb, 1956; Hinton, Linton & Hedges, 1985) while in a litter of 5 piglets (Table 1, Survey No. 15) 4 of 8 *E. coli* strains, differentiated by O-serogroup, biotype and resistance pattern, and isolated from the faeces of the sow during the 5 days prior to farrowing were subsequently isolated from one or more of the piglets within 5 days of birth. In contrast to human infants, farm animals born indoors will find themselves in an environment contaminated, to a greater or lesser extent, by faeces and, as might be expected, the bedding has been found to act as a source of *E. coli* strains for young dairy calves (Hinton & Linton, unpublished data).

Ørskov & Sørensen (1975) noted that the *E. coli* flora of breast-fed infants as judged by O-serogroup, was less complex than that of bottle fed babies while this may also be the case in animals since the rate of isolation of 'new' strains from sucking pigs was lower than it was for calves fed milk substitute (Table 4).

The *E. coli* flora appears to become less complex as the animals grow older since the average number of O-serogroups per swab is lower in adults than it is in young stock (Table 2). Sequential studies of calves during the first few months of life confirm this since both the total number of *E. coli* strains identified per swab, and the rate of isolation of 'new' strains, differentiated by O-serogroup, biotype and resistance pattern, both decreased with increasing age (Hinton *et al.* 1984; Hinton, Linton & Hedges, 1985).

The explanation for both the instability of the *E. coli* faecal flora and its progressive simplification as the animal matures has yet to be sought. A number of factors probably operate and those that require elucidation include (1) the influence of the size and complexity of the *E. coli* population in the environment on these phenomena, (2) the effect of diet on the colonizing ability of *E. coli*, (3) the phenotypic characters of *E. coli* which allow commensal strains to multiply in the lumen of the intestinal tract to form the majority flora, (4) the effect of local immunity on the survival of specific antigen types in the *E. coli* intestinal population and (5) the interaction between *E. coli* and the other bacterial species comprising the intestinal microflora.

CONCLUSIONS

It is possible to differentiate strains of *E. coli* using a variety of different techniques which vary considerably in their technical complexity. Several of these have been applied successfully to the study of the ecology of *E. coli* in the intestinal flora of animals including man. However, a number of important questions still remain to be answered before the ecology of this bacterial species is fully understood.

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