A Viral Gastroenteritis of Ontario Swine

I. Clinical Illness and Recovery of the Virus

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SUMMARY

A severe gastroenteritis affected Ontario pigs in the Spring of 1964 and again in 1966. The mortality of pigs less than a week of age was 100%, in older pigs there were few deaths although morbidity in these pigs approximated 100%. Viruses were isolated from the brains, intestines, and intestinal lymph nodes of baby pigs. One of these isolates, given by mouth to two colostrum deprived piglets, induced a severe enteritis 36 hours after administration and both of the piglets died 24 hours later. The characteristics of the disease and the virus bear a striking resemblance to transmissible gastroenteritis as seen in the United States of America and the United Kingdom.

Introduction

The epidemiology and clinical pattern of an outbreak of vomiting and diarrhea in swine during April 1964, and again in March and April of 1966, suggested that an infectious disease not recognized previously in Ontario was present. The disease, affecting an estimated 100 swine herds in 1966, was characterized by fever, profuse diarrhea, and, occasionally, vomition. Morbidity was extremely high in pigs of all ages but deaths were uniformly restricted to piglets less than two weeks of age in which the mortality rate approached 100%.

In some herds infection was traced to a particular swine sales barn, but in many of the cases no importation of new stock had occurred. Farmers and attending veterinarians were of the uniform opinion that the disease was a new one, and was unfamiliar to them.

This communication will deal with the clinical, pathological, and preliminary virological studies of the disease. Subsequent papers will discuss the classification of the virus isolates and the pathogenesis of the disease in pigs.

Materials and Methods

TISSUE CULTURES:

Two cell lines (PS-Y_{15} and STEC) and primary embryonic pig kidney (PK) were used for the attempted isolation of virus from affected piglets. PS-Y_{15} cells, obtained from Dr. Y. Kanda-Inoue, Institute for Virus Research, Kyoto University, Yoshida-madu, Kyoto, Japan, were initiated in Hanks' balanced salt solution (HBSS) containing 0.5% lactalbumin hydrolysate, 10% tryptose phosphate broth, and 10% fetal calf serum. The maintenance medium consisted of medium H-597 (Connaught Medical Research Laboratories, Toronto) with 5% fetal calf serum added.

The STEC cell line was the gift of Dr. Jan Thorsen, Pitman-Moore Company, Indianapolis, Indiana. These cells were grown in Eagles' minimal essential medium (EME) with 10% added lamb serum and maintained in EME with the lamb serum reduced to 2%.

PK cell cultures were prepared by tryp-

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sinizing embryonic pig kidney, growing the cells in HBSS plus 0.5% lactalbumin hydrolysate (HLa) with 10% fetal serum, and maintaining them in medium 597 with the serum reduced to 5%.

All of the cell cultures were grown in tubes, plastic tissue culture flasks (Falcon Flasks, Becton, Dickinson and Company) or Leighton coverslip tubes, depending upon their use.

PREPARATION OF SPECIMENS:

Virus isolations were attempted from the small intestine, mesenteric lymph node and brain of suckling pigs dying, or dead, from acute enteritis. Approximately two grams of material was emulsified in 15 ml. of HBSS in a 40 ml. centrifuge tube, containing glass beads, by shaking vigorously, or else ground by hand in the same proportion of tissue to HBSS in a Ten-Broeck grinder. The supernatant fluid was strained through two layers of sterile gauze into a 10 ml. Spinco centrifuge tube and centrifuged at 6000 rpm for 15 minutes. The supernatant fluid was collected and treated with 500 i.u. of penicillin, 500 μg of streptomycin, 500 μg of neomycin, and 5 units of bacitracin per ml. (1). This material was inoculated into Falcon flasks of tissue culture and the inoculated flasks were examined daily for the presence of cytopathic effect (CPE).

Histological examination was performed on sections of brain, Gasserion ganglion, intestine, myenteric plexeses in the region of the pylorus, and using routinely prepared paraffin sections stained with hematoxylin and eosin.

In addition routine bacteriological examination was carried out on samples of the intestinal contents and spleen.

EXPERIMENTAL PRODUCTION OF THE DISEASE:

Two caesarian derived piglets, one day of age, were given 1 ml. of one virus isolate (V 147-66) by mouth; this represented approximately 10^3 TCD_{50} of the virus. The piglets were then processed as for the clinical specimens mentioned above.

Results

EPIZOOTIOLOGICAL FINDINGS:

Farm One

A boar was purchased at a local sale and developed a profuse diarrhea several days later. The disease spread rapidly through the herd affecting 20 sows which had just farrowed, or were close to farrowing. Newborn piglets became ill at two days of age and 227 piglets (100%) died within eight days of birth. No adults died and the epidemic ended within three weeks, when all of the sows had farrowed.

Farm Two

This outbreak occurred simultaneously with the outbreak on farm one, however, no connection between the two farms could be established, nor was there any recent importation of swine. Six sows with newborn litters and 15 dry sows were affected. The mortality rate in the piglets was 100% but there were no deaths in the adults or weaned pigs.

Farm Three

The outbreak on this farm was similar in many aspects to those previously described. Mortality rate in piglets under a week of age was 100% but although piglets three to four weeks of age were affected, the mortality rate in this age group was low.

Farm Four

Two pregnant sows were purchased from a local sale barn. One sow had farrowed two days after arrival and her piglets scoured and died. In an attempt to protect 20 other pregnant sows on the premises, the viscera from dead piglets were fed to these sows. They all developed signs of the disease within the next 10 days. Four sows farrowed three weeks after this crude immunization procedure and all their piglets scoured and died. The remaining 16 sows, which were a month or more from farrowing when they were deliberately infected, farrowed normal litters with no signs of the disease.

Farm Five

This farm was a feeder pig operation and all pigs were purchased as weaners. Twenty pigs, 10 weeks of age were purchased from farm four. These pigs were from two of the 16 sows previously described. Seven to 10 days after arrival the pigs in the pen in which they were placed began to scour. The disease spread rapidly through the barn in the next week with a morbidity of 100% in 200 pigs ranging in age from two to four months.

One pig died during the outbreak. At no time did the original 20 pigs show any signs of the disease.

Clinical Findings

The most outstanding clinical feature was a profuse, yellow, watery diarrhea in all age groups. In older pigs, the fluid feces were often propelled one to two feet behind the pig on defecation. Vomition sometimes was present. It was observed in sows and young piglets but not in market pigs. Temperature varied from normal up to 107 F. Affected sows, which had recently farrowed, went completely off feed for up to a week. The subsequent agalactia resulted in hypoglycemia or starvation in the piglets, which, along with scouring, killed them quickly. Market pigs did not go completely off feed but feed intake was reduced about 75%, for a period of a week, with resultant loss in weight.

In the above outbreaks treatment of both the sows and their litters with a variety of antibiotics was completely ineffective.

VIRUS ISOLATIONS:

From specimens received from the 1964 and the 1966 outbreaks, transmissible cytopathic agents were recovered in STEC and PK cell cultures. The agents, subsequently proven to be viruses (2), were isolated from the brains, intestinal lymph nodes, and small intestine of baby pigs. Although the viruses grow in STEC, PSY_{15} and PK cultures, the CPE was minimal on first passage of infected material. Following four or five serial passages in PK the CPE became more defined and in two instances blind passage of material from tissue cultures apparently free of CPE on early passage revealed cytopathic virus.

The cytopathic effect in STEC cells became noticeable after 4-5 days of incubation. Small holes appeared in the cell sheet; these holes were bordered with small, dark, round cells. The centers of infection enlarged over the next 4 days, gradually causing destruction of the cell sheet. Coverslip cultures stained with hematoxylin and eosin revealed margination of the nuclear chromatin and a condensation of the nucleoplasm, giving the appearance of basophilic intranuclear inclusion bodies.

Complete characterization of these viruses will be described in a subsequent communication (2).

HISTOLOGICAL AND BACTERIOLOGICAL EXAMINATION:

No significant lesions were seen in hematoxylin and eosin stained sections of the intestine, brain, Gasserian ganglion, or myenteric plexes in the region of the pylorus. *Escherichia coli* was recovered from the intestines and, occasionally, the spleens of piglets submitted for examination; these organisms were sensitive to chloramphenicol and nitrofurazone both of which had been used in treating most of piglets submitted.

PRODUCTION OF THE DISEASE IN PIGLETS:

The two piglets given V 147-66 virus by mouth, developed a severe diarrhea 36 hours after administration of the virus. Both piglets died 24 hours later. The histological and bacteriological examination of these pigs was essentially negative. V 147-66 virus was recovered from the brain, small intestine, and mesenteric lymph nodes of both pigs by the inoculation of STEC and PK tissue culture cells.

Discussion

On clinical and epidemiological grounds the disease described above appeared to be different from any other previously recognized as an entity in this area. The high morbidity of all age groups, the rapidity with which the disease spread through the district, and the termination of the outbreak several weeks later support the hypothesis that it was due to an infectious agent in a highly susceptible population.

The epizootiological pattern of the disease eventually provided some help in diagnosis. However, early in an outbreak, the disease had to be differentiated from colibacillosis in piglets, coliform enteritis of weaned pigs and vibriosis and salmonellosis in feeder pigs and mature animals.

Clinically, colibacillosis is similar, but vomition is very uncommon and sows are not affected. Early cases of colibacillosis usually respond to antibiotics to which the organism is sensitive. No chemotherapeutic agent appeared to aid in treatment of this disease in any age group.

Coliform enteritis in weaned pigs produces a higher mortality and a more obviously depressed pig on clinical examin-

ation. The response to treatment and the history of recent weaning are useful to establish a diagnosis.

Vibriosis in feeder pigs appears much like this disease in the same age group. Pigs are bright and alert but obviously gaunted in appearance. Dysentry, which can occur in vibriosis, was not observed.

Feeder pigs or sows with salmonellosis are generally more depressed and off feed than are pigs with this condition. The bluish discolouration of the ears and ventral abdomen which is common with salmonellosis has not been observed.

It should be emphasized that the clinical diagnosis of the swine diseases listed should be confirmed by bacteriological, pathological, and if indicated, virological examination.

Although *E. coli* was recovered from the intestines, and sometimes spleens, of piglets dying from the disease, it was considered that its role in the pathogenesis of the infection was secondary to a primary viral agent, especially when tissue culture propagated virus induced the disease in baby pigs.

Viruses which have been recovered from piglets with encephalomyelitis have also been suggested as playing a role in the vomiting and wasting syndrome of young pigs (3). Although no nervous signs were noted in the outbreaks of disease described in this communication, tissues were examined for evidence of encephalomyelitis and ganglionitis. In every case these examinations proved negative, despite repeated isolation of virus from the brains of pigs dying from the disease. Furthermore no viruses related to the enterovirus subgroup of the *Picornavirus* group, or to the hemagglutinating virus of Greig (4) were recovered. In fact one of the isolates, V 52-64, appeared to be morphologically related to the *Herpesvirus* group on preliminary examination (2).

Viruses causing a transmissible gastroenteritis in swine have been isolated in the United Kingdom (5), Japan, and the United States of America (6). The disease in the United Kingdom as described by Cartwright *et al* (5) bears a striking resemblance to the outbreak we have described in this communication, as do the properties of the viruses isolated.

Characteristically classical transmissible gastroenteritis of pigs is a disease affecting all age groups of susceptible pigs and a mortality rate of 100% is often seen in very young piglets (8). This disease picture is hardly likely to be confused with enteritis in pigs due to other causes, such as colibacillosis or vibriosis.

An excellent review of the disease as seen in the United Kingdom has been presented by Saunders (7) in which he gives useful features on which the diagnosis can be made clinically. The rapid onset in all age groups; the graded death rate (100% in baby pigs up to no deaths in older pigs); the consistent clinical sign of diarrhea in all age groups with vomiting in older pigs, particularly sows; the lack of bacterial pathogens; with no signs or histological lesions of central nervous disease; are all typical of the disease as described in the United States, the United Kingdom, and are typical of the clinical syndrome observed in Ontario. The relationships between the viruses isolated in the United States, the United Kingdom, and Ontario, have been investigated and will be reported in a separate communication (2).

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