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The persistance of drug resistant *Escherichia coli* strains in the majority faecal flora of calves

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SUMMARY

Two groups of calves, one of three and the other of two animals, were purchased in markets and reared initially on a commercial veal unit for 1 month and 4 months respectively. They were then moved to the Veterinary School, Langford, and kept for a further 6 and 4 months respectively. The animals were sampled weekly and a continual turnover in the strains forming the majority *Escherichia coli* faecal flora was demonstrated for all calves. Antibacterial-drug resistance, as measured by an Antibiotic Resistance Index (ARI), increased after arrival on the veal unit and persisted at high levels during the whole of their stay. After moving to Langford the ARI fell. Initially there was a reduction in the average number of resistance determinants per resistant strain and then, after a delay of up to 8 weeks, by an increase in the proportion of isolates that were fully sensitive. The source of the sensitive strains was not ascertained, although their appearance was not associated specifically with either weaning or turning out to pasture.

INTRODUCTION

A progressive increase in antibacterial-drug resistance was demonstrated among *Escherichia coli* isolates from the faeces of calves reared on a farm in Somerset and fed a milk-substitute diet (Linton, Timoney & Hinton, 1981; Hinton & Linton, 1983; Hinton, Hedges and Linton, in preparation). This change occurred in all animals irrespective of whether they had received any drug therapy and was due to the strains initially colonizing the gut being displaced by others which were multiply resistant to antibacterial drugs.

It was the policy on this farm to purchase young calves in markets and then to sell them unweaned after a 4- to 5-week rearing period. As a consequence it has not been possible hitherto to determine for how long multiply-resistant *Esch. coli* continued to be excreted in the faeces after the initial rearing period. In the present study calves, reared initially on this farm, were subsequently monitored for several months on a second establishment. In the first part of the investigation three calves

were kept on the farm for 3 to 4 weeks and then for a further 6 months at the Veterinary School, Langford, while in the second, two calves were kept on the farm for 4 months followed by a further 4 months at Langford.

MATERIALS AND METHODS

The calves and their management

Calves about 1 week of age were purchased, either at local markets during March 1981 (calves C1–C3) or markets in the north of England during June 1981 (C4 and C5) and transported by road to the farm in Somerset. The general management policy on the farm has been described elsewhere (Linton, Timoney & Hinton, 1981). The calves were housed in groups of 36, in similarly appointed rooms. They were kept in individual wooden pens, which had solid sides and slatted floors, arranged in two ranks of 18 on each side of the room. Adjacent calves could contact each other at each end of the pen. The calves were subsequently transferred to the Veterinary School, Langford, and housed in artificially ventilated rooms measuring 3 by 4 m. The floor and walls were cement-rendered while the internal pen partitions were constructed of tubular steel and galvanized sheeting.

The calves, when unweaned, were fed twice daily on a milk-substitute diet which contained the growth-promoting antibiotics flavomycin and zinc bacitracin at recommended levels.

Calves C1 to C3

Two of the calves, a Hereford × Friesian (H × F) (C1) and a Friesian (C2) formed part of one group of 36 calves while the third (H × F; C3) came with a second group purchased 7 days later. The sampling protocol and other details of management are summarized in Table 1. Calf 2 suffered diarrhoea on days 6 and 7 and was treated with three doses of antibacterial drugs (1·6 g framycetin, 0·2 g streptomycin sulphate and 1 g each of sulphadiazine, sulphamerazine and sulphapyridine) administered in the milk. In addition, this group of calves, which included C1 and C2, were given a prophylactic course of chlortetracycline in the milk (20 mg/l) on the 2 days before they were due to be sold (Table 1).

All three animals were transferred to the Veterinary School, Langford, 27 days after the start of the survey and were housed in three separate pens (two on one side and one on the other) in a room (see above) which contained no other stock. The calves were kept on wooden slats until weaning (Table 1) after which they were bedded on straw. At weaning the milk ration was reduced gradually over a week. The calves were offered a proprietary concentrate ration, which contained the growth-promoting antibiotic nitrovin (25 mg/kg) (BOCM Silcock, Calfwena pencils), from the commencement of weaning until they were turned out to pasture.

In order to prevent the development of parasitic bronchitis (husk) after 'turnout' to the pasture, which was already being grazed by adult cattle and sheep, the calves were immunized with two oral doses of *Dictyocaulus viviparous* vaccine (Glaxo, *Dictol*) (Table 1).

Calves C4 and C5

These two calves were reared on the farm in Somerset for 18 weeks with four successive groups of 34 newly purchased calves. They were moved three times, after

Table 1. *A summary of the management of calves C1 to C3*

Day of survey	Sampling occasion: Calves C1 and C2	Sampling occasion: Calf C3	
1	1	—	C1 and C2 purchased
6–7	—	—	C2 diarrhoea: treated with antibiotics
7	2	1	C3 purchased
25–26	—	—	C1 and C2 chlortetracycline in milk
27	—	—	All calves to Langford
63	10	9	C1 weaned
84	13	12	All calves 1st husk vaccination
91	14	13	C2 and C3 weaned
112	17	16	All calves 2nd husk vaccination
126	19	18	C1 and C2 turned out to pasture
140	21	20	C3 turned out
198	NE	28	All calves sold

NE = not examined.

the 5th, 9th and 14th week, when each group of calves was sold, into a recently cleansed and disinfected room which housed the newly purchased animals. When they arrived the calves were placed in pens on opposite sides of the room. Later they were kept in adjacent pens at one end of a rank of 18 pens with C4 always being next to the end wall. After the 18th week they were transported to the Veterinary School, Langford, and kept in separate pens in a similar room to that used for C1 to C3. The calves were bedded on straw and fed milk substitute only until they were sold after a further 15 weeks. They received no therapeutic antibacterial drugs during the rearing period except in the 5th week, when a prophylactic course of chlortetracycline was administered in the milk (20 mg/l) to all 36 calves in the room.

Collection of samples

An alginate swab of rectal faeces was obtained from each calf on the day of purchase and then at weekly intervals until they were sold. In all, 28 swabs were examined from C1, 2 and 3 (Table 1) and 32 from C4 and C5. The swabs were placed into Stuart's transport medium and processed on the day of collection or occasionally the next day after storage at 4 °C.

Bacteriological techniques

Studies on Escherichia coli

The swabs were inoculated on to each of two plates of bile lactose agar without salt (BLA; Oxoid, CM7b) that were then incubated overnight at 37 °C in air. Ten lactose-fermenting colonies, typical of *Esch. coli*, were picked from each plate (20 in all) and their identity confirmed by a positive indole and Eijkman test (Hartley *et al.* 1975) and a negative citrate test.

The sensitivity of all isolates to seven antibacterial agents was determined. The drugs were incorporated separately into Isosensitest agar (Oxoid, CM471) to which 1·2% (v/v) lysed horse blood was added. The final concentration of each drug (μg/ml) was ampicillin (Ap) (50), chloramphenicol (Cm) (50), kanamycin (Km) (30), streptomycin (Sm) (10), sulphafurazole (Su) (250), tetracycline (Tc) (50) and

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trimethoprim (Tm) (1·5). Masterplates (BLA) inoculated, with the aid of a template, with up to 35 *Esch. coli* isolates, were incubated for 4 h at 37 °C in air. The antibiotic plates were then inoculated from the masterplates using a multipoint inoculator (Denley, A400) and examined for growth after overnight incubation at 37 °C in air. Any inconclusive results were resolved using a disk diffusion method which had been used originally to calibrate the test (Linton, Howe & Osborne, 1975). All isolates from calves C1 to C3 and isolates from swabs 17 to 32 collected from calves C4 and C5 were also biotyped according to their ability to ferment adonitol, dulcitol, D-raffinose, L-rhamnose and L-sorbose in solid media (Hinton, Allen & Linton, 1982). Up to three of each biotype/resistance pattern combination isolated from each swab were O-serogrouped using a micro-agglutination method (Howe & Linton, 1976).

Salmonella isolation

After inoculation of the BLA plates each swab was cultured for salmonella (Linton *et al.* 1981).

Analysis of the data

In order to compare the incidence of drug resistance among isolates from each swab an Antibiotic Resistance Index was calculated according to the formula y/nx, where y was the number of resistance (R) determinants in a population n and x was the number of drugs included in the sensitivity test (Hinton & Linton, 1983). The *Esch. coli* isolates were differentiated into individual strains on the basis of O-serogroup, biotype and resistance pattern. When a strain was isolated for the first time from a calf it was considered as a 'new' strain for that sampling occasion.

RESULTS

Variation in the incidence of drug resistance with time

Calves C1 to C3. The Antibiotic Resistance Index (ARI) was calculated for the *Esch. coli* isolates obtained on each week of the survey and the results summarized in Fig. 1. The index rose from a minimum of 0·25 initially to exceed 0·70 during weeks 4–6; the maximum value recorded was 0·96. The ARI then fell gradually for all three calves during the next 12 weeks. It was 0·16 or less in 26 of 27 samples collected after the 20th week with all *Esch. coli* isolates being fully sensitive on 13 (48%) occasions. All isolates were fully sensitive in one of the calves (C1) 1 week before being turned out to pasture.

Calves C4 and C5. The ARI rose initially and persisted at high levels (usually >0·60) during the calves residency on the veal unit; the highest value recorded was 0·94. The ARI then fell after the animals were moved to Langford with the fall being most rapid in C4. The ARI was 0·45 or less for the last ten swabs collected from both calves but in neither case were all the *Esch. coli* isolates fully sensitive on any occasion (Fig. 2).

Escherichia coli isolates

The O-typable *Esch. coli* (OTEC) isolates were differentiated into strains on the basis of O-serogroup, biotype and resistance pattern while the non-O-typable *Esch.*

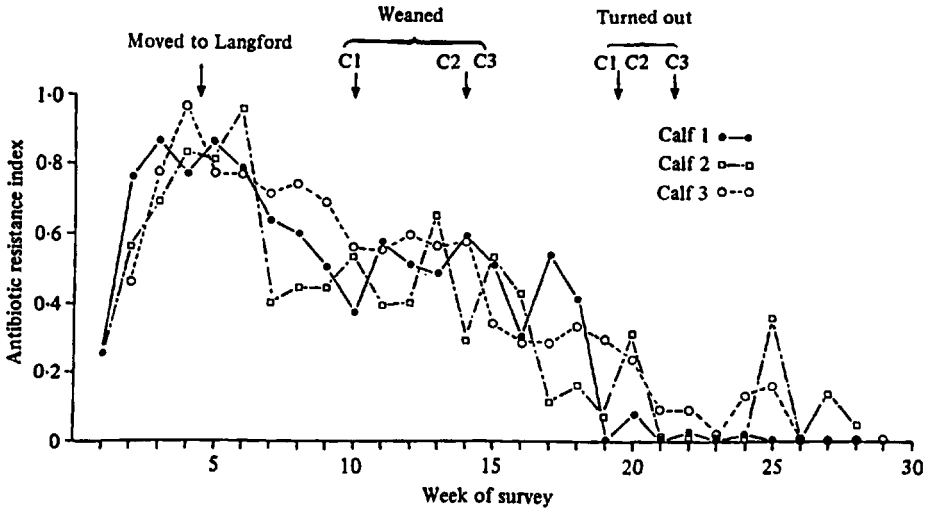

Fig. 1. The Antibiotic Resistance Index for *Escherichia coli* isolates obtained each week from calves C1, C2 and C3.

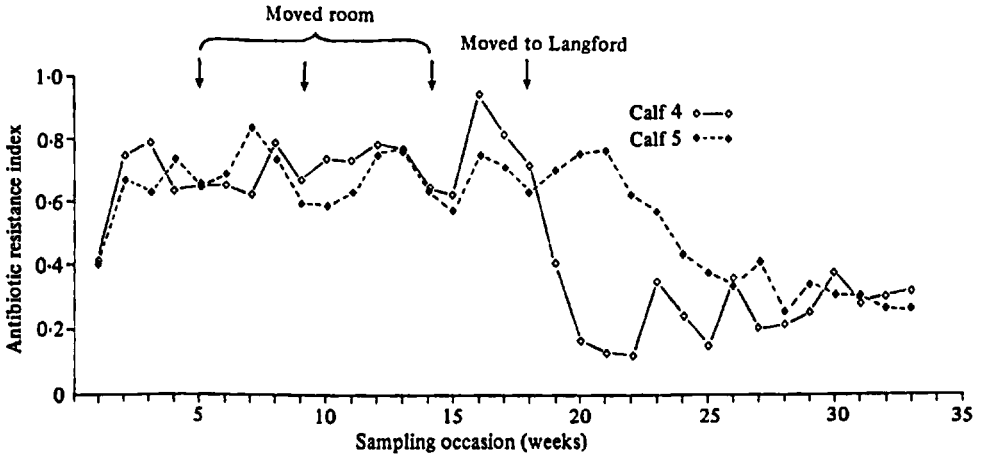

Fig. 2. The Antibiotic Resistance Index for *Escherichia coli* isolates obtained each week from calves C4 and C5.

coli (NTEC) were differentiated using the biotype and resistance pattern only. The total number of strains isolated from calves C1 to C3 (swabs 1 to 28) were 92, 109 and 77 respectively while the numbers for calves C4 and C5 (swabs 17 to 32) were 125 and 115 strains. In each animal the majority (68–76%) of strains were isolated from only a single swab indicating that the period of residency of the strains in the majority flora was generally for a short time only. In each animal a small proportion (5–10%) of strains were isolated from between four and seven swabs while the average number of swabs from which each strain was isolated was remarkably similar for all calves at 1·5–1·7.

Calves C1 to C3. The average numbers of strains (OTEC and NTEC) isolated per swab per calf per month are summarized in Table 2. The grand mean isolation rate per swab increased to 8·1 in the second month before declining thereafter to

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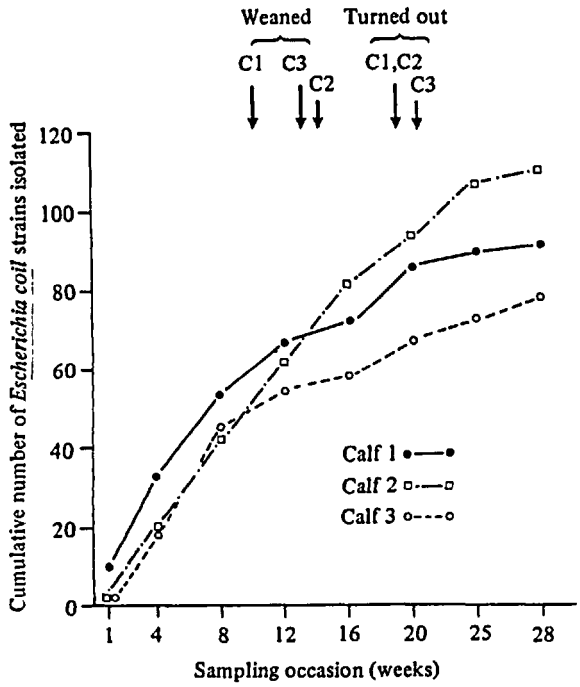

Fig. 3. Cumulative number of *Escherichia coli* strains isolated from calves C1, C2 and C3.

2·25 in the seventh. The differences between means were significant ($P < 0{\cdot}001$). A turnover in strains was recorded for each calf and new strains were isolated continually during the survey period. The cumulative numbers of *Esch. coli* strains isolated per calf are expressed graphically (Fig. 3). The curves are asymptotic presumably towards a finite number of *Esch. coli* strains in the population.

The sampling occasion on which each strain was first isolated was recorded and as expected the grand mean number of 'new' strains isolated per swab declined during the survey period from nearly six per swab during the first two months to about one during the last. The differences between these means were significant ($P < 0{\cdot}001$) (Table 2).

Calves C4 and C5. The *Esch. coli* isolates from these calves were biotyped and O-serogrouped from the 17th sampling occasion only. The grand mean isolation rate of strains per swab per month ranged between 7·25 and 12·9 (Table 3). The cumulative number of strains isolated per calf increased linearly between the 17th and 32nd samples. There were no significant differences between the average isolation rate of 'new' strains either between calves or between months (Table 3).

Drug resistance among strains of Escherichia coli

The changes in the incidence of drug resistance among all *Esch. coli* isolates per sampling occasion, as measured by the ARI, are summarized in Figs. 1 and 2. In this section the composition of the *Esch. coli* flora of each calf is analysed in relation to the number of resistance (R) factors carried by the individual strains.

Calves C1 to C3. The average number of R factors carried by each 'new' strain

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Table 2. *The average number of* Escherichia coli *and strains isolated per swab per month from calves C1, C2 and C3*

Months after purchase	Average no. of strains per swab				Average no. of 'new' strains per swab			
	C1	C2	C3	Grand mean	C1	C2	C3	Grand mean
1	8·5	5·5	5·0	6·33	8·0	5·0	4·5	5·83
2	6·75	8·25	9·25	8·08	5·25	5·5	6·75	5·83
3	5·5	11·5	5·75	7·58	3·5	5·0	2·25	3·58
4	3·25	7·25	3·25	4·58	1·25	4·75	1·0	2·33
5	5·5	4·25	2·5	4·08	3·25	3·0	1·0	2·42
6	1·5	4·25	4·0	3·25	1·0	3·0	2·25	2·08
7	2·0	2·5	2·25	2·25	0·25	1·0	1·25	0·83
Grand mean	4·71	6·21	4·57	5·17	3·21	3·89	2·71	3·29
ANOVA*	D.F.	*F* ratio		*P*	D.F.	*F* ratio		*P*
Month	6	8·01		<0·001	6	9·28		<0·001
Calf	2	3·16		<0·05	2	0·53		NS

* ANOVA, Summary of analysis of variance table. NS = not significant ($P > 0·05$).

Table 3. *The average number of* Escherichia coli *strains isolated per swab per month from calves C4 and C5*

Months after purchase*	Average no. of strains per swab			Average no. of 'new' strains per swab		
	C4	C5	Grand mean	C4	C5	Grand mean
5	8·75	5·75	7·25	7·25	4·5	6·13
6	10·75	11·5	11·13	8·25	8·25	8·25
7	12·25	14·5	13·38	8·0	10·0	9·0
8	13·5	12·25	12·88	7·25	6·0	6·63
Grand mean	11·31	11·0	11·16	7·81	7·19	7·50
ANOVA*	D.F.	*F* ratio	*P*	D.F.	*F* ratio	*P*
Month	3	4·09	<0·025	3	1·13	NS
Calf	1	0·05	NS	1	0·24	NS

* Isolates obtained in months 1–4 were tested for drug resistance only.
† ANOVA, Summary of analysis of variance table. NS = not significant ($P = > 0·05$).

ranged between 3·9 and 5·35 during the first two months and between 3·0 and 3·5 in the third. It was generally lower in the 4th to 6th month while in the 7th all 'new' strains isolated were fully sensitive (Table 4).

The proportions of the 'new' *Esch. coli* strains isolated during each month from each calf resistant to each of the seven drugs included in the sensitivity test are listed in Table 5. The majority of strains resistant to Ap, Cm and Tm were isolated for the first time during the first two months while after the fourth month the most prevalent R determinants in 'new' resistant strains were Sm and Su.

Some sensitive *Esch. coli* strains were isolated at the beginning of the survey period but none were detected during the 2nd and 3rd month. Sensitive strains

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Table 4. *The average number of R determinants per resistant* Escherichia coli *strain* according to the month that the strain was first isolated*

Month after purchase	Calf reference				
	C1	C2	C3	C4	C5
1	5·35	5·16	4·83	ND	ND
2	4·19	3·91	4·85	ND	ND
3	3·0	3·45	3·50	ND	ND
4	2·6	2·56	2·25	ND	ND
5	2·5	1·71	2·07	4·61	4·56
6	3·0	1·0	2·0	2·67	3·97
7	—	—	—	2·57	2·56
8	NE	NE	NE	2·92	2·09

NE, Not examined as calf sold. ND = Not done as the *Esch. coli* isolates were not O-serogrouped or biotyped.

* Differentiated on the basis of O-serogroup, biotype and resistance pattern.

Table 5. *The proportion (%) of 'new'* Escherichia coli *strains isolated from each calf each month resistant to each of seven antibacterial drugs*

Month ...	1	2	3	4	5	6	7
				Calf C1			
Sensitive to all 7 agents	28	—	—	—	38	75	100
Ampicillin	34	43	36	40	8	25	—
Chloramphenicol	41	33	—	—	8	—	—
Kanamycin	59	43	7	80	15	—	—
Streptomycin	72	86	100	100	38	25	—
Sulphafurazole	69	100	93	60	54	25	—
Tetracycline	72	81	71	80	15	—	—
Trimethoprim	41	33	7	20	—	—	—
Total 'new' *Esch. coli* strains isolated	32	21	14	5	13	4	1
				Calf C2			
Sensitive to all 7 agents	5	—	—	5	42	92	100
Ampicillin	45	45	25	5	8	—	—
Chloramphenicol	60	36	5	16	8	—	—
Kanamycin	85	45	45	21	8	—	—
Streptomycin	85	95	100	63	33	—	—
Sulphafurazole	95	95	80	79	42	—	—
Tetracycline	85	68	70	37	8	8	—
Trimethoprim	25	14	15	16	—	—	—
Total 'new' *Esch. coli* strains isolated	20	22	20	19	12	12	4
				Calf C3			
Sensitive to all 7 agents	—	—	—	—	25	89	100
Ampicillin	50	22	22	—	50	—	—
Chloramphenicol	33	37	—	—	50	—	—
Kanamycin	83	74	33	50	25	—	—
Streptomycin	94	100	100	100	25	11	—
Sulphafurazole	100	96	89	75	50	11	—
Tetracycline	67	93	78	—	—	—	—
Trimethoprim	56	63	33	—	—	—	—
Total 'new' *Esch. coli* strains isolated	18	27	9	4	4	9	5

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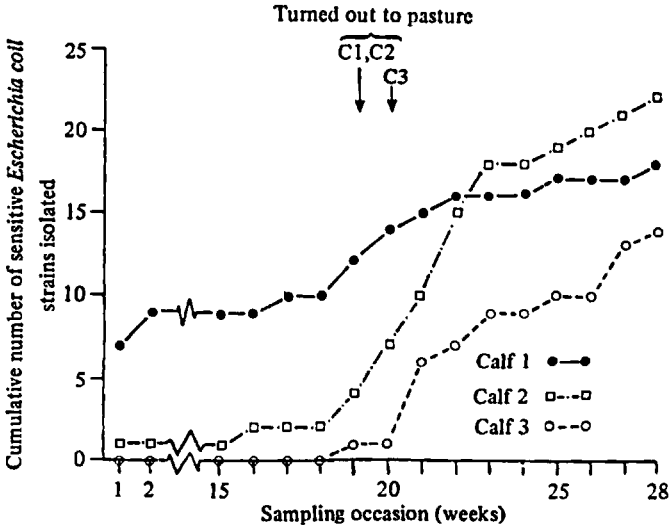

Fig. 4. Cumulative number of antibiotic sensitive *Escherichia coli* strains isolated from calves C1, C2 and C3.

first reappeared in the faecal flora between the 16th and 19th sampling occasions some 3 months after the calves arrived at Langford (Fig. 4). There was a general increase in the rate of appearance of 'new' sensitive strains after the 18th sampling of all three calves with this increase in rate commencing in calves C1 and C2 before they were turned out to pasture (Fig. 4).

Calves C4 and C5. The ARI fell dramatically after calf C4 was transferred to Langford while the fall was more gradual for C5 (Fig. 2). This difference was a reflexion of the fact that the average number of R determinants carried by the 'new' strains isolated each month fell more gradually in C4 than it did in C5 (Table 4). The faecal *Esch. coli* flora of calves C4 and C5 never became fully sensitive but there was a steady increase in the number of 'new' sensitive strains isolated from the 20th and 24th sampling occasion respectively with a total of 21 sensitive strains being isolated from C4 and 10 from calf C5.

Patterns of drug resistance

Calves C1 to C3. Forty-one of the 128 (2^7) possible resistance patterns were identified. Eight patterns occurred in 5% or more of the strains isolated from the individual calves, namely, fully sensitive (3 calves), SmSu (3), SmSuTc (3), KmSmSuTc (1), KmSmSuTcTm (2), ApCmKmSmSuTc (1), CmKmSmSuTcTm (1) and ApCmKmSmSuTcTm (3).

Calves C4 and C5. Thirty-two patterns were identified among the *Esch. coli* strains isolated from the 17th to the 32rd sample. The most common patterns (>5% of the strains isolated per calf) were fully sensitive (2 calves), Sm (1), Tc (2), SmSu (2), SmTc (1), SmSuTc (2), CmKmSmSuTcTm (1) and ApCmKmSmSuTcTm (1).

Salmonella excretion

No salmonella were isolated from any of the swabs collected from the five calves.

DISCUSSION

The five calves were each monitored over a period of several months. A continual turn over in the strains forming the majority faecal *Esch. coli* flora was demonstrated in all calves and this confirmed findings in previous investigations (Hinton, Hedges and Linton, in preparation). It was expected that moving the calves from the veal unit into a relatively clean environment, in which there was no antibiotic selection pressure, would be followed by a simplification in the faecal *Esch. coli* flora. This proved to be the case in calves C1 to C3 and was associated with a decrease, with time, in the isolation rate of 'new' *Esch. coli* strains (Table 2, Fig. 3). On the other hand, this change did not occur in calves C4 and C5 since there was no decrease in the number of strains isolated per swab (Table 3) and 'new' strains were isolated at a constant rate (i.e. linearly) during the four months that they were resident at Langford. The reason for this was not ascertained but it probably reflected the fact that these animals remained longer on the veal unit and that they were moved several times into new environments during that time.

The incidence of antibiotic resistance, as measured by the ARI, increased after the calves arrival on the veal unit and, once established, persisted at high levels during the whole of the 4 months that calves C4 and C5 were kept on that farm. The ARI fell after all calves were moved to Langford. This was associated initially with a reduction in the average number of R determinants per resistant strain and then, after a delay of up to 8 weeks, by an increase in the proportion of isolates that were fully sensitive. The first appearance of sensitive strains was not related to weaning, nor to 'turnout' to pasture, since they appeared before both weaning in calves C4 and C5 and turning out to pasture in calves C1 to C3. When sensitive strains reappeared in the majority flora they tended to be isolated at a regular rate from all calves. The source of these sensitive strains was not determined but, in time, they would have presumably displaced all the resistant strains since it is usual to find a predominantly sensitive *Esch. coli* flora in adult cattle even when the *Esch. coli* isolates from calves on the same farm were generally multiply resistant (Linton, 1977; Hinton, Linton and Hedges, in preparation and unpublished observations).

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