The incidence of antibiotic resistance and other characteristics amongst *Escherichia coli* strains causing fatal infection in chickens: the utilization of these characteristics to study the epidemiology of the infection

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SUMMARY

Of 173 epidemiologically unrelated strains of *Escherichia coli* isolated from the pericardial sac of chickens that had died from infection with these organisms in England in 1972, approximately 1 year after the introduction of legislation forbidding the routine use of feeds containing 'therapeutic' antibiotics, 83·8 % were resistant to sulphonamides, 31·2 % to tetracyclines, 20·8 % to furazolidone, 18·5 % to streptomycin, 2·9 % to spectinomycin and 1·2 % to ampicillin; none of the strains were resistant to chloramphenicol, neomycin, polymixin, trimethoprim or nalidixic acid. The sulphonamide resistance and possibly some of the resistance to other agents might have been the consequence of sulphonamides being exempted from the legislation. Much of the resistance, with the exception of that to furazolidone, was of the transferable type. Many strains possessed transfer factors in the absence of any known transferable characteristic. Colicine production was twice as common in the pathogenic strains as in a collection of strains isolated from the faeces of healthy chickens; about half of it was transferable.

By means of serology, antibiotic resistance and other markers, it was found that several different kinds of *E. coli* were usually incriminated in any one outbreak of *E. coli* infection in broiler chickens. Sometimes the same kinds of *E. coli* were found in outbreaks in consecutive crops of chickens on the same farm. New kinds, too, appeared to be brought in by replacement chickens.

INTRODUCTION

The routine use of tetracyclines as feed additives has given rise to large populations of tetracycline-resistant *Escherichia coli* in the alimentary tract of chickens (Smith & Crabb, 1957). There is little doubt that it has also been responsible, in great part, for the high incidence of tetracycline resistance found amongst strains of *E. coli* pathogenic for poultry (Sojka & Carnaghan, 1961). Following the report of the Swann Committee (Report, 1969), legislation was introduced in Britain in March 1971 prohibiting the use of 'therapeutic' antibiotics, except sulphonamides, as feed additives; their use for disease control on veterinary prescription, however,

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continued. It seemed worth while therefore to study the antibiotic resistance of strains of *E. coli* that had killed broiler chickens in Britain in 1972 to determine whether this legislation had had a more immediate effect and to form a 'base-line' for future assessments of its long-term effect. The results are presented here. The strains were also examined for antibiotic resistance, R factors, non-transmissible but mobilizable resistance determinants, transfer factors, other transferable characters such as colicine (Col) and haemolysin (Hly) production and for lysogenicity. The information gained was then used to augment serological typing in studying the epidemiology of *E. coli* infection, because the value of the latter is limited by the fact that the majority of pathogenic poultry strains belong to one or other of only three serotypes, O2:K1, O1:K1 and O78:K80 (Sojka & Carnaghan, 1961; Harry, 1964).

MATERIALS AND METHODS

Escherichia coli *strains*

These were obtained by culturing on MacConkey's agar the diseased pericardial sac of broiler chickens which, at autopsy, exhibited pathological lesions typical of *E. coli* infection. From the heavy *E. coli* growth that resulted, a single colony was picked, purified by replating and finally cultured on Dorset's egg medium. This was then maintained at 5° C. until required.

Escherichia coli *strains from healthy chickens*

These were obtained from the faeces of chickens kept on different farms; they were not submitted for serotyping.

Serological examination

Glucose agar cultures were submitted to slide agglutination tests with O78:K80 and O2:K1 antisera. Those that agglutinated with the former antiserum were accepted as O78:K80. Those that agglutinated with the latter were made into a thick suspension with nutrient broth and autoclaved at 121° C. for 30 min. to destroy the K antigens and expose the O antigens. The suspensions were then centrifuged, resuspended in broth and submitted to slide agglutination tests employing O1:K1 and O2:K1 antisera suitably diluted to enable O suspensions of known O1:K1 and O2:K1 strains to be differentiated from each other.

Antibiotic-sensitivity tests

These were performed by the disk method described by Smith (1970) using an Oxoid Multodisk (1744 E) composed of eight disks containing (i) streptomycin (Sm) 25 μg., (ii) ampicillin (A) 25 μg., (iii) oxytetracycline (T) 50 μg., (iv) chloramphenicol (Cm) 50 μg., (v) neomycin (Neo) 30 μg., (vi) nalidixic acid (Nal) 30 μg., (vii) furazolidone (F) 15 μg. and (viii) sulphonamides (Su) 300 μg., and three separate disks containing spectinomycin (Sp) 25 μg., polymixin (Pol) 300 units and sulphamethoxazole/tri-methoprim (SXT) 23·75 μg. and 1·25 μg. respectively. No difficulty was experienced in making readings; the zones of bacterial inhibition surrounding the disks were either wide, very narrow or absent.

Transfer of antibiotic resistance in vitro

This was performed by the method described by Smith (1970), the prospective recipient strain being a nalidixic acid-resistant mutant (*nal*^{r}) of *E. coli* K12 th.:t was non-lactose fermenting (*lac*$^-$). The latter property provided an additional method of distinguishing the recipient strain from the prospective donor strains which, apart from being *nal*^{s}, were *lac*$^+$. The concentrations of antibiotics employed in the selection media were as described above except that furazolidone was used at 10 μg/ml. Strains that did not transmit their resistance were retested several times, with in addition a mutant of the K12 recipient strain resistant to several different colicines; this was done because most of the prospective donor strains examined were colicinogenic for the usual recipient strain and sometimes, in mixed culture, had a marked depressant effect on its growth rate.

The identification of strains possessing only transfer factors or only resistance determinants

For this we used the mobilization test of Anderson (1965), the actual technique employed being that described by Smith & Linggood (1970). An *E. coli* K12 strain possessing mobilizable determinants for streptomycin (Sm$^+$) and sulphonamide (Su$^+$) resistance was employed to identify strains possessing only transfer factors and K12 strains possessing transfer factor F or I were used to identify strains possessing only mobilizable determinants.

Haemolysin and colicine production: their transferable nature

Haemolysin production was detected by spot-inoculating broth cultures on plates of 'washed blood' agar (Smith & Halls, 1967) and colicine production by spot-inoculation on plates of tryptose nutrient agar previously evenly spread with a broth culture of *E. coli* K12 suitably diluted to produce just confluent bacterial growth after incubation. All plates were incubated at 37° C. for 24 hr. and read. The methods of Smith & Gyles (1970) were employed to determine whether or not the haemolysin and colicine production of individual strains were transferable, the indicator for colicine production being the *nal*^{r} *lac*$^-$ K12 strain.

Colicine typing

The technique employed was that described by Lewis (1968), using indicator strains received from Professor P. Fredericq.

Identification of lysogenic strains

A broth culture of the *nal*^{r} *lac*$^-$ K12 strain resistant to several different colicines was spread evenly over the surface of a plate of tryptose agar containing 0·02 M-CaCl$_2$ and 20 μg./ml. of sodium nalidixate. When dry, it was 'spot-inoculated' with young broth cultures of the strains of *E. coli* under test. The plates were incubated at 37° C. and examined for evidence of phage action.

RESULTS

Incidence of antibiotic resistance amongst strains of Escherichia coli *isolated from diseased chickens*

The antibiotic resistance found amongst 173 strains of *E. coli* isolated from the pericardial sac of chickens which had died in different outbreaks of clinical *E. coli* infection in England are illustrated in Table 1; over 60 % of the strains belonged either to serotype O78:K80 or O2:K1. Only 13·3 % of the strains were sensitive to all the 11 antibiotics against which they were tested. Sulphonamide resistance was by far the most common, followed by tetracycline, furazolidone and streptomycin resistance. Resistance to tetracyclines and streptomycin was found less frequently amongst the O2:K1 strains than amongst the others; the converse was true in the case of furazolidone resistance. With the exception of a few strains that were resistant to spectinomycin or ampicillin, all 173 were fully susceptible to the other seven antibiotics, including chloramphenicol, against which they were tested.

Sixteen different patterns of resistance were shown by the 173 strains. Apart from the large group that were resistant to sulphonamides only, none of the groups comprised 14 % of the whole; most of them were much smaller. The resistance patterns were usually small, resistance to more than three antibiotics being uncommon.

The incidence of colicine and haemolysin production and lysogenicity amongst chicken strains of Escherichia coli

The results of examining the 173 pathogenic strains of *Escherichia coli* and 168 strains isolated from the faeces of healthy chickens from different farms for colicine production are summarized in Table 2. Most of the O78:K80 and O2:K1 strains and the other pathogenic strains produced colicines, the incidence being almost twice as high in these strains as in those isolated from the faeces of healthy chickens. Difficulty was experienced in identifying the actual colicines produced by the pathogenic strains as many strains produced more than one colicine. However, it was possible to divide all those that were identified into seven fairly well defined groups (1–7) according to the particular indicator strains which were susceptible to their colicinogenic activity. The percentage of the 173 epidemiologically un-related pathogenic strains that fell into each group was: group 1, 28·5 %; group 2, 20·9 %; group 3, 7·6 %; group 4, 13·9 %; group 5, 5·1 %; group 6, 13·3 %; and group 7, 10·1 %.

Only one of the 173 pathogenic strains and one of the 168 strains isolated from the faeces of healthy chickens were haemolytic. Twelve (6·9 %) of the 173 pathogenic strains were lysogenic for *E. coli* K 12. Three belonged to serotype O78:K80 and one to serotype O2:K1.

Antibiotic resistance, colicine and haemolysin production; their transferable nature in the chicken strains of Escherichia coli

The incidence of transferable antibiotic resistance, colicine and haemolysin production in the 173 pathogenic strains of *E. coli* and in the 168 other strains is

Table 1. *Incidence of antibiotic resistance amongst strains of* Escherichia coli *isolated from diseased chickens*

Strains	Serotypes			
	O78:K80	O2:K1*	Other serotypes	All
No. examined . . .	43	63	67	173
% resistant to one or more drugs	83·7	88·9	85·1	86·7
% resistant to				
Sulphonamides	83·7	87·3	80·6	83·8
Tetracyclines	37·2	14·3	43·2	31·2
Furazolidone	18·6	28·6	14·9	20·8
Streptomycin	28·0	3·2	23·9	18·5
Spectinomycin	9·3	1·6	0	2·9
Ampicillin	0	1·6	1·5	1·2
Neomycin	0	0	0	0
Chloramphenicol	0	0	0	0
Nalidixic acid	0	0	0	0
Sulphamethoxazole/trimethoprim	0	0	0	0
Polymixin	0	0	0	0
% with resistance pattern†				
Su	30·0	44·5	35·9	37·6
Su T	18·6	9·5	15·0	13·9
Su F	2·3	22·2	1·5	9·3
Sm Su T	4·7	1·6	15·0	7·5
Sm Su T F	7·0	0	6·0	4·1
Su T F	4·7	3·2	4·5	4·1
Sm Su	4·7	3·2	0	2·3
Sm Su Sp F	2·3	1·6	0	1·2
Sm Su F	2·3	0	1·5	1·2
Sm Su Sp	4·7	0	0	1·2
F	0	1·6	1·5	1·2
A Sm Su T	0	0	1·5	0·6
Sm Su T Sp	2·3	0	0	0·6
A Su	0	1·6	0	0·6
Sm	0	0	1·5	0·6
T	0	0	1·5	0·6

* Includes two O1:K1 strains.

† A = ampicillin; F = furazolidone; Sm = streptomycin, Sp = spectinomycin; Su = sulphonamides; T = tetracyclines.

illustrated in Table 3. So is the incidence of transfer factors in those strains not shown to possess transferable characters. Most of the spectinomycin and ampicillin resistance and about half the tetracycline and streptomycin resistance was transferred to *E. coli* K 12, the incidence of transferable streptomycin resistance being higher in the pathogenic group than in the other group. The incidence of transferable tetracycline resistance was lowest in the untyped pathogenic strains. Less than 20% of the sulphonamide resistance and none of the furazolidone resistance was transferable. Colicine production was transferable in about half the strains possessing this character, the incidence of transferability being similar in both groups. The incidence of transfer factors in strains not possessing known transferable characters was highest in the O78:K80 strains and in the untyped pathogenic strains. There

Table 2. *The incidence of colicine production amongst strains of*
Escherichia coli *isolated from chickens*

	Source of strains				
	Diseased chickens				Healthy chickens
	O78:K80	O2:K1*	Other serotypes	Total	Untyped serologically
No. examined	43	63	67	173	168
No. colicine-positive	38	59	55	152	78
Colicine-positive (%)	88·4	93·6	82·1	87·8	46·4

* Includes two O1:K1 strains.

Table 3. *The incidence of transferable antibiotic resistance, colicine and haemolysin
production and transfer factors in chicken strains of* Escherichia coli

	Source of strains				
	Diseased chickens				Healthy chickens
Characteristic*	O78:K80	O2:K1	Other serotypes	Total	Untyped serologically
Sm	9/12† (75·0)	0/2 (0)	7/17 (41·2)	16/31 (51·6)	7/27 (25·9)
Su	10/36 (27·8)	3/56 (5·4)	11/53 (20·8)	24/145 (16·5)	19/102 (18·6)
T	10/14 (71·4)	8/9 (88·9)	7/28 (25·0)	25/51 (49·0)	28/52 (53·8)
Sp	3/4 (75·0)	1/1 (100)	0/0	4/5 (80)	0/3 (0)
A	0/0	1/1 (100)	1/1 (100)	2/2 (100)	3/3 (100)
F	0/8 (0)	0/18 (0)	0/10 (0)	0/36 (0)	0/8 (0)
Col	22/38 (57·8)	30/59 (50·8)	30/55 (54·5)	82/152 (53·9)	41/78 (42·6)
Hly	0/0	0/0	0/1 (0)	0/1 (0)	0/1 (0)
Tra‡ only	6/12 (50·0)	4/21 (19·1)	15/25 (60·0)	25/58 (43·1)	16/90 (17·8)

The figures in parentheses are percentages.
* Sm, Su, T, Sp, A, F, Col, Hly and Tra are genetic determinants for streptomycin, sulphon-amides, tetracyclines, spectinomycin, ampicillin and furazolidone resistance, colicine and haemolysin production and transfer factor.
† No. of strains in which it was transferable/no. of strains tested.
‡ Presence of transfer factor in absence of any characters shown to be transmissible.

were several examples of strains possessing transfer factors yet being unable to transfer their own tetracycline, streptomycin and sulphonamide resistance and their colicine production. By means of transfer factors F and I, it was possible to mobilize the non-transfering resistance possessed by three strains.

The isolation of different types of Escherichia coli *from outbreaks of* Escherichia coli *infection in broiler chickens*

The results of examining strains of *E. coli* isolated from the hearts of chickens that had died from *E. coli* infection on three broiler farms are summarized in Tables 4–6; the chickens belonged to one or other of three successive crops. Table 6 also shows the results for strains isolated from the infected yolk sacs of 20 one-day-old chicks belonging to the second crop of farm C which had died immediately before they were taken to the farm. None of the chickens on the farms had been given antibiotics or any other agents active against *E. coli*. Each farm consisted of 10–17 broiler houses sited close together and, although one particular kind of *E. coli* was sometimes isolated more commonly from dead chickens from one house than from another, this occurrence did not seem sufficiently common to justify considering each house as a separate unit.

Strains belonging to serotype O2:K1, O78:K80 and to other, unidentified, serotypes were present amongst those isolated from chickens from all three crops on all three farms. According to antibiotic sensitivity, production of colicine and haemolysin and lysogenicity, these could be further classified into a comparatively large number of different kinds, many of which were incriminated in the deaths that occurred in each crop, e.g. the number of kinds isolated from the dead chickens in the first crops examined from farms A, B and C were 10, 9 and 4 respectively. It was not unusual for the same kinds of *E. coli* to be isolated from some of the chickens in all three crops on each farm. A notable example of this was the finding of an antibiotic-sensitive, colicine-producing strain of O78:K80 *E. coli*, to be the most common kind causing disease in all three crops on farm B. A kind of *E. coli* O78:K80, sulphonamide and tetracycline-resistant, colicine-producing, and also lysogenic, was responsible for most of the deaths in the first and second crop on farm C; it was also isolated from dead chickens of the third crop but most of the deaths in this crop were caused by an O2:K1 kind of *E. coli*. These two kinds of *E. coli* O78:K80 were not commonly found amongst the epidemiologically unrelated strains illustrated in Table 1. Several kinds of *E. coli* isolated from chickens in the second and third crops of farm C resembled some of those that had been isolated from the infected yolk sacs of the dead chicks that would have formed part of the second crop. The colicine produced by the O2:K1 strain isolated from one of these chicks was the same as that produced by some of the O2:K1 strains that were isolated later from dead chickens of this crop and of the third crop.

A total of 198 strains of *E. coli* isolated from the hearts of chickens, one per chicken, that had died from *E. coli* infection on 36 other farms, 4–10 (average 5·5) per farm, were also examined. The number of kinds of *E. coli* amongst the strains from each farm varied from 1 to 6 (average 2·5); those from each of ten of the farms were of the same kind.

Table 4. *The different kinds of* Escherichia coli *isolated from 236 dead chickens in successive broiler crops on farm A*

	No. of strains isolated from dead chickens in crop no.		
Kind of *E. coli**	1	2	3
O2:K1			
Su Col	2	14	4
Su T Col	0	0	4
Su F Col	0	1	0
O78:K80			
Col	22	4	9
S Su Sp Col	34	4	0
Untyped†			
Col	4	8	0
Su	6	1	2
Su Col	15	39	0
Su T	0	7	0
Su T Col	8	7	8
Su T F Col	1	5	0
Su F Col	0	5	0
S Su T Col	3	5	10
S Su T F Col	1	3	0
All kinds	96	103	37

One strain from the heart of each dead chicken was examined.

* S, Sp, Su, T and F = resistant to streptomycin, spectinomycin, sulphonamides, tetracycline and furazolidone. Col = colicine-producing.

† Neither O78:K80, O1:K1 or O2:K1.

DISCUSSION

Despite the introduction of legislation, some 12 months previously, banning the use of 'therapeutic' agents as feed additives, the incidence of tetracycline and furazolidone resistance found in the present survey amongst strains of *Escherichia coli* pathogenic for poultry was still 31·2 and 20·8% respectively. It will be of interest to see whether or not these figures will decrease significantly with time, now that the use of these two agents in feeds has been restricted. The high incidence of resistance to sulphonamides, 83·8%, was not unexpected because it is still permissible to feed diets containing these agents continuously to poultry to prevent coccidiosis. It is doubtful whether the streptomycin resistance possessed by 18·5% of the pathogenic strains can be attributed to the selection pressure of streptomycin itself because this antibiotic is rarely given to poultry. It may well be a reflexion of the use of sulphonamides, as the genetic determinants for resistance to streptomycin and sulphonamides are often located on the same plasmid. It is possible, too, that the use of sulphonamides may be playing a part in maintaining the high incidence of tetracycline resistance because practically all the tetracycline-resistant *E. coli* examined were also resistant to these agents. If sulphonamides are acting in this manner it calls into question the advisability of

Table 5. *The different kinds of* Escherichia coli *isolated from 274 dead chickens in successive crops on farm B*

	No. of strains isolated from dead chickens in crop no.		
Kind of *E. coli**	1	2	3
O2:K1			
Su	0	0	7
Su Col	1	30	4
Su T Col	0	3	0
O78:K80			
Su	1	0	0
Col	38	42	24
S Su T Col	0	2	1
Untyped			
Col	1	0	0
Su Col	7	15	2
T	1	0	0
Su T Col	2	6	3
Su T F Col	0	3	0
S Su T	0	4	0
Su F Col	0	5	0
S Su T Col	2	29	5
S Su T F Col	19	0	0
Su Col Hly	0	15	2
All kinds	72	154	48

* Hly = haemolytic. For other details see Table 4.

Table 6. *The different kinds of* Escherichia coli *isolated from 200 dead chickens in successive broiler crops on farm C*

	No. of strains of this kind isolated from dead chickens in crop no.			
Kind of *E. coli**	1	2A†	2B	3
O2:K1				
Su Col	0	1	2	46
Su F Col	3	0	0	0
O78:K80 Su T Col Lys	73	0	10	12
Untyped				
Unclassifiable	0	1	2	1
Col	0	3	0	9
Su	1	4	1	1
Su Col	0	7	1	7
Su T Col	0	1	2	6
Su F Col	2	3	0	0
S Su T Col	0	0	0	1
All kinds	79	20	18	83

* Lys = lysogenic for *E. coli* K 12. For other details see Table 4.
† Strains from infected yolk sacs of 1-day-old chicks of crop no. 2; they had died immediately before being brought to farm C.

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their continued use as feed additives unless, of course, it can be shown, as alleged, that they are essential in coccidiosis control. The complete absence of strains resistant to neomycin, chloramphenicol, nalidixic acid, trimethoprim and poly-mixin is in keeping with the fact that these agents are seldom, if ever, administered to poultry.

The failure to transfer furazolidone resistance from all 44 strains of *E. coli* examined confirms the observations of Smith (1966), from which it appears that transferable furazolidone resistance in *E. coli*, in contrast to resistance to most other antibiotics, must be uncommon. The high incidence of pathogenic strains, other than those of serotype O2:K1, possessing transfer factors and no identifiable transferable characteristics suggests that these strains, in fact, may possess transferable characteristics that, in some way, are associated with pathogenicity, as was found in the case of strains enteropathogenic for pigs (Smith & Linggood, 1970). It is noteworthy, too, that colicinogenicity, shown to be transferable in over 50 % of the strains examined, was twice as common in the pathogenic strains as in those isolated from the faeces of healthy chickens.

The results of classifying strains from different outbreaks according to serology, antibiotic resistance pattern, colicine and haemolysin production and lysogenicity have served to augment those previously made by Harry (1964), Harry & Hemsley (1965) and Hemsley & Harry (1965) using serological methods only. They show, for example, that several different strains may be involved in any outbreak. This finding stresses the desirability of performing antibiotic sensitivity tests on a number of strains before selecting an antibiotic with which to treat a particular outbreak. It suggests, too, that vaccines giving coverage against a wide variety of strains would be essential if immunological procedures were to be successful in preventing such outbreaks. It is also apparent from the examination of strains from farms A, B and C that (1) pathogenic strains may persist in broiler houses and thus give rise to outbreaks of clinical infection in consecutive crops of birds and (2) new pathogenic strains can be brought in by the replacement chicks themselves.

REFERENCES

ANDERSON, E. S. (1965). A rapid screening test for transfer factor in drug sensitive Entero-bacteriaceae. *Nature, London* **208**, 1016–17.

HARRY, E. G. (1964). A study of 119 outbreaks of coli septicaemia in broiler flocks. *Veterinary Record* **76**, 443–8.

HARRY, E. G. & HEMSLEY, L. A. (1965). The relationship between environmental contamination with septicaemia strains of *Escherichia coli* and their incidence in chickens. *Veterinary Record* **77**, 241–5.

HEMSLEY, L. A. & HARRY, E. G. (1965). Coliform pericarditis (colisepticaemia) in broiler chickens: a three year study on one farm. *Veterinary Record* **77**, 103–7.

LEWIS, M. J. (1968). Transferable drug resistance and other transferable agents in strains of *Escherichia coli* from two human populations. *Lancet* i, 1389–93.

REPORT (1969). *Report of the Joint Committee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine.* London: H.M.S.O.

SMITH, H. W. (1966). The incidence of infective drug resistance in strains of *Escherichia coli* isolated from diseased human beings and domestic animals. *Journal of Hygiene* **64**, 465–74.

SMITH, H. W. (1970). The transfer of antibiotic resistance between strains of enterobacteria in chickens, calves and pigs. *Journal of Medical Microbiology* **3**, 165–80.

SMITH, H. W. & CRABB, W. E. (1957). The effect of the continuous administration of diet containing low levels of tetracycline on the incidence of drug resistant *Bacterium coli* in the faeces of pigs and chickens: the sensitivity of the *Bact. coli* to other chemotherapeutic agents. *Veterinary Record* **69**, 24–30.

SMITH, H. W. & GYLES, C. L. (1970). The relationship between different transmissible plasmids introduced by F into the same strain of *Escherichia coli* K 12. *Journal of General Microbiology* **62**, 277–85.

SMITH, H. W. & HALLS, S. (1967). The transmissible nature of the genetic factor of *Escherichia coli* that controls haemolysin production. *Journal of General Microbiology* **47**, 153–61.

SMITH, H. W. & LINGGOOD, M. A. (1970). Transfer factors in *Escherichia coli* with particular regard to their incidence in enteropathogenic strains. *Journal of General Microbiology* **62**, 287–99.

SOJKA, W. J. & CARNAGHAN, R. B. A. (1961). *Escherichia coli* infection in poultry. *Research in Veterinary Science* **2**, 340–52.