Before we had identified the viruses we isolated, Dr. I. A. Galloway, of the Foot and Mouth Research Centre at Pirbright, kindly examined sera from two patients for us, but found no evidence of any antibodies against any of the standard types of foot-and-mouth disease virus.

Summary

An outbreak of mild infection characterized by vesicular lesions in the mouth and on the hands and feet occurred in Birmingham in the summer of 1959. A few patients also had a maculo-papular rash on the buttocks. Coxsackie group A type 16 virus was isolated from vesicle fluid from the hands of one. The disease was similar to that described by Robinson *et al.* (1958) as occurring in Toronto in 1957.

Our thanks are due to Dr. D. Hobson for the cultures of baboon-kidney cells, and to Dr. J. Hotchin for the prototype coxsackie A 16 virus. The technical work described will form part of a thesis for the F.I.M.L.T. by one of us (J. R. F.). Our thanks are also due to the College of General Practitioners, whose Epidemic Observation Unit enabled us to collect as many cases as we did.

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CANICOLA FEVER IN MAN FROM CONTACT WITH INFECTED PIGS

FURTHER OBSERVATIONS

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Outbreaks of human leptospirosis due to *Leptospira canicola* affecting workers on pig farms have been reported in Israel (van der Hoeden, 1956) and in Edinburgh (Seiler, Norval, and Coghlan, 1956). That apparently normal pigs were harbouring the organisms and were probably responsible for the outbreaks in the Edinburgh farms was finally established by Coghlan, Norval, and Seiler (1957) when they reported the isolation of *L. canicola* from specimens of urine collected from pigs at one of the farms where human cases had occurred. Surveys detailed in the same report revealed from the results of serological tests that 19 out of 47 piggery workers had been infected during the years 1950-5 and that over 40% of the pigs at three farms where 12 of the cases had occurred were or had been infected. In Glasgow, Michna (1959) found serological evidence of infection by *L. canicola* in the pigs at seven different farms and succeeded in isolating the leptospires from kidneys of naturally infected pigs.

Since the 1950-5 survey further sporadic cases of canicola fever have occurred among pig contacts in

Edinburgh, and the present communication serves both to summarize the salient features of these cases and to show how a leptospiral infection in a herd of pigs may be maintained for many years, thereby constituting a hazard to any person coming in contact with the animals.

Laboratory Methods

The methods employed were essentially the same as those described previously (Coghlan *et al.*, 1957); live cultures of leptospires were used for the agglutination-lysis test.

Significant Agglutination Titres.—Titres of 1 in 100 may be indicative of past or newly acquired leptospiral infection of some kind, but in this investigation only titres of 1 in 1,000 and over are considered to be significant of present active infection in a herd of pigs. To confirm the diagnosis of human cases it was essential to show, during the course of the disease, a rise in the leptospire-agglutinating titre of the patient's serum.

Observations

With one exception all the cases dealt with here were ill enough to require hospital treatment. They were all of the meningeal type of leptospirosis; none was icteric. Since 1956 there have been 16 cases of canicola fever in the Edinburgh area, and 11 of these persons had been in contact with pigs. One was an abattoir worker engaged in slaughtering pigs, one was a child who played around a piggery, and the other nine were employed in tending pigs.

Five pig farms were involved—namely, C, D, E, F, and G, two of which (F and G) had been previously incriminated in human infections of *L. canicola*. Specimens of pig blood taken from a number of animals at each of the five piggeries at the time of the human outbreaks were found to contain antibodies to *L. canicola* in titres high enough to signify recent infection. The details of the 11 human cases are given in Table I and the results of the serological examination of the pig sera in Table II (these include some tests carried out more recently as well as at the time of the human infections).

Farm C.—In addition to the two cases of canicola fever at farm C, there was one apparently subclinical infection—Mr. U, who remained symptomless, although his serum agglutinated *L. canicola* to a titre of at least 1 in 30,000 at the time when his fellow worker, Mr. S, was in hospital suffering from a severe attack of canicola fever with classical symptoms. Serological tests revealed a 36% infection rate among the pigs.

Farm D.—The piggery owner became ill with pyrexia, polyarthritides, frontal headache, and vomiting. His serum at the time of admission into hospital failed to agglutinate *L. icterohaemorrhagiae*, and *L. canicola*. Nine days later the serum had a titre of 1 in 1,000 for *L. canicola* and 1 in 30 for *L. icterohaemorrhagiae*, and by the end of the third week the titres had reached their maximum of at least 1 in 30,000 and 1 in 300 for *L. canicola* and *L. icterohaemorrhagiae* respectively. At the same time the pigs were examined for serological evidence of infection, and it was found that 9 of the 11 sera examined had titres of 1 in 1,000 and over.

Farm E.—There were four cases of canicola fever—three in July-August, 1958, and one a year later. It should be noted that three of the patients were youths under 20 years of age who had only recently taken up

TABLE I.—*Details of 11 Cases of Canicola Fever and One Apparently Symptomless Infection Which Occurred Among Human Contacts of Pigs in Edinburgh During 1956-9*

Date of Onset	Patient	Age	Where Employed	Maximum Agglutination Titres	
				Against <i>L. canicola</i>	Against <i>L. icterohaemorrhagiae</i>
21/4/56	Mr. P	40	Abattoir	1/10,000	1/1,000
30/7/56	Mr. Q	15	Farm F 1	1/30,000	1/1,000
9/10/56	Mr. R	27	" C	1/3,000	1/1,000
12/2/57	Mr. S	27	" C	*	*
9/2/57	Mr. T	30	" D	1/30,000	1/300
7/3/57	Mr. U†	29	" C	1/30,000	1/10
25/7/57	Miss V	11	Played at Farm F 3	1/3,000	1/10
17/7/58	Mr. A W	26	Farm E	1/100,000	1/10,000
18/7/58	Mr. B W	19	" E	1/10,000	1/300
7/8/58	Mr. X	18	" E	1/3,000	Neg. at 1/10
3/10/58	Mr. M Y‡	21	" G		
14/9/59	Mr. Z	15	" E	1/10,000	1/1,000

* Case diagnosed elsewhere; results not available.

† Symptomless infection (see text).

‡ This may have been a case of *L. icterohaemorrhagiae* (see Table III).TABLE II.—*Agglutination Titres of Sera from Pigs Taken from the Five Farms Where 11 Human Cases of Canicola Occurred*

Farm	Total No. of Sera Tested	No. of Sera giving the Following Agglutination Titres Against <i>L. canicola</i>			
		<1/100	1/100 to 1/300	1/1,000 to 1/3,000	1/10,000 to 1/30,000 and over
C	25	14	2	3	6
D	11	0	2	6	3
E	12	0	0	2	10
F1, F2, F3*	73	40	9	21	3
G	44	1	8	25	10

* Three farms owned by Mr. A treated as one herd (F) (see text).

piggery work. All of the 12 pig sera examined agglutinated *L. canicola* strongly enough to indicate active infection among the animals; in fact, the titres of 10 of them were as high as 1 in 10,000 and over.

Farm F.—At farm F1 a 15-year-old boy who worked with pigs developed canicola fever in July, 1956. This farm is one of three owned by Mr. A and the conditions at all three of them are similar. Some sows are kept for breeding, but, in order to maintain an even number of pigs in the herds, purchases of young pigs at the age of three months are made at the local market at weekly or fortnightly intervals. Occasionally pigs are transferred from one farm to another, and for this reason the three are treated as one herd. The pigs are fed mostly on swill with the addition of a certain amount of meal. The premises are fairly old, and, although kept clean, the fact that the floors are uneven tends to result in the formation of pools of liquid consisting of urine, cleaning-water, and swill. The pigs are kept in small pens with approximately 10 in each. At farm F2 one of the workers had a history suggestive of canicola fever prior to 1955 (this was mentioned in the previous report) and more recently, in July, 1957, an 11-year-old girl developed canicola fever after playing around Farm F3.

Of the 73 pig sera from the three farms, 33% had titres indicative of infection by *L. canicola*.

Farm G

At this farm in October, 1958, one of the farmer's sons, Mr. MY aged 21, contracted an illness with symptoms described by the clinician as "photophobia, headache, and muscle pains." Since he worked on a piggery the possibility that this might be a case of canicola fever was considered and specimens of the patient's serum taken at intervals were examined. The

results are shown in Table III. Thus it appeared that the infection was of *L. icterohaemorrhagiae* and not *L. canicola*. Unfortunately, attempts to isolate the organism in order to confirm its identity, by culture and animal inoculation of the patient's urine, failed.

Serum from a rat caught on the farm failed to agglutinate *L. icterohaemorrhagiae* and *L. canicola*. Cultures of rat-kidney tissue inoculated into Stuart's medium failed to produce a growth of leptospirae.

This case was of particular interest in view of the fact that one of the patient's brothers, Mr. DY, had suffered from canicola fever several years before and that serological tests carried out in 1955 during the survey of piggery workers had revealed that a third member of the family, Mr. RY, had also been infected by *L. canicola*. The results of the serological tests carried out in 1955 are shown in Table IV.

TABLE III

Duration of Illness	Agglutination Titres against	
	<i>L. icterohaemorrhagiae</i>	<i>L. canicola</i>
4 days	1 in 100	1 in 30
8 "	1 " 1,000	1 " 1,000
11 "	1 " 10,000	1 " 1,000
18 "	1 " 3,000	1 " 1,000

TABLE IV

Serum	Agglutination Titres Against	
	<i>L. canicola</i>	<i>L. icterohaemorrhagiae</i>
Mr. DY	1 in 3,000	1 in 30
Mr. RY	1 " 1,000	Negative at 1 in 10

There was therefore no doubt that identity of the infecting organism responsible for these two cases was *L. canicola*.

As shown in Table II 80% of the pig sera from farm G agglutinated *L. canicola* strongly enough to indicate recent infection. During October and November, 1958, when the third case (Mr. MY) was being investigated, 23 out of 26 pig sera had titres of 1 in 1,000 or more, while *L. icterohaemorrhagiae* agglutination did not exceed 1 in 300. The details of these serological tests are shown in Table V.

TABLE V.—*Examination of Pig Sera from Farm G During October and November, 1958*

No. of Sera Examined	Agglutination Titres					
	Against <i>L. canicola</i>				Against <i>L. icterohaemorrhagiae</i>	
	<1/300	1/300	1/1,000 to 1/3,000	1/10,000 to 1/30,000	<1/300	1/300
26	0	3	17	6	21	5

Leptospirae were seen by dark-ground microscopy in 7 out of 10 specimens of urine taken at random from pigs at farm G during October, 1958; they were seen in the urine from the bladders of two pigs whose agglutination titres against *L. canicola* were 1 in 30,000, and were also seen in three specimens of bulked pig urine from pigs in three separate pens. The organisms were isolated from one of these latter specimens by inoculating two hamsters with the untreated urine and culturing small particles of kidney tissue from both hamsters killed one week after inoculation. Leptospirae were also isolated by culturing particles of tissue from

the kidneys of two out of three pigs from this farm, chosen at random at the time of slaughter at the abattoir in October, 1958. These were identified as *L. canicola*.

In February, 1960, over one year later, five pig bloods were examined to determine whether the infection was still present in the herd. Four of the sera agglutinated *L. canicola* to a titre of 1 in 3,000 and one to 1 in 1,000. *L. icterohaemorrhagiae* was agglutinated to a slight degree by all five sera, but in only one case did the titre reach 1 in 300.

At no time had any of the pigs shown any abnormal symptoms. Whether or not Mr. MY had indeed suffered from an infection by *L. icterohaemorrhagiae* of unknown origin or whether it was a case of canicola fever with an unusual serological response, the case revealed that the pigs on farm G are maintaining a *L. canicola* infection which has probably continued for many years.

Method of Husbandry at Farm G.—On this farm there are usually 500 to 600 pigs. No breeding is done and the pigs are purchased at the age of 3 to 4 months at the local market. They are mostly kept in three large courts and when they are small there may be 75 to 100 pigs in each. As they approach bacon weight, however, they are placed in smaller pens holding approximately 5 to 10 pigs. Under this system, with so many pigs running together, it is obvious that one infected animal may spread the infection throughout the herd, and this is demonstrated by the fact that 80% of the pig sera from this farm showed evidence of active infection. The premises are in good condition and are kept very clean, but the pigs are fed on swill, which is inclined to keep the floors moist, thereby predisposing to the survival of leptospire shed in the urine of infected animals.

Farms A and B, Where Cases of Human Canicola Fever Occurred in 1955

Finally, in order to determine whether the pigs were still infected in farms A and B, where the two original outbreaks of canicola fever occurred in 1955 (Coghlan *et al.*, 1957), a few specimens of blood from pigs chosen at random were examined in February and March, 1960. The results of the agglutination tests with *L. canicola* and *L. icterohaemorrhagiae* are shown in Table VI. These results make it quite clear that at both these farms the pigs are still actively infected by *L. canicola*.

TABLE VI

Farm	No. of Sera Tested	No. of Sera giving the Following Agglutination Titres						
		Against <i>L. canicola</i>				Against <i>L. icterohaemorrhagiae</i>		
		<1/100	1/300	1/1,000 to 1/3,000	1/10,000 to 1/30,000	<1/100	1/100	1/300
A	13	1	1	6	5	6	5	2
B	10	1	0	2	7	10	0	0

Although it is possible that reinfection of the herds may have occurred from some outside sources (dogs or pigs) during the time subsequent to the previous survey, it seems more likely that the infection has been maintained during the intervening period by passage from pig to pig. It may have existed on these farms for many years prior to 1955, when the diagnosis of several cases of canicola fever in piggery workers and our failure to find any infected dogs as the source of infection finally

led us to suspect, and prove, that the pigs were acting as carriers of *L. canicola*.

These findings bear out those of our previous work, which indicated that the pig may be as effective a carrier of *L. canicola* as the dog. The infections previously found to exist in the piggeries five years ago have been shown not to be transient chance occurrences, but to have been maintained through the years, probably as a result of external conditions favourable to the survival of the leptospire. When several pigs all at the same stage of infection are excreting leptospire in their urine the organisms must be present on the floor of the pen in very large numbers, and the moist conditions which prevail in piggeries using swill will allow them to survive for a time outside the animal body, thereby constituting a hazard to any non-infected animals coming into contact with them.

It would be interesting to know if infections such as these are occurring in similarly run pig farms in other parts of the country. Evaluation of the results of serological tests should, of course, be made on a herd basis to obtain an accurate assessment of the infection rate.

Summary

Of the 16 cases of canicola fever in the Edinburgh area, 11 were found to be among pig-farm contacts. They occurred subsequent to our previous communication (Coghlan *et al.*, 1957) reporting a number of similar cases which had apparently resulted from contact with infected pigs.

Five farms were involved in the present outbreaks. A high percentage of the pigs at all of them were shown by serological tests to be probably actively infected.

Leptospira canicola was isolated from the urine and kidneys of pigs at one of the five farms where the infection appeared to have been maintained in the herd for many years.

At two farms incriminated five years ago in human outbreaks 87% of sera of pigs were found to have antibody levels high enough to signify that active infection is still present in the herd.

These observations indicate that the infection of a herd of pigs by *L. canicola* may be maintained for many years with the risk of human cases resulting from contact with the contaminated urine. This is especially apt to occur in young persons employed for the first time in work with pigs.

Similar investigations carried out on a herd basis to determine whether such infections are occurring in pig farms in other parts of the country are recommended.

Our thanks are due to Professor Robert Cruickshank for his interest in this work and for his helpful advice. We are also indebted to Dr. H. E. Seiler, M.O.H. Edinburgh, and to the clinicians who attended the cases for their interest and co-operation, also to Dr. J. P. Duguid for reading through the manuscript.

ADDENDUM.—Since this article was submitted for publication two more cases of canicola fever have occurred in recently employed piggery workers, one working on farm F (June 29, 1960) and the other on farm B (September 23, 1960).

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