OBSERVATIONS ON PLEUROPNEUMONIA-LIKE ORGANISMS RECOVERED FROM SWINE WITH INFECTIOUS ATROPHIC RHINITIS AND GLASSER'S DISEASE

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The isolation of pleuropneumonia-like organisms (PPLO) from the nasal passages of pigs affected with infectious atrophic rhinitis (IAR) was reported recently (1). Since that report a number of baby pigs have been exposed to suspensions of PPLO with a view to determining what role if any they have in IAR.

Because these experiments were negative they have been summarized briefly in Table 1. By filtrate culture is meant a 10-20 per cent serum-enriched thioglycollate culture initiated with a filtrate obtained in the manner described previously (1). The PPLO suspensions employed in Experiments C and D were secondary broth cultures. No attempt was made to recover the PPLO from the exposed pigs.

TABLE 1

DETAILS OF EXPERIMENTS IN WHICH BABY PIGS WERE EXPOSED VIA THE NASAL PASSAGES TO THE PPLO

Expt. No.	Instilled with*	No. of Pigs	age at Exposure	Age Examined	Results
A	Filtrate culture	5	5 days	41 days	Negative
B	Solid medium culture	4	7 days	36 days	Negative
C	*Past. multocida*+PPLO	6	5 days	68 days	Negative
D	*Past. multocida*+PPLO	5	5 days	70 days	Negative

*Only one instillation given throughout

It became evident during the investigation that the media employed for the propagation of the swine strains of PPLO were not completely adequate. Without the use of fresh brewer's yeast extract the larger, characteristic PPLO colonies were not regularly seen. The medium which gave the best results was essentially the same as that described by Edward (2).

With this improved medium PPLO were isolated from pigs with a disease which appeared to be identical with that described by Glasser (3). The affected pigs were from three different herds in which Glasser's disease had ap-

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peared. Unfortunately complete histories were not available on each herd; however, the data presented below will give some indication of the clinical nature of this disease.

Herd 719.—The farmer lost 10 out of 26 pigs approximately one month old. The principal symptoms were anorexia, laboured breathing, lameness, depression and some scouring. Two pigs were presented for necropsy, 719 (1) and 719 (2). *Herd* 762.—One hundred and fifty pigs ranging in age from two to four months were involved. The majority of them were sick and the owner

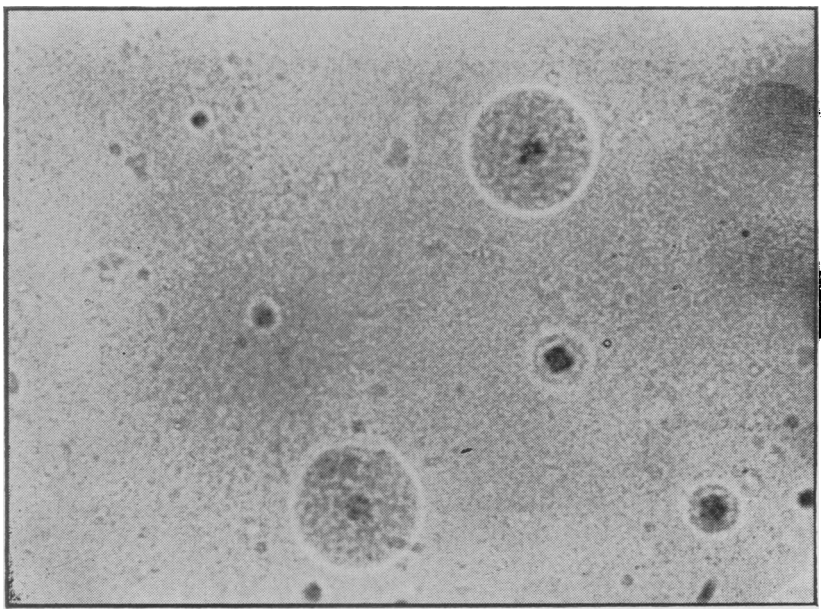


FIG. I

Colonies of the PPLO after incubation for three days. Transmitted light, X120.

believed their illness was a sequel to worming. The principal symptoms were coughing and difficult respiration. Only three of the pigs had died at the time one (762) was submitted for examination. *Herd* 817.—Nine pigs out of 36 in the age range of two to four months were affected. They had recently been weaned and the nutrition was considered poor. Two pigs died but the remainder responded to improved nutrition and antibiotic-sulphonamide therapy. One animal (817) was submitted for necropsy.

PPLO were isolated from lesions in the four pigs referred to above. The sources of the isolations were: 719 (1)—lung; 719 (2)—lung; 762—spleen, lung and epicardium; and 817—lung, heart's blood, and epicardium. The lesions observed were: 719 (1), 719 (2) and 762—confluent bronchopneumonia, peritonitis and pericarditis; and 817—bronchopneumonia, pericarditis

and peritonitis. The inflammation observed was characterized by an abundant fibrinous exudate.

Characteristic PPLO colonies appeared on the serum-agar plates after 72 hours' incubation (see Fig. 1). They were indistinguishable structurally from those obtained from the nasal passages of swine. That they represented PPLO and not *Hemophilus suis* was attested by the fact that characteristic *Hemophilus* colonies did not appear beside staphylococcal colonies on blood agar, and bacterial elements were not demonstrable in cultures from either solid or fluid media.

TABLE 2

DETAILS OF INOCULATIONS OF PPLO FROM CASES OF GLASSER'S DISEASE

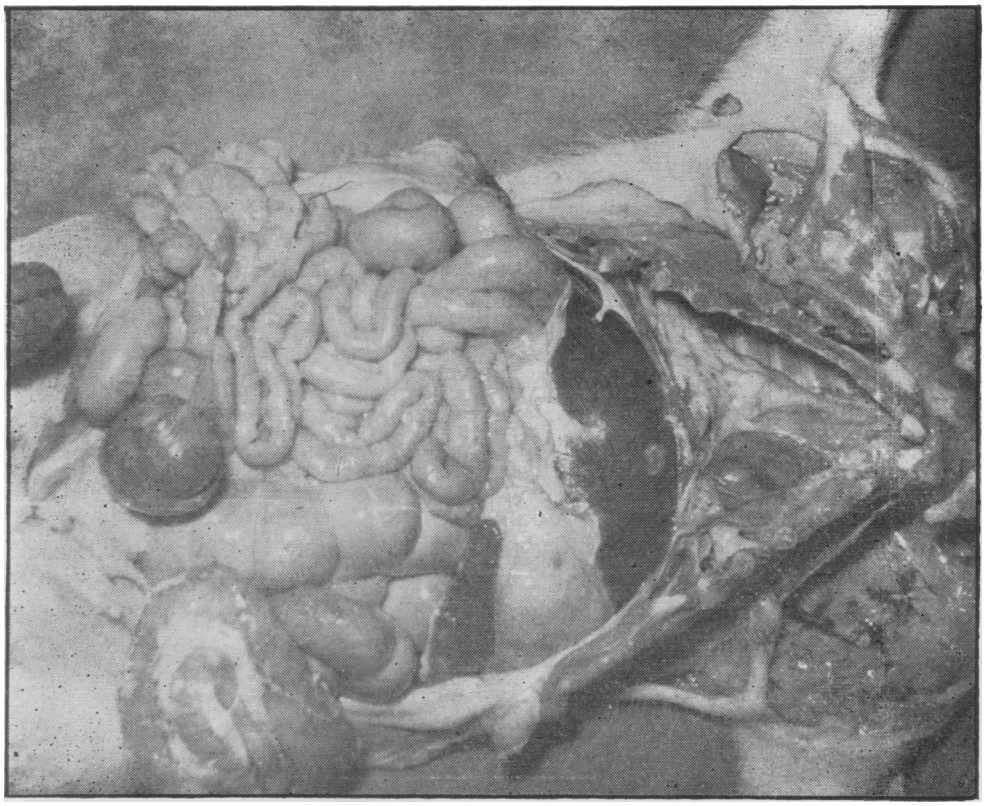
Strain Injected	Route of Inoculation	Dose	No. of Pigs and Age
719 (lung)	intraperitoneal	5.0 ml.	2 — 36 days 1 — 48 days
762 (spleen)	intraperitoneal	5.0 ml.	4 — 48 days

An experiment was conducted in which three pigs were inoculated with one of the PPLO cultures and four pigs were injected with another. The experimental subjects were taken from a herd with no history of Glasser's disease. The cultures employed were initiated from the primary 20 per cent horse serum-agar plates with an inoculum consisting of several colonies on a section of agar. The 719(1) lung culture was incubated 48 hours and the 762 spleen culture was incubated for 72 hours. After incubation all of the cultures employed were checked for purity. In none of the tubes was bacterial contamination observed. Further details of the experiment are given in Table 2.

Within 48 hours of the inoculation of the PPLO all of the pigs but one showed temperatures ranging from 104.6°F. to 105.8°F. Twenty-four hours after the inoculations the pigs were noticeably depressed and showed little interest in feeding. At 48 hours post-inoculation they refused to eat and breathed with difficulty. It was not until the seventh day that they began to eat and move around normally. By this time their temperatures had returned to normal.

One pig was necropsied at six days after inoculation and displayed a severe serofibrinous inflammation involving the serous membranes of the thoracic and abdominal cavities (see Fig. 2). The remaining six killed on the ninth, tenth and eleventh days showed similar lesions, and definite adhesions were observed between the pericardium and epicardium, and between the peri-

FIGURE II



Pig 719A killed six days after inoculation with the PPLO. of the spleen and liver.

toneum covering the liver and the contiguous parietal peritoneum. There was some inflammatory swelling of the synovial membrane but lameness was not observed. The PPLO was recovered from all of the experimental subjects.

A second experiment was conducted for the purpose of determining whether or not the infectious agent was filterable. Peritoneal washings were made with normal saline of experimental pig 719A. Fifty millilitres of the washings were filtered through an 02 Selas candle under vacuum which at no time exceeded five pounds. Each of the three pigs, approximately two months of age, received 10.0 ml. of the filtrate via the intraperitoneal route. The symptoms and lesions exhibited by two of the pigs were essentially the same as those observed in the experimental subjects listed in Table 3. The remaining pig was normal after seven days' observation. The interval between inoculation and onset of the disease was longer than in the first experiment, and this was probably due to the smaller number of PPLO. The incubation periods were four and five days.

In experiment 3, three pigs approximately two months of age were placed with the pigs of the first experiment, after the latter had contracted Glasser's disease. After a period of observation of eight days there was no evidence that the disease had spread to the three normal contacts.

DISCUSSION

It would appear beyond doubt that the disease produced in the nine pigs was Glasser s disease. However, it seems unlikely that the organism referred to by Hjane and Wramby (4) as the cause of the disease is identical with the PPLO of this study. There is considerable evidence that the filterable agents of Switzer (5, 6) and McNutt, Leith and Underjberg (7) are identical with the agent referred to in this study.

If the PPLO recovered from cases of Glasser's disease is the same as the PPLO isolated from the nasal passages of swine—and it would appear that such is the case in view of Switzer's findings—we can infer in a speculative way some interesting epizoological facts about this disease. The organism is apparently found occasionally in the respiratory passages of normal as well as rhinitis-affected swine. Natural infection would appear to be the result of some predisposing factor such as weaning and poor nutrition, or shipment as pointed out by Hutyra, Marek and Manninger (8). That such is the case is suggested by the failure to transmit the disease to normal contact pigs.

Severe arthritis and meningitis were not observed in any of the experimental subjects. McNutt and associates observed that the predilection their agent evinced for certain sites varied under different circumstances.

If we assume, as we have done above, that the PPLO is identical with Switzer's agent, then the antibiotic studies carried out by him on infected chicken embryos may have practical significance in this disease. The antibiotics which he tested are listed in order of their activity: terramycin, aureomycin and streptomycin. Penicillin and bacitracin were ineffective in the amounts used. Good results have been reported in the field with sulfonamide therapy.

SUMMARY

1. Baby pigs instilled with cultures of PPLO from rhinitis-affected swine did not develop infectious atrophic, rhinitis.

2. PPLO were isolated from four pigs from three different outbreaks of Glasser's disease in swine.

3. Lesions characteristic of Glasser's disease, viz., serofibrinous pericarditis, pleuritis and peritonitis were produced in seven pigs which received secondary cultures of the PPLO intraperitoneally. PPLO were recovered from all of the experimental subjects after necropsy.

4. A bacteria-free filtrate of the peritoneal washings of one of the pigs

with the experimental disease produced the characteristic disease in two of three pigs.

5. The disease was not spread from three experimentally infected pigs to three normal subjects after contact for a period of eight days.

6. It is suggested that the PPLO is identical with the filterable agents referred to by other workers.

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REFERENCES

(1) CARTER, G. R. A pleuropneumonia-like organism associated with infectious atrophic rhinitis of swine. Can. J. Comp. Med. 17: 413-416, 1953.

(2) EDWARD, D. G. A selective medium for pleuropneumonia-like organisms. J. Gen. Microbiol. 1: 238-243, 1947.

(3) GLASSER, K., HUPKA, E., and WETZEL, R. Die Krankheiten des Schweines, Fifth Ed. Verlag, M. and H. Schaper, Hanover, 1950.

(4) HJARRE, A. and WRAMBY, G. Om fibrinos serosa-ledinflammation (Glasser) hos svin. Skan . Vet. Tidskr. 32: 257-289, 1942. (Abstract, Vet. Bull. 13; 5, 1943.)

(5) SWITZER, W. P. Studies on infectious atrophic rhinitis of swine. I. Isolation of a filterable agent from the nasal cavity of swine with infectious atrophic rhinitis. J. Am. Vet. Med. Assoc. 123: 45-47, 1953.

(6) SWITZER, W. P. Studies on infectious atrophic rhinitis of swine. II. Intraperitoneal and intra nasal inoculation of young pigs with a filterable agent isolated from nasal mucosa of swine. Vet. Med. 98: 392-394, 1953.

(7) MCNUTT, S. H. and LEITH, T. S. and UNDERBJERG, G. K. An active agent isolated from hogs affected with arthritis (preliminary report). Am. J. Vet. Res. 6: 247-251, 1945.

(8) HUTYRA, F., MAREK, J. and MANNINGER, R. Special Pathology and Therapeutics of the Diseases of Domestic Animals, Fifth English Ed. Alexander Eger, Chicago, 1938.