Studies on Pathogenic Porcine Enteroviruses

I. Preliminary Investigations

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Introduction

Following reports (1) on the occurrence of a disease of suckling piglets in Southwestern Ontario during the Fall of 1957, a similar condition was encountered in Eastern Ontario commencing in the Spring of 1958 (2). At this time twelve outbreaks were investigated and others were reported by veterinary practitioners. In all cases the symptoms resembled those described by Roe and Alexander in their original paper (3), viz. vomiting, anorexia, constipation, progressive wasting and death. At that time no pathognomonic lesions were found in the affected piglets examined and limited transmission studies indicated that the condition was distinct from transmissible gastro-enteritis (T.G.E.). No further outbreaks were investigated until April of 1959 when lesions of a viral polioencephalomyelitis were observed in the brains of two piglets from an affected litter. Subsequent papers by Alexander et al (4) and Richards and Savan (5) described the finding of a viral encephalomyelitis in 84 piglets from 44 herds in Southern Ontario. They stated that the disease was characterized by high morbidity and mortality and that affected piglets showed marked clinical signs of a central nervous system disturbance. The authors suggested the cause to be a virus and after further study they proposed that the disease belonged to the same group of non-suppurative encephalomyelitides of swine as Teschen disease, Talfan disease and poliomyelitis suum.

Since our initial observation of lesions indicative of a viral polioencephalomyelitis a number of outbreaks of disease in pigs, where the clinical histories were suggestive of this condition, have been investigated in more detail. The purpose of this paper is to describe and discuss preliminary findings with respect to the clinical history, histopathology and experimental transmission of this disease in five herds.

A project was initiated with the object of attempting to isolate a causative agent from outbreaks of the disease in the Ottawa area and also to investigate the serological relationship between this and other porcine enteroviruses. The isolation of cytopathogenic agents from four of these outbreaks and the results of virus neutralization tests in tissue culture will be reported in later papers (6, 7).

Clinical Histories

Herd 1

This outbreak occurred in a herd containing seven sows on a small mixed farm. An old boar had been sold in January and a young boar brought in on March 27 as a replacement. This boar had a slight cough and nasal discharge when purchased and had been kept apart from the sows.

On April 2, 1959, sow A farrowed ten piglets and on April 6 this litter suddenly became ill exhibiting anorexia, diarrhea, shivering and vomiting. The whole litter died within three days following the onset of symptoms. On April 6, sow B farrowed fourteen apparently normal piglets. Three were crushed by the sow shortly after birth. On April 12 the remaining 11 developed symptoms similar to those seen in the first litter although vomiting was not so marked. Two of these piglets died on April 12 and on the next day the two most severely affected of the survivors were submitted for laboratory examination. The remaining seven survived until April 18 but showed rapid, progressive emaciation, ataxia, diarrhea and excessive thirst. Six more died during the next three days and on April 21 the sole survivor and one which had died the previous evening were submitted to the laboratory. At no time did the temperature of any of the affected piglets exceed 103.6°F.

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Sow C farrowed nine piglets on April 9 in a pen adjacent to sow A and despite the fact that this litter suckled sow A on April 15 they remained normal and healthy. Sow D farrowed eleven piglets on April 11 in a pen adjacent to sow B and with the exception of a single runt all the piglets in this litter remained normal and healthy. Sow E had previously farrowed a litter of ten piglets on February 17 and these were weaned normally on April 16. On April 16 this litter developed diarrhea and slight inappetance. A simple astringent was administered and their condition rapidly returned to normal. The other two sows in the herd were running outside and had not been bred at the time of the outbreak. This herd has been under periodic scrutiny since but no further trouble of this nature has occurred.

Herd 2

This herd contained fourteen sows and the owner reported that he had bought 24 newly weaned pigs at a local livestock auction two weeks prior to the first visit on April 17, 1960.

On April 6, a sow farrowed 12 piglets; all were healthy until the evening of April 13 when the owner noticed that they appeared gaunt and huddled in a corner. The next day he took two of these piglets to a local veterinarian, their temperatures were 103.5 and 101°F. respectively at this time and one of the piglets showed marked ataxia. The following day two of the piglets from the affected litter were submitted for laboratory examination. During the next three days six of the litter died having shown only symptoms of emaciation and ataxia. The four survivors were submitted to the laboratory on April 17 (Figure 1). These four animals were put on an artificial milk diet but died on April 20, May 4, May 9 and May 11 respectively. Daily temperatures of these animals were recorded but never exceeded 104°F.

Three other sows farrowed in pens adjacent to the affected litter on March 16, March 17 and April 23 respectively; these litters remained healthy. No further evidence of this condition has been seen in this herd.

Herd 3

There was only a single sow on this farm which had no direct contact with other pigs. The owner reported that he worked at a local livestock auction.

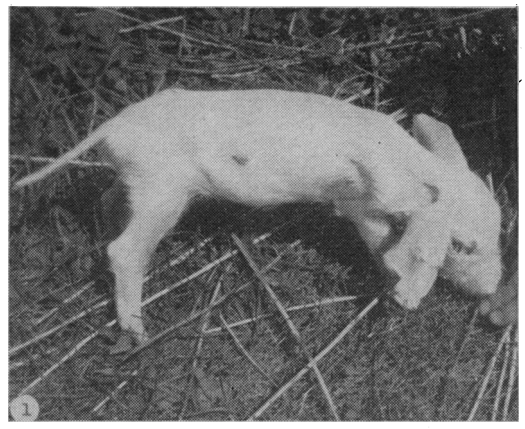


Fig. 1. Naturally affected piglet (Herd 2) — four days after onset of symptoms.

On June 6, 1960, this sow farrowed fourteen live piglets all of which were apparently normal until July 12 when one piglet was found dead. The next day a second piglet died and it was noticed that the remainder of the litter were shivering and one had developed posterior paralysis. On July 14, two of the piglets were submitted to the laboratory, one of these was alive and one dead. During the following four days all but five of the survivors died. Another death occurred on July 27 and two of the remaining four were submitted alive to the laboratory on August 3 but died a few days after admission. On August 22, the two survivors were brought to the laboratory for observation. They were placed in isolation and challenged on September 19 with Teschen disease virus. Both of these animals developed typical symptoms of Teschen disease and examination of the brains and spinal cords revealed lesions of a polioencephalomyelitis.

Herd 4

There were five sows in this herd, one had recently weaned a normal litter and one had not been bred at the time of the outbreak.

On July 29, 1960, two sows farrowed litters of eleven piglets each (litters A and B). On July 30, a third sow farrowed 12 piglets (litter C). On August 5 litter A was noticed shivering, gaunt and apparently not suckling. The next day litter B was noticed to be similarly affected and on August 14 litter C also became ill. One af-

fected piglet was seen by the owner to have a convulsion and to vomit. Although frothing at the mouth was observed in the other piglets no vomiting occurred. All the piglets in litters A and B died within three weeks of the onset of symptoms, the majority dying within the first five days. Five piglets in litter C survived but all showed a marked interruption in growth rate.

All three of these sows had been bred by a neighbour's boar but this was the only direct contact with other pigs. Seven of the affected piglets were submitted for laboratory examination. No further evidence of this condition has been reported in this herd.

Herd 5

There were thirteen sows in this herd at the time of the outbreak.

On October 24, 1960, seven litters containing a total of seventy-five piglets between five and ten days of age suddenly became ill. They huddled in a corner, did not appear keen to suckle and when handled squealed loudly. A roughness of the coat, shivering, ataxia and a degree of hyperaesthaesia were the salient symptoms observed. The piglets in one litter were seen to vomit but no diarrhea occurred and none of the sows were affected. The two youngest litters all died within three days after the appearance of symptoms. The first piglets died within 36 hours. By November 3rd only 19 of the 75 piglets were alive and on November 9th all but four had died. These four survivors were taken to the laboratory on that date and sacrificed. A total of nine affected piglets were submitted for laboratory examination.

A litter born on October 30 and litters born on November 29 and December 9 have remained healthy. No pigs have been brought on to this premises for some years but the owner does transport his boar to several neighbouring farms to breed sows. Two eight week old litters were sold a few days prior to the outbreak.

Summary

Of the forty sows in these five herds fourteen had litters which contracted the disease. These litters contained 156 normal piglets prior to the onset of symptoms and only seven of these survived. As can be seen the only constant findings were the sudden onset, shivering or trembling, variable degrees of ataxia and incoordination and a rapid progressive loss of condition. Marked signs of a central nervous system (CNS) disturbance were seen in only a small number of piglets although many developed a somewhat stiff, tip-toeing gait suggesting the presence of a degree of brain or cord involvement. Vomiting occurred in four litters, frothing at the mouth in two litters and diarrhea in three litters. None of the sows appeared to be affected and several have given birth to one or more normal litters since.

Materials and Methods

Pathology

A total of twenty-four piglets were received, twenty-one of these being alive on submission. Portions of the (CNS) from an additional five piglets were forwarded as initial specimens from three of the herds by the Regional Veterinary Laboratory at Kemptville.

The living piglets were killed by exsanguination. Routine post-mortem examinations were conducted and one half of the brain and portions of the spinal cord with attached paravertebral ganglia were placed directly into 10 per cent formalin in physiological saline for histological examination. The other half of the brain with portions of the intestinal tract were harvested and frozen for subsequent virological examination. Representative sections of the brain and spinal cord were prepared and stained with hematoxylin and eosin.

Virology

Portions of brain and other tissues were frozen at —30°C until experiments could be conducted. After thawing, approximately 1 gm. of brain tissue was ground in a Ten Broeck grinder with beef infusion broth to make a 10 per cent suspension. Penicillin and streptomycin were then added. Additional inocula were prepared from defibrinated blood to which antibiotics were added and from portions of ileum and intestinal content which were ground in beef infusion broth and passed through Steriflo filter pad D-8.

Experimental piglets were routinely anesthetized with trichlorethylene* prior to inoculation. Intracranial inoculation was

*"Trilene" — Ayerst McKenna and Harrison Limited, Montreal, Quebec.

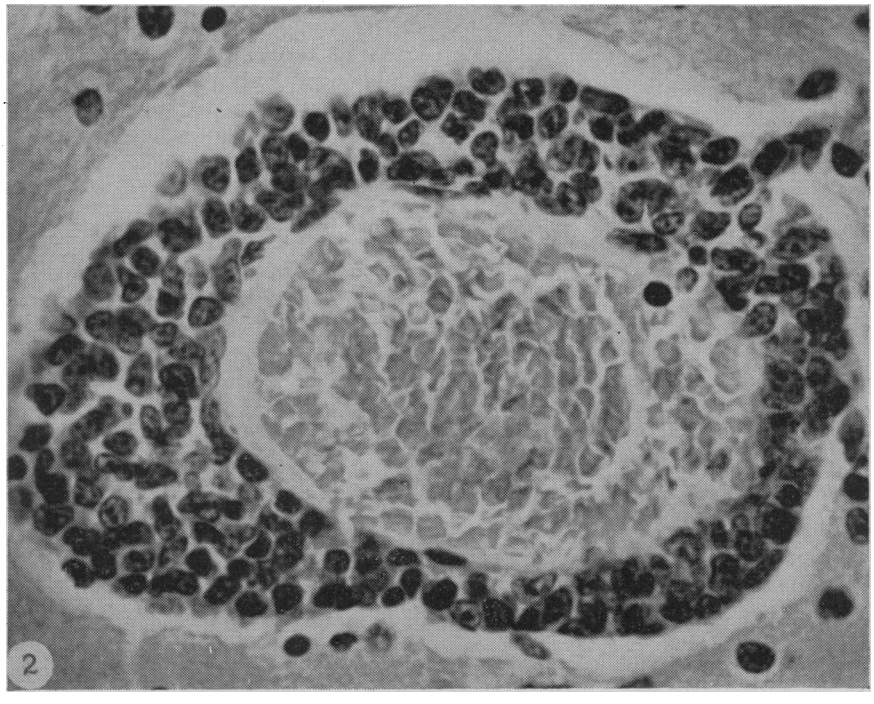
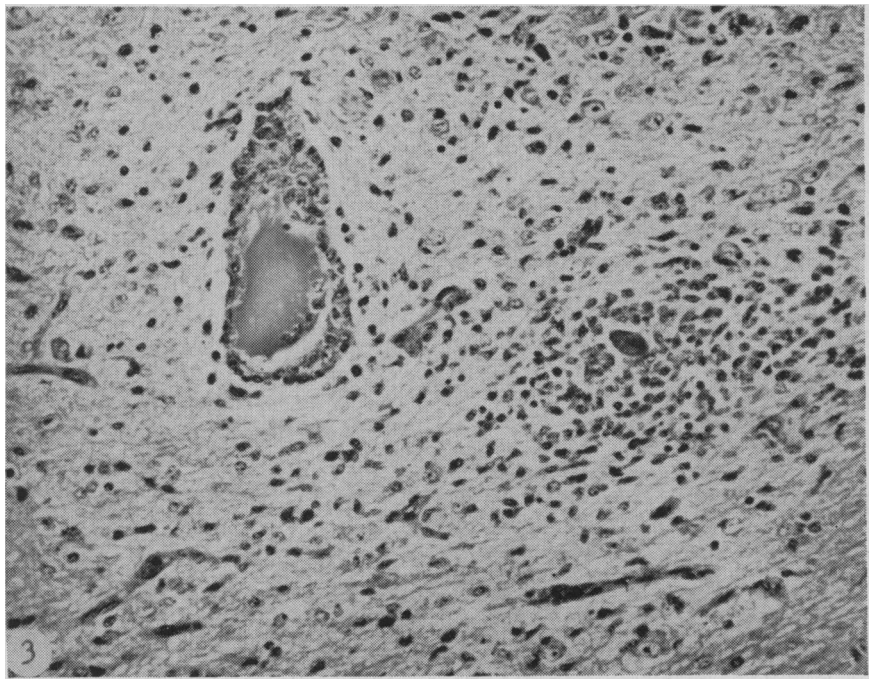


Fig. 2 Perivascular cuffing in hippocampus. H. and E. X 780.

Fig. 3. Perivascular cuffing and glial node containing degenerate neuron in medulla oblongata. H. and E. X 234.



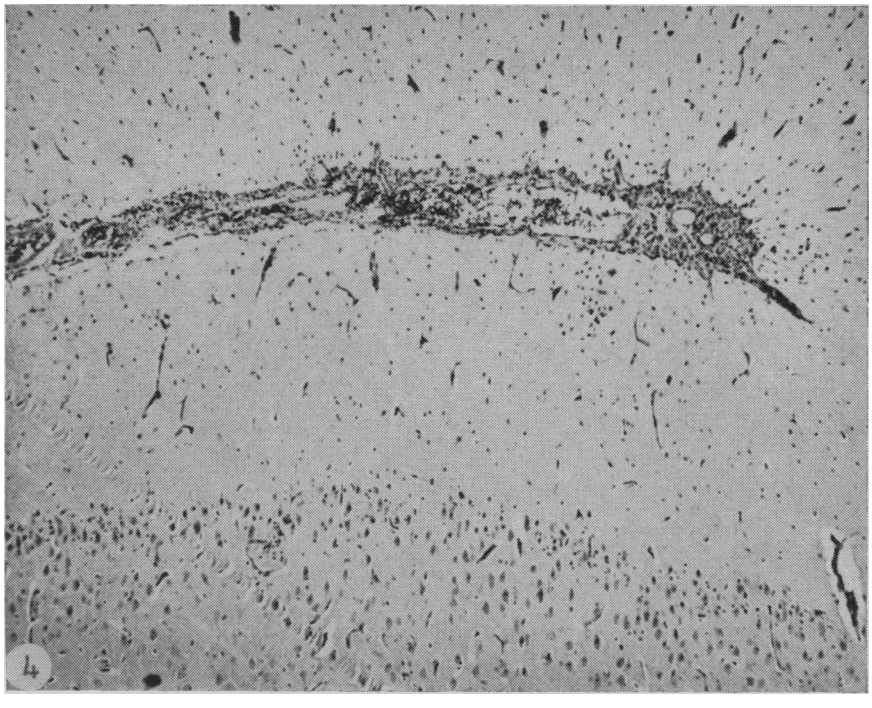


Fig. 4. Mild cerebral meningitis. H. and E. X 78.

Fig. 5. Typical reaction in paravertebral ganglion. H. and E. X 780.

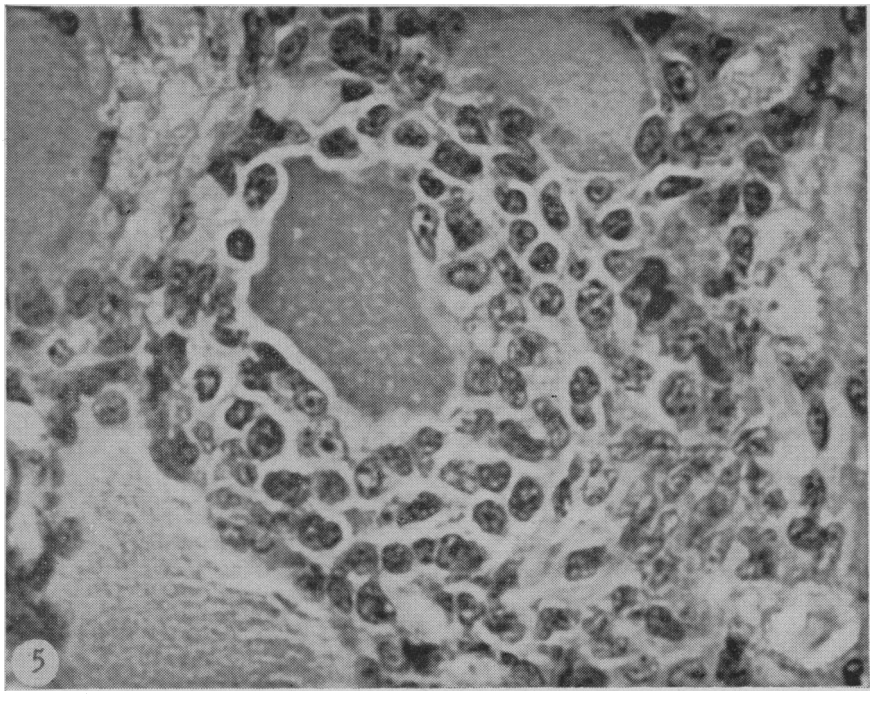


TABLE I

Summary of Clinical and Pathological Findings

Herd No.	Number of Sows on Premises	Number of Litters Affected	Age at Onset of Symptoms (in days)	Piglets Affected	Deaths	Number of Specimens Examined	Number with Lesions of Ence-phalomyelitis	Number with Perivascular Haemorrhage in Cerebellum	Number with no Lesions
1	7	2	4-6	21	21	4	2	0	2
2	14	1	7	12	12	6	3	3	0
3	1	1	6	14	12	4	2	0	2
4	5	3	7-15	34	29	6	2	0	4
5	13	7	5-10	75	75	9	5	3	1
Totals	40	14	4-15	156	149	29	14	6	9

carried out using a small bore needle with a second needle employed as a canula. Two-tenths ml. of the various inocula were employed. These animals were housed thereafter in standard Horsfall cages usually with two animals in each cage. All of the cages were in a single room and were looked after by a single animal attendant.

Two preliminary experiments were carried out and although the results are not conclusive they are reported here in order to demonstrate some of the problems encountered.

Experiment 1: Portions of brain from the two original specimens submitted on April 13 from herd 1 were prepared and inoculated intracranially into three one day old piglets which had been purchased at random. In addition six guinea pigs, two rabbits, ten 21 day old mice, a litter of suckling mice and two chickens were inoculated intracranially; two rabbits were inoculated subcutaneously; ten 21 day old mice were inoculated intraperitoneally; and embryonated chicken eggs were inoculated by various routes.

Experiment 2: Three litters containing 29 three day old piglets were employed in this experiment. They were divided as equally as possible into six groups and inoculated (as outlined in Table II) with material harvested from two of the pigs used in experiment 1.

Results

Pathology

No gross lesions of pathological significance were encountered except for a slight increase in the amount of cerebro-spinal fluid in some cases. This appeared cloudy and contained flakes. Of the 29 specimens examined histologically 14 showed lesions of viral polioencephalomyelitis characterized by perivascular mononuclear cuffing (Figures 2 and 3), the formation of glial nodes, neuronal degeneration (Figure 3) and meningitis (Figure 4). Lesions were most severe and constant in the mesencephalon, pons and medulla oblongata. They diminished in severity both rostrally and caudally but were quite constant in the brachii and roof nuclei of the cerebellum, the thalamus, corpus striatum, rhinencephalic cortex, cervical cord and paravertebral ganglia (Figure 5). Figure 6 shows typical lesions in the dorsal horn of the thoracic cord.

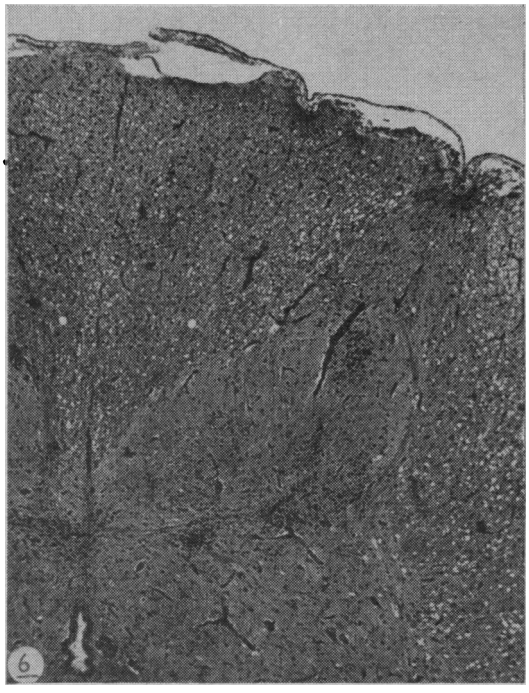


Fig. 6. Perivascular cuffing and glial node formation in dorsal horn of thoracic cord. H. and E. X 60.

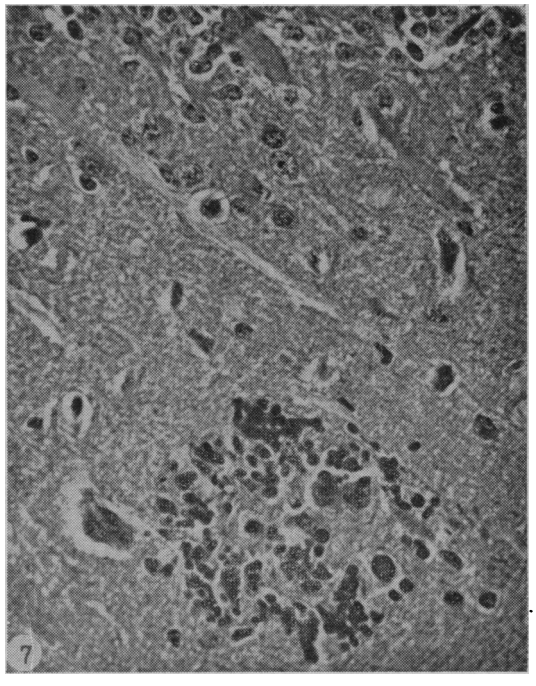


Fig. 7. Swollen endothelial cells and perivascular haemorrhage in molecular layer of cerebellum. H. and E. X 357.

The distribution and character of these lesions was essentially similar to those reported by Richards and Savan (5) except that meningitis involving the cerebellar meninges was not found to be nearly as constant. A cerebellar meningitis of very mild proportions was noted in two specimens and a rather more severe but still mild cerebral meningitis was present in eight other animals. Six of the animals which had survived for some time showed swollen endothelial cells and perivascular hemorrhages in the molecular layer of the cerebellum (Figure 7). These six specimens came from two of the five herds involved. Table I summarizes the salient clinical and pathological findings.

Virology

Experiment 1: None of the laboratory animals or embryonated eggs inoculated showed any deviation from the normal.

On the second day two of the inoculated pigs developed diarrhea, marked emaciation and the stilted gait seen in many of the naturally infected animals. These piglets were killed on the third day after inoculation. Examination of brain sections however revealed no significant histological changes. The third piglet in this experiment died seven days post-inoculation without showing any definite symptoms. Histopathological study revealed the presence of encephalitis at the site of inoculation and this specimen was discarded.

Experiment 2: As can be seen from Table II the results of this experiment are difficult to interpret. It is felt that this is in some measure due to an accidental cross contamination between the isolation cages and this is supported by the finding of lesions in two of the control animals. It was also found difficult to maintain these animals and further experiments were postponed until suitable quarters and colostrum-deprived piglets were available.

It is significant that none of the animals from litter three in any of the six groups showed lesions, suggesting that these animals were not susceptible.

In group 2 all four of the animals from the apparently susceptible litters inoculated intracranially with brain emulsion showed lesions. These animals died on the third and fourth day post-inoculation.

In group 3 none of the animals showed lesions. This would suggest either that there was no infective material present in the defibrinated blood or that the intraperitoneal route was not suitable for transmission purposes.

TABLE II

Outline of Virus Transmission Experiment 2

Group	Exposure Material	Route of Exposure	Litter No.	Pig No.	Time of Death (Post-Inoculation Day)	Pathology
1	Controls	—	1	622	6	Positive
			1	661	1	Not Examined
			2	670	3	Positive
			2	671	6	Not Examined
			3	775	3	Negative
			3	800	3	Negative
2	Brain Emulsion	Intra-Cranial	1	620	4	Positive
			1	621	3	Positive
			2	668	3	Positive
			2	669	4	Positive
			3	579	3	Negative
			3	724	4	Negative
			3	774	7	Negative
3	Defibrinated Blood	Intra-Peritoneal	1	616	6	Negative
			1	617	7	Negative
			2	667	6	Negative
			3	674	3	Negative
4	Ileum and Intestinal Content	Intranasal and Oral	1	618	6	Negative
			2	664	5	Positive
			2	666	5	Positive
			3	675	11	Negative
5	Intestinal Filtrate	Intra-Cranial	1	619	7	Positive
			2	667	6	Negative
			3	577	7	Negative
			3	578	6	Not Examined
6	Contaminated Cage	Contact	1	615	5	Positive
			2	662	5	Positive
			3	672	11	Negative
			3	673	7	Negative

In group 4 both of the piglets from litter 2 developed lesions although the single animal from litter 1 was negative. It would therefore appear that the agent was infective by the intranasaloral route and it is interesting to note that the animals which showed lesions did not die until the fifth day post-inoculation.

In group 5 the results must be regarded as inconclusive.

In group 6 both of the animals from susceptible litters developed lesions following exposure to the contaminated cage which had housed the pigs used in experiment 1 and it is again interesting to note that these two animals both died on the fifth day suggesting that infection occurred by oral or intranasal introduction.

Discussion

It is apparent from these findings that a disease or diseases of swine which may produce lesions of a viral polioencephalomyelitis in a proportion of the affected animals, is present in Eastern Ontario. As observed in this paper it has been primarily a disease of young suckling pigs and although it is not known how or when this condition first appeared, it has probably existed for some years. Its exact relationship to the encephalomyelitis described by Roe and Alexander (3) and Richards and Savan (5) has not yet been definitely established but it would appear to be similar if not identical.

In the five outbreaks described the mortality amongst affected litters was close to 100 per cent with only seven of 156 piglets surviving. In all cases the onset of the disease was sudden and the majority of deaths occurred during the first few days although some of the animals survived for 3 to 4 weeks. The only constant findings were: the sudden onset, shivering or trem-

bling, varying degrees of ataxia and incoordination, a somewhat stilted gait and rapid progressive emaciation. Definite signs of a CNS disturbance were seen in only a few animals and this finding was supported by the absence of lesions in the CNS of 14 of the 29 animals examined.

The presence of perivascular hemorrhages in the molecular layer of the cerebellum in six animals, all of which had survived for at least seven days, was also observed. It is possible that these lesions could have been associated with secondary bacterial infection. They have been seen in the brains of suckling pigs from other herds where the clinical history suggested that a disease similar to that described had been present previously.

In none of the herds has the condition recurred and it has been noted in three cases (herds 1, 2 and 5) that litters born even a few days after the appearance of the disease may not become affected. Older animals did not show clinical evidence of infection.

Virus transmission studies have been hampered by the fact that normally raised suckling pigs often appear to be resistant to experimental infection. However, the results of the limited trials described indicate that the condition can be reproduced in suitable experimental animals with the production of characteristic histopathological changes in the central nervous system. Two survivors from one of the affected litters were challenged with Teschen virus two months later and both developed the typical symptoms and lesions associated with this disease.

The results of attempts to isolate an infective agent in tissue culture will be reported in a subsequent paper (6). Cytopathogenic agents have been isolated from four of these outbreaks and comparative studies together with the results of further transmission trials will be described.

Summary

The clinical and pathological findings in a disease of suckling pigs in five herds in Eastern Ontario are described. The disease occurred in 14 litters from 4 to 15 days of age and was characterized by sudden onset, shivering, ataxia, rapid emaciation and high mortality. Lesions of a viral polioencephalomyelitis were seen in fourteen of twenty-nine affected piglets examined. Limited virus transmission studies were not conclusive but it appears that the condition can be transmitted by intracranial as well as intranasal-oral inoculation. Further studies and the isolation of cytopathogenic agents will be described in later papers.

Acknowledgements

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Rabies Down In 1960

With only 154 cases of rabies reported in Ontario, Department of Agriculture authorities feel confident that there is little or no danger of an epidemic. The picture is equally bright elsewhere in Canada, rabies having been reported in only two other provinces. In one area of Manitoba the disease has been reported in skunks but in no other species of wildlife, and in Megantic County, Quebec, four cases involving a fox and four cattle have been reported. The 154 cases of rabies compares favourably with 3,005 cases in 1958-59 in Ontario. This year there were no heavy concentrations of the disease.