THE DIAGNOSIS OF CORONAVIRUS-LIKE AGENT (CVLA) DIARRHEA
IN SUCKLING PIGS

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ABSTRACT

Two techniques are evaluated for the diagnosis of CVLA enteritis in suckling pigs. CVLA antigens can be demonstrated in the absorptive epithelial cells of the small intestine by the direct immunofluorescent staining test (IF). Very reliable results were obtained with this technique in piglets having diarrhea for only 3-4 days. Coronavirus particles can also be detected by electron microscopy (EM) in the feces of scouring pigs, but this should be followed by a specific identification test since there is no morphological difference between CVLA and transmissible gastroenteritis (TGE) virus. The EM is furthermore less sensitive than the IF test for the diagnosis of CVLA enteritis.

INTRODUCTION

The coronavirus-like agent (CVLA) has been described since 1977 as a pathogen for swine, causing enteritis in pigs of all ages (Pensaert, 1978; Debouck, 1980). The electron microscopical appearance of this virus indicates that it probably belongs to the Coronavirus family. The CVLA was also shown to be antigenically distinct from the 2 known porcine coronaviruses, Transmissible gastroenteritis (TGE) virus and Hemagglutinating encephalomyelitis virus. When a sudden outbreak of diarrhea occurs on a swine breeding farm, involving the piglets as well as the sows, then one should make a differential diagnosis between TGE and CVLA infection. This is very difficult to perform on the basis of the clinical signs. Up to now, it is impossible to grow the CVLA in tissue culture. The etiologic diagnosis of a CVLA infection should therefore be made by direct demonstration of the virus in sections of intestinal mucosa or in the feces of scouring pigs.

METHODS AND RESULTS

The immunofluorescent staining technique (IF) on cryostat sections of small intestine from piglets with diarrhea, is routinely used for the

diagnosis of TGE (Pensaert *et al.*, 1968). This technique was adapted for the diagnosis of CVLA enteritis in young pigs. A hyperimmune serum against CVLA was raised in a SPF pig and the globulin fraction of the serum was conjugated with FITC. The specificity of the conjugate was assured by an inhibition test and by the absence of fluorescence when used on TGEV and rotaviral containing intestinal sections. The method of the IF technique is described in the manual. The sensitivity and reliability of the IF as a diagnostic test in CVLA diarrhea were controlled by staining intestinal sections of experimentally infected pigs. Thirteen pigs were inoculated when 2 days old and they were killed at different intervals during the first 4 days of diarrhea. Specific fluorescence was observed in epithelial cells covering the small intestinal villi of all the pigs. Intestinal sections from the jejunum of an uninoculated control pig showed no fluorescence.

We also looked for another diagnostic test for which it was not necessary to kill the animals. The only test that is working up to now is the electron microscopic examination of the feces of scouring piglets. However, the detection of a coronavirus particle in the feces of a pig with diarrhea does not tell us whether it is CVLA or TGE virus. Both particles cannot be distinguished morphologically. This implies that the EM is only of limited value for a diagnosis of coronaviral diarrhea in *naturally* infected pigs, unless it is followed by a specific identification test. Nevertheless, EM may be very useful in the detection of CVLA in feces of *experimentally* inoculated piglets. The sensitivity of the EM was tested in piglets experimentally inoculated with CVLA when younger than 20 days by examination of feces collected at different moments after the onset of diarrhea. The results are shown in table 1. The highest percentage of positive fecal samples (maximally 73 %) was obtained at the first day after the beginning of the diarrhea. Consecutive samples collected from the same pig were sometimes alternately positive and negative. If several fecal samples from a same piglet were examined, then the sensitivity of the EM increased to about 90 %. In another experiment, CVLA particles were never detected in feces collected from fattening swine having diarrhea after an experimental inoculation with the CVLA.

CONCLUSIONS

At present, the field diagnosis of a CVLA enteritis can only be made by the IF technique on cryostat sections of young pigs having diarrhea

for less than 3 days. The sensitivity and the reliability of this technique are very high provided that it is carried out on intestinal segments collected from killed pigs, as described in the manual. The electron microscopic examination of feces is limited in sensitivity and is not specific. It can, therefore, only be used in experimentally inoculated pigs from which several fecal samples should be collected. Fecal samples of naturally infected piglets should be examined by immune EM. The feasibility of the immune EM on random collected fecal specimens remains, however, to be established.

We are yet unable to perform the diagnosis of a CVLA infection in pigs older than 2 weeks, until other techniques i.e. serology are established.

TABLE 1 - ELECTRON MICROSCOPICAL EXAMINATION OF FECES FROM 38 PIGLETS, EXPERIMENTALLY INOCULATED WITH THE CORONAVIRUS-LIKE AGENT (CVLA)

Days after onset of diarrhea	Number examined	Number positive for CVLA	(%)
0	37	26	(70)
1	15	11	(73)
2	14	9	(64)
3	13	5	(38)
4	8	0	
5	2	1	

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