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Observations on leptospiral antibodies occurring in pig sera

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INTRODUCTION

The occurrence of cases of infection with *Leptospira canicola* among workers in piggeries (Coghlan, Norval & Seiler, 1957) suggested a search for antibodies in pig sera to various leptospira. The only serotypes recognized in leptospirosis in Great Britain were *L. canicola* and *L. icterohaemorrhagiae* (Broom, 1951), which were believed to have as their hosts only the dog and the rat, respectively, until the occurrence of *L. icterohaemorrhagiae* in calves was reported in England by Field & Sellers (1950) and Ingram, Jack & Smith (1952) and in Northern Ireland by Baxter & Pearson (1956). Its occurrence in pigs in Scotland was demonstrated by Nisbet (1951). Antibodies to *L. hyos* (*mitis* Johnson) and *L. pomona* were demonstrated in dogs (Davies, 1957), to *L. pomona* in pigs (Michna, 1958) and to *L. ballum*, *L. bataviae* and *L. sejroe* in small rodents (Broom & Coghlan, 1958). The last named authors, in addition, isolated *L. ballum* from *Apodemus sylvaticus*, *Microtus hirtus* and *Clethrionomys glareolus*, and also strains antigenically related to *L. sejroe* from *M. hirtus* and *C. glareolus*.

These observations indicate the existence in this country of leptospiral serotypes, and hosts or reservoirs for them, other than those hitherto considered.

MATERIAL AND METHODS

A total of 849 samples of pig sera collected at random were examined. Of these, 518 were from animals reared in and around Edinburgh, while the remaining 331 samples were from animals from the Belfast area of Northern Ireland.

The agglutination technique of Schüffner & Mochtar (1927) was used in screening tests on all samples, at a final dilution of 1/30. The eight leptospira used as antigens were *L. canicola*, *L. icterohaemorrhagiae*, *L. grippotyphosa*, *L. hyos* (*mitis* Johnson), *L. pomona*, *L. ballum*, *L. bataviae* and *L. sejroe*. These were grown in Stuart's (1946) medium, enriched with either rabbit or new-born (pre-colostrum) calf serum, at 30° C. The cultures were formalinized at the period of maximum growth, i.e. between 7 and 10 days. All sera showing agglutination in the screening test were retested against their specific antigen, in dilutions from 1 in 10 to 1 in 3000.

RESULTS

Edinburgh area

Agglutinating sera were found in 25 of the 518 samples tested, 4·8 %. Of these reactors, 3 agglutinated with *L. canicola*, 15 with *L. icterohaemorrhagiae*, 5 with *L. ballum*, 1 with *L. pomona* and 1 with *L. sejroe*.

Belfast area

In this group, agglutinating reactions occurred in 32 of the 331 samples tested, 9·7 %. Of these, 9 agglutinated *L. icterohaemorrhagiae*, 4 *L. ballum* and 19 *L. pomona*. These observations in detail, and the serum titres, are shown in Table 1.

Table 1. *Distribution of leptospiral agglutinins in fifty-seven samples of pig serum*

		Edinburgh area	Belfast area
L. canicola	1:30	1	0
	1:100	1	0
	1:1000	1	0
L. icterohaemorrhagiae	1:30	10	5
	1:100	3	4
	1:300	1	0
	1:1000	1	0
L. ballum	1:30	3	3
	1:100	2	1
L. pomona	1:30	1	12
	1:100	0	7
L. sejroe	1:30	1	0

DISCUSSION

The interpretation of the above observations and the assessment of the possible significance of the low titre observed in the greater proportion of the samples is difficult.

In studying clinical cases a titre of 1:100 is the minimum usually accepted as being of practical significance at the time of testing, but nevertheless, lower titres are frequently encountered and some workers, notably Field & Sellers (1950) and Smith & Davidson (1936) have accepted much lower titres as evidence of past or present infection. Since none of the pigs tested were known to have shown any signs of disease, the leptospiral antibody titre of the serum would be expected to be that of the carrier state and, therefore, probably of a comparatively low level.

A second problem arising from the diversity of leptospiral serotypes encountered is that of possible cross-agglutination, especially in the lower dilutions. The possible extent of such cross-agglutination with heterologous strains of leptospira has been illustrated by Wolff & Broom (1954) who showed that cross-agglutination may occur to any extent varying from 0·3 to 100 % of the homologous titre. No

agglutinin absorption tests, such as would be necessary to eliminate this possibility, were carried out in this survey nor were any kidney tissues available for attempts to isolate leptospira.

When considered in relation to the observations of Coghlan *et al.* (1957), the results of the present survey showed a lower proportion of *L. canicola* compared with *L. icterohaemorrhagiae* reactors in the Edinburgh area. Comparing the results of the Edinburgh area with those of the Belfast area, the outstanding difference is in the high incidence of *L. pomona* in samples from Belfast. In both groups of serum samples, a comparable number showed positive results with *L. icterohaemorrhagiae*, 2·9 % in Edinburgh and 2·7 % in Belfast.

The possible significance of the two single positive reactors in the Edinburgh group of sera is difficult to assess. One of these, number 402, reacted with *L. sejroe*, while another, number 74, reacted with *L. pomona*. According to Hoag (1957) these two organisms are unrelated serologically, and therefore no cross-agglutination should be expected to occur.

The possibility of such positive reactions being false must be considered. Such false positives could be due to the presence of either antibodies to other leptospiral serotypes or to antibodies to other micro-organisms. This problem is unlikely to be solved satisfactorily, however, until further investigations show a wider incidence of leptospiral types, if these do prove to exist in Great Britain.

CONCLUSION

When considered in relation to the observations of other workers, particularly with reference to the sporadic occurrence of unsuspected serotypes, the results of this survey would seem to suggest that a greater incidence of serological types of leptospira exists than has hitherto been expected. They would also indicate that animal species other than rodents and the dog, may be serving as reservoirs for these serotypes, and that further investigation of serum of the larger animals might lead to useful results.

SUMMARY

A total of 849 samples of pig sera were tested for antibodies to *L. canicola*, *L. icterohaemorrhagiae*, *L. pomona*, *L. ballum*, *L. sejroe*, *L. grippotyphosa*, *L. hyos* (*mitis* Johnson), and *L. bataviae*. Positive results were obtained in 57 samples to all but the last three serotypes.

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REFERENCES

BAXTER, J. T. & PEARSON, J. K. L. (1956). *Vet. Rec.* **68**, 6.
BROOM, J. C. (1951). *Brit. med. J.* ii, 689.
BROOM, J. C. & COGHLAN, J. D. (1958). *Lancet*, ii, 1041.
COGHLAN, J. D., NORVAL, J. & SEILER, H. E. (1957). *Brit. med. J.* i, 257.
DAVIES, M. E. (1957). *Vet. Rec.* **69**, 93.
FIELD, H. I. & SELLERS, K. C. (1950). *Vet. Rec.* **62**, 311.
HOAG, W. G. (1957). *Amer. J. vet. Res.* **18**, 50.
INGRAM, P. L., JACK, E. J. & SMITH, J. E. (1952). *Vet. Rec.* **64**, 865.
MICHNA, S. W. (1958). *Vet. Rec.* **70**, 80.
NISBET, D. I. (1951). *J. comp. Path.* **61**, 155.
SCHÜFFNER, W. & MOCHTAR, A. (1927). *Zbl. Bakt.* **101**, 405.
SMITH, J. & DAVIDSON, L. S. P. (1936). *J. Hyg., Camb.*, **36**, 438.
STUART, R. D. (1946). *J. Path. Bact.* **58**, 343.
WOLFF, J. W. & BROOM, J. C. (1954). *Docum. med. Geogr.* **6**, 78.