VOMITING AND WASTING DISEASE,

A CORONAVIRUS INFECTION OF PIGS

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I. THE DISEASE

In 1957, an epizootic disease of nursing pigs characterized by high morbidity, vomiting, anorexia, constipation and severe progressive emaciation was observed in Canadian swine herds.[1] In acute cases, vomiting and severe depression were the only symptoms noted before death. More frequently the disease tended to become chronic. The affected suckling pigs became emaciated and usually died of starvation after a few weeks. Pigs that survived were permanently stunted. The condition was called "vomiting and wasting disease" because of its salient characteristics.

A second condition, called viral encephalomyelitis, appeared almost concurrently and caused some confusion as to whether one or two disease entities existed.[2] The initial clinical signs also consisted of anorexia, vomiting and constipation, but in this second condition, they progressed after one to three days to an acute encephalomyelitis. At that time hyperesthesia, muscle tremor, ataxia, blindness and paddling of the legs could be observed. The mortality rate was low in older litters (over 3 weeks of age) but it often approached 100 % in very young litters. The Canadian workers Greig et al.[3] isolated in 1961 a virus from the brains of such pigs with encephalomyelitis. It was called hemagglutinating encephalomyelitis virus (HEV) because of its hemagglutinating properties.

In 1969, Cartwright et al.[4] isolated a virus from pigs in England with vomiting and wasting disease. This isolate was later classified as a coronavirus[5] which turned out to be antigenically similar if not identical with HEV.[4] In repeated trials, Mengeling and Cutlip could reproduce the vomiting and wasting syndrome as

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well as the motoric disorders, using American field isolates.[6] It was, therefore, concluded that the two diseases are different manifestations of the same virus.

Meanwhile, outbreaks of vomiting and wasting disease (VWD) were reported in many European countries.[7-11] However, the epizootic character of the disease seems now to have disappeared. Clinical outbreaks on larger breeding farms are rare and usually occur only in a few litters from gilts. Only on very small breeding farms, outbreaks are still observed in pigs from sows of all ages.[12]

II. EPIZOOTIOLOGY

The spread of VWD virus in the pig population has been studied in several countries by means of serological surveys. In fattening swine, 31 per cent of the sera were positive in Canada,[13] 49 per cent in England,[14] 46 per cent in N.-Ireland,[15] 52 per cent in Japan,[16] 0 to 89 per cent in the United States[17] and 75 per cent in W. Germany.[18] The percentage of sows with antibodies at slaughter varied from 43 per cent in N.-Ireland[15] to 98 per cent in the United States.[17]

From these data, it can be concluded that VWD virus is widely spread among the swine population in different countries. To determine the incidence of infection in Belgium, 140 sow sera were collected in slaughterhouses in 1974 and again in 1979.[12] Seroneutralising antibodies were present in about 93 per cent of these sera. This indicates that most Belgian breeding farms either become regularly infected or harbour the virus persistently. To obtain a better understanding of the normal pattern of infection by the VWD virus, the seroepizootiologic study was extented to younger animals, kept under various circumstances.

The rate of decline of maternal antibodies, was examined in three litters consisting of 29 pigs which were suckling their immune mothers, while kept in isolation. During 4 months, 464 sera were collected at various time intervals after birth. The geometric mean seroneutralisation titer was 192 during their first week of life. At the age of 8 weeks, 4 out of 27 pigs had become negative and at the age of 14 weeks all the pigs were seronegative.

The decline of maternal immunity was also studied in twenty-one weaned pigs, housed in a small stable at the age of 7 weeks. They represented the only pigs on the farm. The geometric mean titer declined until the age of 15 weeks. Four weeks later, the geometric mean titer had risen to 64. The infection which apparently had taken place, passed without clinical signs. The source of infection remained unknown.

On two breeding farms with animals of different ages and a high turnover of pigs, the course of development of seroneutralising antibodies was different from that in the former group of animals. The titers first declined until the age of ten to twelve weeks, but the animals did not become seronegative. A gradual increase in average titer indicated that the passive immunity subsequently was converted into active immunity. On one of these farms, the VWD virus was isolated from nasal discharge of healthy pigs at the age of 2 to 5 weeks. These observations confirmed that the VWD virus was continuously present on these farms and that the pigs became subclinically infected in the presence of maternal antibodies.

Field outbreaks of VWD in piglets seldom occur on conventional breeding farms. This observation can be explained by the fact that most sows have seroneutralising antibodies and that their litters obtain a colostral immunity which protects them against clinical signs during the susceptible period. Suckling pigs will only become sick if they are born from seronegative mothers. On large breeding farms, clinical outbreaks are usually limited to a few litters from gilts. These gilts have probably, through circumstances, insufficient active immunity at the time of parturition. On very small breeding farms, outbreaks are observed in pigs from sows of all ages. These sows can be seronegative, because viral persistence is not likely to occur in such a small animal populations.

III. THE SPREAD OF VWD VIRUS IN PIGS

Preliminary studies on the pathogenicity of VWD virus using different routes of inoculation provided evidence that viral spread occurs along nerve pathways.[9] Typical disease was obtained in pigs after the virus was inoculated into oral and nasal cavities or into the infraorbital nerve but not after intravenous inoculation. In oronasally inoculated pigs killed during the incubation period, the virus could be isolated regularly from the tonsils and the respiratory tract, irregularly from the digestive tract, rarely from the blood and never from lymph nodes, spleen and kidney. In pigs which were killed when ill, the brainstem practically always contained virus while other parts of the brain and the vagal nerve were inconsistently positive.[20]

In a recent experiment, the route of viral spread and the exact sites of viral replication in oronasally infected pigs were examined in detail.[21] Fourteen colostrum deprived pigs were inoculated oronasally within 6 hours after birth with a Belgian VWD virus isolate earlier described.[22] They were killed between post inoculation day (PID) 1 and 7 and frozen sections of different tissues were examined using the direct fluorescent antibody technique.

The incubation period lasted four days in all the pigs which were not yet killed at that time. Illness was characterised by inappetence and listlessness, accompanied or quickly followed by vomition.

In the respiratory tract and tonsils, fluorescent antigens were detected in epithelial cells of the nasal mucosa, tonsillary crypts and greater bronchi of a pig killed on PID 1. The epithelium of terminal and respiratory bronchioli and the pneumocytes of the alveoli became positive starting at PID 2.

In the gastrointestinal tract viral antigens could be detected in the small intestine starting at PID 2. They were located in the cytoplasm of a few cells in the epithelial layer on the villi and in neurons of the submucosal plexus. Neurons of the myenteric plexus became infected at PID 4. Fluorescence was not present in the submucosal and myenteric plexuses of colon and rectum of pigs killed during the incubation period but was found in a pig killed at PID 7. The stomach remained also negative during the incubation period but was positive in 6 of the 7 pigs which were killed when ill. The fluorescence in the stomach was always restricted to the perikaryon of neurons.

In the peripheral nervous system, the trigeminal ganglion became infected at PID 2. The inferior vagal ganglion, the superior cervical ganglion and the solar ganglion (fig. 1) contained viral antigens starting at PID 3. The fluorescence in these ganglia was always restricted to the perikaryon of neurons.

In the lower thoracic region (Th 12-16), a few dorsal root ganglion cells exhibited cytoplasmic fluorescence starting at PID 3. Ganglia at higher and lower levels of the spinal cord were negative in a pig killed at PID 4 but had become positive in a pig killed at PID 7, together with a few neurons of the cervical cord and the thoracic spinal cord.

In the brain, the viral infection started in the sensory nuclei of the trigeminal and the vagal nerve, located in the medulla oblongata. In pigs killed at PID 3 and PID 4, viral antigens were mainly found in neurons of the nucleus spinalis nervi trigemini. In pigs killed after the appearance of the clinical signs, the sensory nucleus of the vagal nerve, the nucleus solitarius, also contained several infected neurons. In pigs killed 2 to 3 days later, the virus had spread into other parts of the brainstem, and sometimes also into the cerebrum and the cerebellum. The medulla oblongata remained, however, the most heavily infected part of the brain. Fluorescence in the brain always remained restricted to the neurons.

Based on the results of the studies presently reported, the following concepts on the spread of VWD virus in pigs can be put forward. After the oronasal inoculation of the virus, the nasal

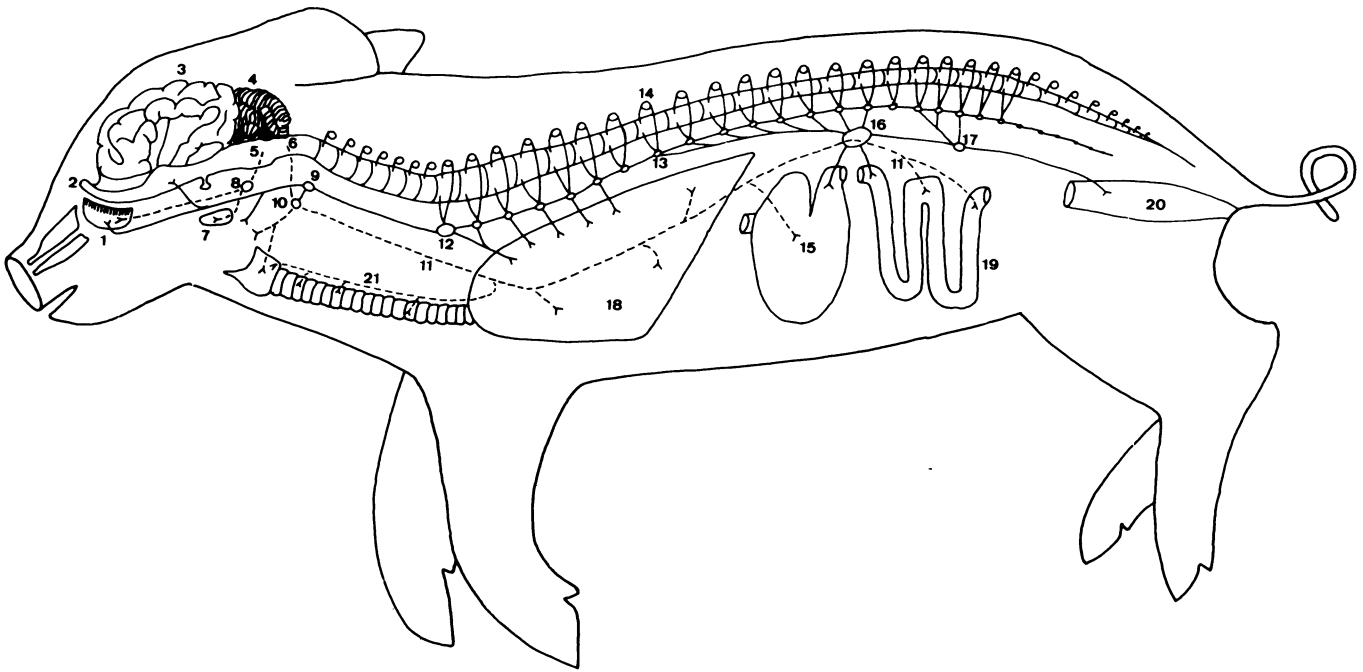

Fig. 1. Scheme of the tissues, nerve paths and ganglia involved in the pathogenesis of Vomiting and Wasting disease
1. nasal mucosa, 2. olfactory bulb, 3. cerebrum, 4. cerebellum, 5. pons varoli, 6. medulla oblongata, 7. tonsils, 8. trigeminal ganglion, 9. cranial cervical ganglion, 10. inferior vagal ganglion, 11. vagal nerve, 12. stellate ganglion, 13. sympathic trunc, 14. dorsal root ganglia, 15. stomach, 16. solar ganglion, 17. caudal mesenteric ganglion, 18. lungs, 19. small intestine, 20. rectum, 21. recurrent vagal nerve.

mucosa, the tonsils, the lungs and the small intestine served as primary sites of replication. The VWD virus then progressed via nerves corresponding to these areas towards the associated peripheral ganglia and further to the central nervous system. At least three pathways appeared to be involved (fig. 1). A first pathway to the central nervous system led from nasal mucosa and tonsils to the trigeminal ganglion and to the nucleus spinalis nervi trigemini in the medulla oblongata. A second pathway occurred along the vagal nerves via the inferior vagal ganglion, towards the nucleus solitarius in the medulla oblongata. A third pathway led from the intestinal plexuses to the spinal cord, either by way of sensory fibers which have their cell bodies in the dorsal root ganglion or after viral replication in the solar ganglion. In the central nervous system, the infection started in well defined nuclei of the medulla oblongata but progressed later into the entire brainstem, the spinal cord and sometimes also into the cerebrum and the cerebellum.

The question whether the vomition is induced centrally by viral replication in the brainstem or is due to viral replication in the peripheral nervous tissue (gastric or intestinal plexuses, solar ganglion, dorsal root ganglia, distal vagal ganglion) remains unanswered after these experiments. In fact, the invasion of the neurons in these tissues almost coincided with the start of the clinical signs.

IV. THE PATHOGENESIS OF THE VOMITING

From the results of the oronasally inoculated pigs, it was concluded that there are six possible target-tissues : the brainstem, the stomach plexuses, the intestinal plexuses, the dorsal root ganglia in the lower thoracic region, the solar ganglion and the distal vagal ganglion. The vomiting is induced by viral replication in one or more of these target-tissues. Further experiments were performed to study the relation between viral replication in these tissues and the appearance of clinical signs. Eleven colostrum deprived piglets were inoculated at one of the following sites : intragastrally (wall) or intraintestinally (wall + lumen) or intramuscularly (neck) or into the cerebrospinal fluid. They were always killed within 12 hours after the appearance of the clinical signs. Viral replication in the candidate target-tissues was examined by immunofluorescent tracing. The results are presented in table 1. All the inoculated piglets developed typical clinical signs after an incubation period of 3 to 5 days. Several candidate target-tissues were consistently negative by immunofluorescence after inoculation at a particular site. In the brainstem,fluorescing neurons were never found after the intraintestinal inoculation. In the stomach plexuses, no fluorescence was seen after the inoculations

Table 1 - Fluorescence in candidate target tissues of pigs sick upon inoculation of vomiting and wasting disease virus at different sites

Inoculation sites	Candidate Target Tissues					
	Brainstem	Stomach Plexuses	Intestinal Plexuses	Dorsal Root Ganglia	Solar Ganglion	Distal Vagal Ganglion
Intraintestinal	0/3	1/3	3/3	2/3	3/3	3/3
Intramuscular	2/2	0/3	2/3	1/3	2/3	2/3
Cerebrospinal fluid	1/1	0/2	1/2	2/2	1/2	2/2
Intragastral (wall)	3/3	3/3	0/3	1/3	2/3	3/3

Data are expressed as No. pigs positive / No. tested

in the cerebrospinal fluid or in the neck muscles. Finally, the intestinal plexuses contained no viral antigens after inoculation into the gastric wall. However, also the three remaining candidates were sometimes negative. Fluorescence was not found in the dorsal root ganglia of five piglets, in the solar ganglion of three piglets and in the vagal ganglion of one piglet.

The present results do not allow a definite conclusion concerning the target-tissue for the vomiting. Nevertheless, they demonstrate that at least five of the six candidates were regularly negative for viral antigens at the time the pigs were sick. Two explanations are still possible. First, the distal vagal ganglion, which was only once negative in the eleven pigs, can still turn out to be the "one and only" target-tissue. However, it is also possible that the vomiting is not induced by viral replication in one target-tissue only. Infected neurons at different sites could give impulses to the vomiting center. The phenomena of emesis could be induced at the moment that sufficient afferent stimuli reach this center. In this hypothesis, it is not necessary that the same target-tissue is infected in each vomiting pig. Vomiting might be induced when a sufficient number of infected neurons are present in one or more target-tissues. Further studies on this question are in progress.

V. THE PATHOGENESIS OF THE WASTING

Chronically affected pigs have lost their appetite and rapidly become emaciated. They suffer from a paralytic ileus and often present a large distended abdomen.[23] This unthrifty state may persist for several weeks until they die of starvation or secondary disease.[1] The stomach of such pigs is dilated and contains much gas, together with a yellow-green fluid.[23]

Recent experiments showed that the wasting syndrome can be experimentally reproduced by inoculating colostrum deprived pigs around the fifth day of age. Radiologic studies were performed to follow the passage of food through the alimentary tract of two chronically infected pigs and one control animal. Thirty-five to fifty ml of micropaque were brought into the stomach lumen via a gastric tube. In the control pig the stomach was empty after 13 to 17 hours whereas the barium was retained in the stomach lumen for 5 to 7 days in the two diseased pigs. Although only a few animals were tested until now, the difference can be considered as remarkable.

Based on the present knowledge, the following concepts on the pathogenesis of the wasting can be put forward. After infection of certain neurons in the brainstem, the stomach itself and/or the associated ganglia, the gastric emptying mechanism is greatly disturbed. The food stagnation in the stomach reduces the appetite

and can cause gastric dilation. Furthermore, the few swallows of milk which are taken up are retained in the stomach for several days and its nutritive value may have become very low when entering the intestine. The pigs have to use their own body protein and glycogen to stay alive, but die of emaciation after a few weeks.

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