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The comparative susceptibility of hysterectomy-produced, colostrum-deprived pigs and naturally born, enzootic-pneumonia-free pigs to enzootic pneumonia

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SUMMARY

Hysterectomy-produced, colostrum-deprived (HPCD) pigs and naturally born, enzootic-pneumonia-free (EPF) pigs were compared with respect to their susceptibility to two strains of enzootic pneumonia induced by intranasal inoculation of suspensions of ground pneumonic tissue. All but one of the HPCD pigs developed enzootic pneumonia, whereas the EPF pigs commonly failed to develop the disease; secondly, the pneumonic lesions were more extensive in the HPCD pigs.

When the dose of inoculum was increased in EPF pigs, the resulting pneumonic areas were larger.

In a small, in-contact experiment the disease was also more readily transmitted to HPCD pigs than to EPF pigs.

INTRODUCTION

The hysterectomy-produced, colostrum-deprived (HPCD) pig is particularly useful in research on infectious diseases, and such animals were used extensively in this laboratory to elucidate the aetiology of enzootic pneumonia. Now that the disease has been shown to be caused by *Mycoplasma suipneumoniae* (Goodwin, Pomeroy & Whittlestone, 1965, 1967), other aspects of this important condition – such as its epidemiology and immunology – can be studied more precisely. Although we have already used HPCD pigs to investigate some aspects of natural and artificial immunity in enzootic pneumonia (Goodwin, Hodgson, Whittlestone & Woodhams, 1969*a*, *b*), these animals are clearly different from naturally born pigs, and they may give misleading results if used in experiments designed to elucidate the behaviour of the disease in the field. Furthermore, HPCD pigs are expensive to produce and maintain in strict isolation, and hence naturally born pigs from an enzootic-pneumonia-free herd (EPF pigs) could have a cost advantage if large groups were to be used experimentally. Before using EPF pigs in greater numbers, however, we wished to know how they compared with HPCD pigs in their susceptibility to enzootic pneumonia.

MATERIALS AND METHODS

The HPCD pigs were produced in a manner close to that described by Betts, Lamont & Littlewort (1960). The EPF pigs came from two herds: both of these were formed originally from HPCD pigs and their offspring, and they had been maintained as closed, isolated herds thereafter. The lungs of routinely slaughtered pigs from both herds are regularly checked, and they have been found to be free from lesions of enzootic pneumonia. All the pigs were housed during experiments in a specially designed isolation building, as previously described (Goodwin, Pomeroy & Whittlestone, 1968).

Two strains of enzootic pneumonia were used: the J strain (Goodwin & Whittlestone, 1963) and the CZ strain (Goodwin *et al.* 1968). The pigs were inoculated by slow intranasal instillation of suspensions of ground pneumonic lesions in broth. The diagnosis of enzootic pneumonia in the experimental cases was based on the examination of touch preparations for organisms with the morphology of *M. suipneumoniae* (Whittlestone, 1967) and on the nature of the gross lesions and the histological picture as previously summarized (Goodwin *et al.* 1969*a*). In addition, 11 cases of pneumonia induced with the J strain and 12 cases induced with the CZ strain were cultured in other work (Goodwin *et al.* 1968); all the 11 J cases and 10 of the CZ cases yielded *M. suipneumoniae*, but no other mycoplasmas. The scoring system for recording the extent of the consolidated lesions was related to the fact that, in enzootic pneumonia, such lesions occur almost entirely in the apical and cardiac lobes of the lung, in the intermediate lobe, and the leading edges of the diaphragmatic lobes. Ten points were allocated to each apical or cardiac lobe, five points to the intermediate lobe and five points to each leading edge of the diaphragmatic lobes; thus, if all this tissue were totally consolidated (which would be an unusually severe case) the pneumonic score would be 55.

RESULTS

Inoculation of HPCD pigs

A total of 42 pigs were infected in a series of 24 separate experiments, using 18 different litters; 36 of these pigs received the J strain, and 6 received the CZ strain of enzootic pneumonia. The dose ranges and the scores for the macroscopic lesions produced are shown in Table 1. Only one pig failed to develop any macroscopic lesions; this was one of the four pigs given the smallest dose. The remainder all showed lesions which were confirmed as cases of enzootic pneumonia microscopically, although in eight of the pigs there was also some foreign-body giant-cell reaction. The average pneumonic score for all 42 pigs was 13·4. Hence, the HPCD pigs almost invariably developed macroscopic lesions and, in the main, substantial areas of the lungs were affected.

The average score for the CZ strain pneumonias was 23 but, as only six pigs were involved, and as they received some of the biggest doses, it would not be justifiable to conclude that the CZ strain was more pathogenic.

If the dose of lung inoculated into individual pigs is plotted against the pneu-

monic score, there is no clear dosage effect. However, as the four pigs which received the lowest dose (0·03–0·04 g. of pneumonic tissue) had a mean score of 5, and as there was a slight tendency for the mean score to increase with dose in the other three groups, this might indicate some relationship between the dose given intranasally and the extent of the lesions. This suggestion was taken up later, by observing the effect of increasing the dose of inoculum in EPF pigs.

Table 1. *Relation between dose of pneumonic tissue and extent of lesions in 42 hysterectomy-produced, colostrum-deprived pigs*

Dose range of tissue (g.)	No. of pigs	Lesion score*	
		Range	Average
0·03–0·04	4†	0–8·5	5
0·15–0·5	20	2–38	13·1
	(2)	(21–32)	(26·5)
0·58–1·17	12	1–27	14·4
2·67–8·0	6	7–34	17·8
	(4)	(7–34)	(21·7)

The figures in parentheses refer only to pigs inoculated with the CZ strain. The main figures refer to all the pigs; that is, those given either the CZ strain or the J strain. Apart from two pigs inoculated with the J strain in the group of 20, which were killed after 12 days, all the pigs were killed between 16 and 26 days after inoculation.

* See Materials and Methods. † One pig had no pneumonia when killed.

Inoculation of EPF pigs

In the first part of the work using EPF pigs, 33 animals were inoculated in eight separate experiments; 26 pigs received the CZ strain, and 7 received the J strain of enzootic pneumonia. The dose ranges, the scores for the macroscopic lesions produced, and the time intervals between infection and slaughter are shown in Table 2. It can be seen that 10 pigs did not have gross pneumonic lesions when killed; the gross lesions in the other 23 were confirmed as cases of enzootic pneumonia microscopically. Although 12 of these 33 pigs were killed longer after infection than any of the HPCD pigs in Table 1, four of the six EPF pigs with pneumonia killed late still had quite active lesions, and the six pigs without lung lesions showed no indication of any previous lesions that might have healed completely by the time they were killed; furthermore, there were more negative results among the six pigs killed after 45 days than among those killed after 61 days. The average score for all 33 pigs was 6·2, the average score for the 23 pigs showing lesions was 8·9, and the average score for the 21 pigs that were killed at similar intervals after inoculation to the HPCD pigs in Table 1 was 7·4. Thus, only 70 % of the EPF pigs had macroscopic lesions, and for those that were positive and killed at a comparable time after inoculation to the pigs in Table 1, the average score was only 69 % of the mean score for all the HPCD pigs. As the incidence and extent of the lesions in EPF pigs was less than is desirable in experimental work, an attempt was made to increase both the incidence and the extent of the lesions by giving larger doses of inoculum.

Effect of increasing dose

Pigs from three litters were distributed into three groups, which were given increasing doses of inoculum prepared from the J strain of enzootic pneumonia (Table 3). The inoculum for each pig in group 2 was divided into two doses which were given 1½ hr. apart; the inoculum for each pig in group 3 was divided into three doses, which were given with 1½ hr. intervals. It can be seen that as the dose of the inoculum was increased, the average score for the extent of the lesions also

Table 2. *Relation between dose of pneumonic tissue and extent of lesions in 33 naturally born, enzootic-pneumonia-free pigs*

Dose range of tissue (g.)	No. of pigs	Post infection interval when killed (days)	Pigs without pneumonia	Lesion score*	
				Range	Average
0·01–0·04	5	18–19	1	0–18·5	8·5
0·55–1·14	4	17–19	1	0–7	2·3
3·0–4·33	12	19–20	2	0–27·5	8·8
	6	45	4	0–23	6·1
	6	61	2	0–8	1·9

* See Materials and Methods.

Table 3. *Effect of increasing the dose of inoculum on the extent of pneumonic lesions in naturally born, enzootic-pneumonia-free pigs*

Group	Dose ranges of inoculum		No. of pigs	Lesion score*	
	Volume (ml.)	Tissue (g.)		Range	Average
1	3·5–5	0·58–0·83	4†	0–16·5	5·9
2	19–25	3·17–4·17	4	6–10·5	7·4
3	62–66	10·33–11·0	3	3·5–16	11·7

All the pigs received the same inoculum, a 1 in 6 dilution of the J strain, and all were killed 20 days later. Two uninoculated control pigs killed on the same day had no pneumonia.

* See Materials and Methods. † One pig had no pneumonia when killed.

increased. Even so, when very large doses of inoculum were given (average 64 ml. containing 10·6 g. of pneumonic tissue) the lesions were not quite as extensive as the average for all the HPCD pigs in Table 1. All the lung lesions were confirmed as cases of enzootic pneumonia microscopically.

Inoculation of HPCD and EPF pigs in parallel

While the work summarized in Tables 1 and 2 was still in progress, it was becoming apparent that there was probably a substantial difference in susceptibility between HPCD pigs and EPF pigs, and this was confirmed when the two series were completed. In the meantime, we wished to establish that a similar difference occurred when HPCD and EPF pigs were inoculated in parallel on the same day with the same inoculum under similar experimental conditions.

A comparison of this type was obtained by giving 10 ml. of the inoculum used for

the EPF pigs in Table 3 to two HPCD pigs in parallel. When killed on the same day as the EPF pigs, their lesions (which were confirmed as enzootic pneumonia microscopically) had scores of 16 and 18, giving a mean of 17. This was considerably greater than the average for any of the EPF groups, even those that received over 60 ml. of the same inoculum, although three out of the 11 EPF pigs had lesions of about this extent. This experiment confirmed the difference in susceptibility to the J strain of enzootic pneumonia, and a similar comparison was made using the CZ strain.

Table 4. *Parallel inoculation of hysterectomy-produced, colostrum-deprived pigs and naturally born, enzootic-pneumonia-free pigs, with subsequent in-contact exposure of pigs of the same type*

HPCD pigs			EPF pigs		
Pig no.	Lesion score	Days after infection when killed	Pig no.	Lesion score	Days after infection when killed
2910	21	21	2912	1·5	21
2911	32		2913	6	
2932	3·5	59	2922	5·5	31
2933	1·5		2923	3	
2917	0	Controls*	2936	1·5	62
2918	0		2937	2	
			2939	0·5	
			2941	9	
			2924	0	Control†
	In contact			In contact	
2930	0	23‡	2934	0	29‡
2931	17	37‡	2935	1	
			2938	0	
			2940	0	

The scoring system for lesions is described under Materials and Methods.
* Killed on same day as 2910 and 2911.
† Killed two days after 2922 and 2923.
‡ Days after first contact when killed.

In this other experiment not only were HPCD pigs inoculated in parallel with EPF pigs, but a comparison was made of the natural transmission of the disease in these two types of pig. For the primary infection all the pigs received 0·5 g. of pneumonic tissue as 3 ml. of a 1 in 6 dilution. Two pigs from each group were killed after 21 days, when the HPCD pigs were found to have much more extensive lesions (Table 4); all four cases were confirmed as enzootic pneumonia microscopically, as were the other cases in this experiment, apart from 2941 (see below) and 2939. Although the results for the 12 pigs killed after 45 and 61 days in Table 2 had indicated that the pneumonic lesions in EPF pigs were not less extensive because they developed more slowly – so that they might become comparable with the lesions in HPCD pigs at a different time after infection – this possibility was re-examined by killing two more HPCD pigs at 59 days, two more EPF pigs at 31 days, and a further four EPF pigs at 62 days (see Table 4). Again, there was no

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evidence to suggest a delayed development of more extensive lesions of enzootic pneumonia in EPF pigs, for the pneumonic lesions in pig 2941 did not, for the most part, resemble enzootic pneumonia microscopically. The pneumonic lesions in the others remained small despite the longer post-inoculation intervals.

Having established that EPF pigs were considerably less susceptible to a single intranasal inoculation, we wished to see whether this difference also obtained with in-contact infection, for it had been observed in the field that enzootic pneumonia was often not a highly infective disease, in that apparently susceptible pigs could be housed with affected pigs for varying periods of time without developing pneumonia.

Two HPCD pigs (2930 and 2931) were added to the isolation room the day following the removal of pigs 2910 and 2911; they were thus living continuously with pigs 2932 and 2933 for 23 and 37 days, respectively (Table 4). One of the two pigs housed in contact (2931) developed substantial areas of enzootic pneumonia; the other pig (2930), although having no gross lesions, had a catarrhal tracheal exudate containing organisms with the morphology of *M. suipneumoniae*.

Four EPF pigs (2934, 2935, 2938 and 2940) were added to the second isolation room 2 days after the removal of pigs 2922 and 2923; they were thus closely housed with the EPF pigs 2936, 2937, 2939 and 2941 for 29 days before being killed (Table 4). Only one of the four added pigs (2935) had any macroscopic lesions; these were two very small areas in each cardiac lobe; the histological picture was not inconsistent with the early changes in enzootic pneumonia, but no mycoplasmas could be seen in touch preparations. The low degree of transmission in the pigs placed in contact, if indeed enzootic pneumonia had been induced in these pigs, could not be explained by the presence of inactive lesions in the experimentally inoculated pigs; for the two pigs (2922 and 2923) that were probably representative of the degree of infection remaining in the inoculated EPF group showed active lesions histologically with many organisms of the *M. suipneumoniae* type in touch preparations.

DISCUSSION

These experiments show a considerable difference between HPCD pigs and EPF pigs in their susceptibility to intranasal inoculation with two strains of enzootic pneumonia. First, HPCD pigs almost invariably developed gross lung lesions, whereas the failure rate in EPF pigs was considerably higher; secondly, the pneumonic areas were more extensive in the HPCD pigs. It is clearly important to appreciate this difference in susceptibility to intranasal inoculation in experimental work, particularly in studies concerned with natural or artificial protection against this disease; for a degree of immunity or protection that might be inadequate for the more susceptible HPCD pig could be sufficient for the more natural EPF pig.

It seems that the increased resistance of EPF pigs might be partly overcome by increasing the dose of intranasal inoculum, but such heavy dosing could soon exhaust stocks of tested pneumonic tissue.

Why the respiratory tract of EPF pigs should be more resistant to enzootic pneumonia is not clear. Although it is theoretically possible that the herds which

supplied the EPF pigs had at some time been infected subclinically with *M. suipneumoniae*, there is no evidence to suggest that such infection had occurred and both the herds are still free from enzootic pneumonia clinically and pathologically. It is more likely therefore to be a non-specific effect, possibly associated with natural suckling or with some other aspect of the more normal rearing of these animals.

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REFERENCES

BETTS, A. O., LAMONT, P. H. & LITTLEWORT, M. C. G. (1960). The production by hysterectomy of pathogen-free, colostrum-deprived pigs and the foundation of a minimal-disease herd. *Veterinary Record* **72**, 461.

GOODWIN, R. F. W., HODGSON, R. G., WHITTLESTONE, P. & WOODHAMS, R. L. (1969*a*). Immunity in experimentally induced enzootic pneumonia of pigs. *Journal of Hygiene* **67**, 193.

GOODWIN, R. F. W., HODGSON, R. G., WHITTLESTONE, P. & WOODHAMS, R. L. (1969*b*). Some experiments relating to artificial immunity in enzootic pneumonia of pigs. *Journal of Hygiene* **67**, 465.

GOODWIN, R. F. W., POMEROY, A. P. & WHITTLESTONE, P. (1965). Production of enzootic pneumonia in pigs with a mycoplasma. *Veterinary Record* **77**, 1247.

GOODWIN, R. F. W., POMEROY, A. P. & WHITTLESTONE, P. (1967). Characterization of *Mycoplasma suipneumoniae*: a mycoplasma causing enzootic pneumonia of pigs. *Journal of Hygiene* **65**, 85.

GOODWIN, R. F. W., POMEROY, A. P. & WHITTLESTONE, P. (1968). Attempts to recover *Mycoplasma suipneumoniae* from experimental and natural cases of enzootic pneumonia in pigs. *Journal of Hygiene* **66**, 595.

GOODWIN, R. F. W. & WHITTLESTONE, P. (1963). Production of enzootic pneumonia in pigs with an agent grown in tissue culture from the natural disease. *British Journal of Experimental Pathology* **44**, 291.

WHITTLESTONE, P. (1967). Mycoplasma in enzootic pneumonia of pigs. *Annals of the New York Academy of Sciences* **143** (art. 1), 271.