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**Pathogenicity of Hemagglutinating Encephalomyelitis
(Vomiting and Wasting Disease) Virus of Pigs,
using Different Routes of Inoculation**

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With 2 tables

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1. Introduction

In 1961, an infectious encephalomyelitis of viral origin was observed in pigs in Canada (7). The clinical illness was characterised by a prodromal stage with anorexia, depression, retching and vomiting, followed by motor disorders (1). The virus responsible for this disease was later found to be a porcine coronavirus (8; 13) which, because of its hemagglutinating properties, was called hemagglutinating encephalomyelitis virus or HEV (7).

An infectious disease of suckling pigs, characterised by vomiting and wasting, was described in England in 1969 (2) and later observed in other European countries (4; 6; 11; 15; 16). The disease, called vomiting and wasting disease, was shown to be caused by a virus antigenically and structurally similar if not identical to the HEV from Canada (3; 12; 13). A similar coronavirus, which caused mainly vomiting and wasting symptoms in hysterectomy-derived colostrum-deprived pigs was also reported from the U.S.A. (9). Using the same field isolates MENGELING was able to reproduce experimentally the two clinical entities, the encephalitic form and the vomiting and wasting form (10). It was, therefore, concluded that both types of the disease, are caused by the same virus.

Few studies have been performed on the pathogenesis of this viral infection. Transmission experiments have proved that reproducible results could be obtained only if colostrum-deprived pigs were used (7; 14). Clinical disease has been successfully reproduced after inoculation of the virus by oral, intranasal, intramuscular and intracerebral routes (7). In other studies, the virus was reisolated with regularity from brain, respiratory tract and oronasal swabs, but only if the pigs were examined during the early stages of the disease (3; 12). Hemagglutination inhibiting and seroneutralizing antibodies were demonstrable starting at 6 and 9 days after inoculation respectively (12). These antibodies are suspected to preclude virus isolation at later stages of the disease (5; 9; 12).

The present studies were primarily designed to determine whether a virus isolate, obtained from pigs with the vomiting and wasting syndrome only, could produce clinical signs after inoculation by different routes. At the same time, some information was obtained on the optimal conditions for virus reisolation and on the distribution of the virus in different organs particularly after oronasal inoculation.

2. Material and Methods

2.1. Virus

The virus strain, designated VW 572, was isolated in Belgium in 1972 as previously described (12). The virus stock used represented the 10th passage in primary pig kidney (PPK) cells and contained $10^{6.7}$ TCID₅₀ per ml.

2.2. Pigs and inoculation routes

The studies were performed in hysterectomy-derived-colostrum-deprived (HDCD) pigs which were individually housed in Horsfall type units. They were inoculated at the age of 3 to 5 days using different routes of inoculation. A blood sample was withdrawn prior to inoculation to assure the absence of specific viral antibodies. Sixteen piglets were inoculated by combined oral and nasal (ON) route, using 2 ml. of stock virus. In seven piglets, 0.7 to 1 ml. of stock virus was inoculated directly into the stomach wall after laparotomy around the pyloric sphincter at four inoculation sites. Five pigs were inoculated with 1 ml. of stock virus directly into the gastric lumen after laparotomy by inserting a needle through a canula which perforated the stomach wall. In two pigs, 1 ml. of stock virus was brought into the abdominal cavity after laparotomy. Two pigs were inoculated with 1 ml. of stock virus intravenously into the ear vein. Five pigs were inoculated in the cerebral cortex, using 1 ml. of stock virus, by perforating the skull with a needle. Four pigs were inoculated into the infraorbital nerve with 0.5 ml. of stock virus by insertion of the needle into the infraorbital canal. Eight pigs were inoculated with 1 ml. of stock virus intramuscularly either in the neck muscles or in the foreleg muscles or in the hindleg muscles.

All pigs were observed three times a day for clinical signs. When sick, pigs were killed at time intervals varying from one to five days after the appearance of clinical signs and different tissues were collected for virus isolation. From the ON inoculated pigs, serum was collected at the time of killing for detection of hemagglutination inhibiting (HI) and seroneutralizing (SN) antibodies. The pigs which did not become sick during the present study were followed clinically until 3 weeks after the inoculation. During the last week of the observation period, a serum sample was collected and examined for the presence of HI-antibodies.

2.3. Virus isolation and serology

From the pigs killed at different time intervals after inoculation, the following tissues were collected for virus isolation: nasal mucosa, tonsils, lungs (apical and cardiac lobes), pyloric region of the stomach, pons and medulla combined, cerebrum, cerebellum and blood clot. From each organ, a 20 per cent suspension (w/v) was prepared in phosphate buffered saline by homogenisation. After centrifugation at $7500 \times g$, the supernatant fluid was collected and 0.1 ml. inoculated in each of 10 tubes with fully sheeted primary pig kidney (PPK) cells. After four days, hemadsorption with chicken erythrocytes was attempted on 5 tubes. The 5 remaining tubes were used for making

a second passage. The cells and culture medium were subjected to two cycles of freezing and thawing and, after centrifugation at $700 \times g$, the supernatant was inoculated in 5 tubes with fresh monolayers of PPK-cells. Hemadsorption was performed on all tubes 4 days later.

The procedures used for the hemagglutination inhibition and seroneutralisation test were previously described (12).

3. Results

In the presentation of the results, differentiation will be made between the ON inoculated pigs and the pigs inoculated by other routes. The number of pigs in the ON inoculated group was sufficiently high to allow a more complete study since animals could be killed at several time intervals after the appearance of clinical signs. For the other routes of inoculation, it was the main purpose to see whether clinical illness could be obtained or not.

3.1. Oronasally inoculated pigs

The overall results obtained after ON inoculation of the 16 pigs are presented in Table 1. The individual results of these pigs are presented in Table 2. All pigs were free of HI- and SN-antibodies at the time of inoculation. It can be seen from the tables that all ON inoculated pigs became ill. The incubation period varied from 3 to 7 days with an average of 5 days. The earliest clinical signs were characterised by anorexia and vomiting which suddenly appeared. The vomiting usually started after the uptake of milk. The vomit first consisted of milk curds and changed, after a few hours, to a yellow foamy fluid. As soon as the piglets took up more milk, curds were seen again. Vomiting and retching movements were very regularly present during the first 24 hours. Afterwards, the frequency of vomiting decreased but the appetite remained markedly reduced. The smaller pigs became emaciated and moribund after 3 to 4 days. The course of the disease was somewhat slower in the stronger pigs.

Virus could be isolated from 11 of the 16 pigs. As shown in Table 2, the virus was isolated most consistently from the lungs and tonsils. It was sometimes isolated from the pons + medulla and nasal mucosa, and only in 2 pigs from the stomach wall. Virus was not isolated from the cerebrum, the cerebel-

Table 1
Results of Inoculation of pigs with VW 572 Virus by different Routes

Inoculation route	Nr. of pigs sick / Nr. of pigs inoculated	Incubation period (days)		Virus reisolation		HI-antibodies**	
		Range	Average	Nr. of pigs positive* / Nr. of pigs sick	Nr. of pigs positive / Nr. of pigs without clinical signs.		
Oral + nasal	16 / 16	4 - 9	5	11 / 16	-		
Stomach wall	6 / 7	2 - 4	3	4 / 6	1 / 1		
Stomach lumen	0 / 5	-	-	-	4 / 5		
Abdominal cavity	0 / 2	-	-	-	2 / 2		
Intravenous	0 / 2	-	-	-	2 / 2		
Intracerebral	3 / 5	3 - 4	3.5	3 / 3	2 / 2		
Infraorbital nerve	3 / 3	4 - 7	5	3 / 3	-		
Intramuscular	6 / 8	2 - 5	3.5	5 / 5***	2 / 2		

* Pigs were considered positive if virus was isolated from tissues different from the inoculation site.

** Hemagglutination — inhibiting antibodies.

*** Virus isolation was not performed in one pig.

lum and the blood clot. All pigs which were killed 6 days or later after inoculation had HI-antibodies, whereas SN-antibodies were detected starting 7 days after inoculation.

Table 2

Virus isolation and serological reaction in experimental pigs after oronasal inoculation with VW 572 Virus

Pig nr.	Incubation period (days)	Killed after inoculation (days)	Virus isolation								Serological reaction	
			Nasal mucosa	Tonsils	Lung	Stomach wall	Pons + medulla	Cerebral cortex	Cerebellum	Blood clot	HI*	SN**
202	3.5	4	NT	+	+	-	-	-	-	-	< 2	< 5
203	4	4	NT	+	+	-	-	-	-	-	< 2	< 5
296	3	4	+	+	+	+	-	-	-	NT	< 2	< 5
209	4	5	NT	+	+	-	+	-	-	-	< 2	< 5
121	5	6	+	+	+	NT	+	-	-	-	4	< 5
122	4.5	6	-	+	+	NT	+	-	-	-	4	< 5
211	3.5	6	NT	+	+	-	+	-	-	-	64	< 5
298	6	6	+	+	+	-	-	-	-	-	16	< 5
221	6.5	7	-	NT	+	+	NT	-	-	-	64	< 5
299	7	7	-	+	+	-	-	-	-	-	256	64
220	6.5	7	-	NT	-	-	NT***	-	-	-	128	< 5
214	5.5	7	-	NT	-	-	NT***	-	-	-	128	40
204	4	7	NT	-	-	-	-	-	-	-	256	10
183	6	9	NT	+	-	-	-	-	-	NT	64	< 5
120	4.5	9	-	-	-	NT	-	-	-	-	128	80
118	4.5	9	-	-	-	-	-	-	-	-	256	320

* HI: hemagglutination inhibiting antibodies.

** SN: seroneutralizing antibodies

*** The pons + medulla of these pigs was positive by immunofluorescence.

3.2. Pigs inoculated by other routes

The overall results obtained in the pigs inoculated by other routes are presented in Table 1. None of the pigs inoculated into the stomach lumen, the abdominal cavity or the ear vein had become sick at 3 weeks after inoculation. All these animals, except one of the pigs inoculated into the gastric lumen, had HI-antibodies at that time. A variable number of pigs became sick after inoculation by other routes. The clinical disease was not different from that observed after ON inoculation. In Table 2, the sick pigs were considered to be positive for virus when it was isolated from one or more of the collected organs, the inoculated organ not included. Six of the seven pigs inoculated in the pyloric region of the stomach became sick after an average incubation period of 3 days. From two of the six pigs, the virus was reisolated from the stomach wall only whereas, in the remaining four, the virus could be detected in other tissues also. HI-antibodies (titer 64) were present in the serum of the pig without clinical signs at the end of the observation period. Of the intracerebrally and intramuscularly inoculated pigs, three of the five and six of the eight respectively became sick after an average incubation period of 3½ days. All the diseased pigs were positive for virus. The pigs which remained healthy had built up HI-antibodies at titers varying from 6 to 128 at the end

of the experiment. All three pigs inoculated into the infraorbital nerve became sick after an average incubation period of 5 days and were positive for virus.

4. Discussion

The clinical disease and the results of virus isolation obtained upon combined oral and nasal (ON) inoculation were similar to those previously described (12). The virus was most consistently reisolated from the respiratory tract which is probably the natural way of infection. Seroneutralizing antibodies appear soon after the beginning of the clinical symptoms and may be a limiting factor for the further successful isolation of the virus from organs. For the diagnosis of hemagglutinating encephalomyelitis by virus isolation, pigs should therefore be examined preferably within two days after the start of clinical signs. The negative results obtained in pigs 220 and 214, killed within this period of time, are probably due to the fact that tonsils and pons + medulla were not collected for virus isolation. The pons + medulla of these pigs was later found to be positive by immunofluorescence.

The irregularity with which virus was isolated from the stomach wall and pons + medulla does not allow any conclusions to be drawn about the exact target organ, if it is supposed that virus replication in one of these organs is responsible for clinical signs. This irregularity may be due to the lack of sensitivity of the virus isolation technique. Recently, it has been found that centrifugation of tissue suspensions at $7500 \times g$. instead of $2000 \times g$. can remove up to $1 \log_{10}$ TCID₅₀ of hemagglutinating encephalomyelitis virus. In this way, virus isolation from a tissue suspension with little infectious virus could give a false negative result. It is known from electron microscopic studies that HEV during intracellular replication is mainly present in cytoplasmic vesicles with a large amount of membranous material (8). The removal of virus by centrifugation may, therefore, be due to sedimentation of particles associated with subcellular fragments.

Upon inoculation into the stomach lumen, clinical signs were not observed. This finding indicates that virus reaching the gastric lumen after swallowing does not initiate clinical disease. However, the seroconversion observed in 4 of the 5 pigs indicates that a subclinical infection has occurred.

The ability to reproduce typical disease after a very short incubation period in 6 of the 7 pigs inoculated in the stomach wall may be significant in the light of the pathogenesis. In two of these piglets, virus was isolated from the stomach wall only. This points to the stomach wall as a possible target organ for the virus. It is, however, not excluded that the isolated virus is a residue of the inoculum rather than a result of virus replication. The first possibility is rather improbable since these pigs were killed 3 and 4 days after inoculation respectively.

In the present experiments, not all the pigs became sick after intracerebral inoculation and the disease consisted of the vomiting and wasting syndrome. These results are somewhat different from those described by GREIG, who obtained clinical signs consisting of motor central nervous disorders in all inoculated pigs (7). This may be related to the observation that, with the present virus isolate, only the vomiting and wasting syndrome has been observed, not only in the original outbreak but also in experimental pigs. It should be mentioned, however, that MENGELING was able to reproduce both clinical entities with the same virus isolate (10). The inability to find the virus in the cerebral cortex of ON inoculated pigs may indicate that the present isolate has little or no affinity for this part of the central nervous system. It is not impossible that, in intracerebrally inoculated pigs, the appear-

ance of clinical signs is determined by the ability of the virus to reach the pons + medulla, another possible target organ, from the deposition site.

The findings that none of the pigs became sick after intravenous inoculation and that the virus was never isolated from the blood clot of pigs killed at different time intervals after ON inoculation indicate that the target organ is not reached through viraemia. On the other hand, the ability to obtain typical disease after inoculation into the infraorbital nerve suggests that virus spread within the body occurs via nerve pathways. Whether clinical signs are obtained in pigs after intramuscular inoculation may be dependent on the ability of the virus to enter into nerves.

From the present studies, it was impossible to determine the exact target organ of the VW 572 virus. The results indicate that virus replication, either in the stomach wall or in the pons + medulla or possibly in both, may be responsible for causing the vomiting and wasting syndrome in pigs.

Summary

Forty-eight pigs were inoculated by different routes with the VW 572 isolate of the hemagglutinating encephalomyelitis (vomiting and wasting disease) virus. All piglets inoculated by the combined oral — nasal route (16) or into the infraorbital nerve (3) became sick after an incubation period of 5 days. Six of the 7 pigs inoculated into the stomach wall, 6 of the 8 pigs inoculated intramuscularly and 3 of the 5 pigs inoculated intracerebrally became ill after an incubation period of 3—3.5 days. None of the pigs inoculated either intravenously or into the abdominal cavity or into the stomach lumen became sick. All diseased pigs showed the vomiting and wasting syndrome. In oronasally inoculated pigs, killed during the early stages of disease, the virus was reisolated consistently from the tonsils and respiratory tract but irregularly from the pons + medulla and the stomach wall. Pigs inoculated by other routes were positive for virus when sick. All except one of the pigs which remained healthy had seroconverted. The site of virus replication which gives rise to the vomition could not be determined. It was concluded from the present studies that virus spread within the body occurs along nerve pathways.

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Zusammenfassung

Die Pathogenität von hämagglutinierendem Enzephalomyelitis-Virus (Kümmern und Erbrechen) bei Schweinen nach Infektion über verschiedene Inokulationswege

Achtundvierzig Schweine wurden über verschiedene Inokulationswege mit dem VW 572-Isolat des hämagglutinierenden Enzephalomyelitis-Virus (Kümmern und Erbrechen) infiziert. Alle Schweine, die entweder kombiniert oro-nasal (16) oder über den Infraorbitalnerv (3) infiziert wurden, erkrankten nach einer Inkubationszeit von 5 Tagen. Sechs der sieben über die Magenwand inokulierten, 6 oder 8 intramuskulär und 3 der 5 intrazerebral infizierten Tiere wurden nach einer Inkubationszeit von 3—3,5 Tagen krank. Bei den Schwei-

nen, die intravenös oder in die Bauchhöhle bzw. direkt in den Magen inokuliert wurden, kamen Erkrankungsfälle nicht vor.

Alle erkrankten Schweine zeigten das Syndrom des Kümmerns und Erbrechen. Von oro-nasal infizierten Schweinen, die während des Frühstadiums der Erkrankung getötet wurden, konnte das Virus regelmäßig von den Tonsillen und dem Respirationstrakt und gelegentlich vom Gewebe des Pons-Medulla-Bereiches sowie aus der Magenwand reisoliert werden. Von Schweinen, die nach Infektion über andere Routen erkrankten, ließ sich immer Virus isolieren. Alle Tiere, die nicht erkrankten (mit Ausnahme eines Ferkels) bildeten jedoch Antikörper. Der Ort der Virusvermehrung, mit dem das Erbrechen zusammenhängt, ließ sich nicht ermitteln. Die Ergebnisse der vorgelegten Untersuchungen lassen den Schluß zu, daß die Virusausbreitung im Körper über die Nervenbahnen erfolgt.

Résumé

Pathogénicité du virus hémagglutinant de l'encéphalomyélite du porc (déperissement et vomissement) après infection par différents modes d'inoculation

48 porcs ont été infectés selon différents procédés d'inoculation avec l'isolement VW 572 du virus hémagglutinant de l'encéphalomyélite (déperissement et vomissement). Tous les porcs infectés soit par la voie combinée oro-nasale (16) soit par le nerf infraorbital (3) tombèrent malades après une incubation de 5 jours. 6 des 7 animaux infectés par la paroi stomacale, 6 des 8 par voie intramusculaire et 3 des 5 intracérébralement tombèrent malades après un temps d'incubation de 3—3,5 jours. Il n'y a pas eu de maladie chez les porcs inoculés par voie intraveineuse, dans l'abdomen ou directement dans l'estomac.

Tous les porcs malades ont présenté le syndrome de déperissement et de vomissement. Chez les animaux infectés par voie oro-nasale et sacrifiés au début de la maladie, on a pu régulièrement réisoler le virus à partir des amygdales et de l'appareil respiratoire, parfois du tissu de la région «Pons-Medulla» et de la paroi stomacale. Le virus a toujours été isolé chez les porcs tombés malades après un mode d'infection différent. Tous les animaux qui ne furent pas malades (à l'exception d'un porcelet) formèrent des anticorps. L'endroit de multiplication du virus lié au syndrome de vomissement n'a pas été déterminé. Les résultats de ces essais permettent de conclure que la propagation du virus dans le corps se fait par voie nerveuse.

Resumen

La patogenicidad del virus hemoaglutinante de la encéfalomielitis (hipotrepia y vómitos) en el cerdo tras infección a través de vías diversas de inoculación

Se infectaron cuarenta y ocho cerdos a través de diferentes vías de inoculación con el aislamiento VW 572 del virus hemoaglutinante de la encéfalomielitis (hipotrepia y vómitos). Todos los cerdos infectados bien con arreglo al procedimiento combinado buco-nasal (16) o bien a través del nervio infraorbitario (3) enfermaron tras un tiempo de incubación de 5 días. Seis de siete animales inoculados a través de la pared gástrica, 6 de 8 por vía intramuscular y 3 de 5 por vía intracerebral enfermaron tras un tiempo de incubación de 3—3,5 días. No se registraron casos de enfermedad en los cerdos inoculados por vía intravenosa o en la cavidad abdominal resp. directamente en el estómago.

Todos los cerdos que enfermaron presentaban el síndrome de hipotrepisia y vómitos. De los cerdos infectados por vía buco-nasal, que se sacrificaron durante el estadio precoz de la enfermedad, se pudo reaislar el virus con regularidad de las tonsilas y del tracto respiratorio y, en ocasiones, del tejido correspondiente al ámbito puente de Varolio-medula, así como de la pared gástrica. Se logró siempre aislar virus de cerdos que enfermaron tras infección por otras vías. Sin embargo, todos los animales que no enfermaron (excepción hecha por un lechón) produjeron anticuerpos. No se logró descubrir el lugar en donde se multiplicaba el virus, hecho relacionado con los vómitos. Los resultados de los estudios presentados permiten llegar a la conclusión de que la propagación del virus en el organismo acontece a través de las vías nerviosas.

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