DIAGNOSIS OF HOG CHOLERA

E. A. CARBREY, VMD, MS; G. A. ERICKSON, DVM, PHD; and C. A. METZ
USDA, APHIS, VS, NVSL, DVL, P.O. Box 844, Ames, Iowa 50010 USA

ABSTRACT


Hog cholera (HC), or classical swine fever, cannot be accurately differentiated from African swine fever (ASF) by clinical signs or lesions observed at necropsy. A well-equipped diagnostic laboratory with a trained staff is the only solution to this problem. Specimens should be submitted to the laboratory from pigs with lesions suspicious of HC or ASF. The best method for confirming HC in a laboratory with limited facilities is the fluorescent antibody tissue section technique. This procedure requires only three major pieces of equipment: microtome-cryostat, incubator, and fluorescence microscope. Tonsil is the specimen of choice and a biopsy of tonsil tissue may be obtained without killing the pig. Hog cholera virus may be isolated in pig kidney cell cultures (PK-15 cell line) from spleen or lymph node. The presence of the virus is detected by staining the inoculated cell cultures with anti-HC fluorescent antibody conjugate. The neutralization test (Nt) may be used to detect HC antibody titers in the sera of pigs surviving infection with strains of low virulence. However, bovine viral diarrhea virus infection will cause heterologous titers against HC virus. In addition, antibody titers produced by vaccination cannot be distinguished from those caused by natural infection.

INTRODUCTION

The clinical signs of hog cholera (HC) (classical swine fever) are often confused with those of African swine fever (ASF) although the two similar diseases of swine are caused by different viruses. A firm diagnosis of HC can only be made by using laboratory procedures that permit the detection of the virus, its antigens, or antibodies produced in response to infection with it (Stewart, 1981). Laboratory assistance is also necessary to distinguish HC from some cases of salmonellosis, erysipelas, transmissible gastroenteritis, and pseudorabies.

Another problem in the diagnosis of HC is the natural occurrence of viral strains of reduced virulence that cause chronic disease (Carbrey et al, 1980). These strains may cause abortions, disease in baby pigs, or clinical signs in older pigs as part of a mixed infection with pathogenic bacteria. All of the infected pigs do not die. At the National Veterinary Services Laboratories pigs have died following inoculation with a splenic suspension that yielded both HC virus and Salmonella choleraesuis on culture. When either of these agents was administered separately, the pigs survived after a mild course of disease.
DIAGNOSIS

A well-equipped diagnostic laboratory with a trained staff capable of confirming viral infections is needed to differentiate HC from ASF. The laboratory should be part of a government agency that has the responsibility for animal health in that state or country. The budget of the laboratory must be maintained on an annual basis without fluctuations in support. The supporting government agency must have people capable of procuring the variety of supplies needed by the laboratory. Contracts must be negotiated for the maintenance of sophisticated equipment. In view of the expense involved, it is advisable to concentrate virologic diagnosis in one laboratory that is centrally located or for several states to support one facility.

LABORATORY TECHNIQUES

Fluorescent Antibody Tissue Section Test (FATST) (Cottral, 1978)

Tonsil is the specimen of choice for this technique and may be obtained without killing the pig. Pigs have flattened areas of tonsillar tissue located on each side of the midline in the posterio-dorsal part of the oral cavity. A small but adequate piece of tonsil may be taken using a human rectal biopsy forceps. Using a speculum to force open the mouth, the forceps is inserted along the hard palate and onto the soft palate a little to either side of the midline. The forceps is opened, the upper jaw of the forceps is pushed into the tonsil, and the jaws are closed. The instrument is removed and the jaws are opened. The biopsy should be teased from the forceps with a needle so as not to macerate the tissue. It should be examined for the pores of the crypts to confirm that tonsillar tissue was obtained. The tissue may be sent to the laboratory on dry or wet ice. If pigs are necropsied, the entire tonsil, mandibular lymph nodes, and spleen from 4 to 6 pigs should be submitted. An advantage of the tonsil biopsy is that a larger number of pigs may be sampled. In addition, a blood sample for a serum antibody test can be obtained while the pig is restrained.

Only three major or expensive pieces of laboratory equipment are required for the FATST: microtome-cryostat, incubator and fluorescence microscope. Blocks of tissue 10 mm square by 3 mm thick are cut from the tonsil, mandibular lymph nodes and spleen and are frozen on microtome chucks with OCT Compound or water.

1 Yoemans Rectal Biopsy Forceps 110AM-663, 14½", rounded end, American Medical Instrument Corp., 133-14 39th Avenue, Flushing, NY 11354

2 Lab-Tek Tissue Culture Chamber/Slides, Lab-Tek Products, Naperville, IL 60540
The biopsy specimens are mounted intact. Tissue sections 8 micrometers thick are cut, mounted on glass slides, fixed in acetone for 10 minutes and dried. The sections are covered with HC fluorescent antibody conjugate (Mengeling et al., 1963; Carbrey et al., 1971; Cottral, 1978) and incubated in a moist chamber at 37°C for 30 minutes. For control purposes, additional sections may be stained with a mixture of equal parts of HC antiserum and HC conjugate to block specific staining and other sections stained with normal serum and HC conjugate to detect nonspecific staining.

Conjugate is decanted from the slides, the sections are rinsed with PBS and distilled water, and coverslips are applied with buffered glycerine. Using a fluorescence microscope, cells infected with HC virus are bright green while uninfected cells are dark green or brown. Usually infected cells are found in patches or clusters, but occasionally the entire section will fluoresce. In the latter case, careful comparison with the control slides may be necessary. The specific viral fluorescence is most easily detected in the tonsillar epithelial cells while fluorescence in the germinal centers may be caused by nonspecific staining of the macrophages and other leukocytes.

During the last extensive outbreak of HC in the U.S. in 1976 (Young et al., 1976) the examination of tonsil biopsies was found to be a highly effective method for detecting HC infection. Management practices involved garbage feeding and the indiscriminate use of illegal HC vaccines and antiserum. Herdsmen were uncooperative and often did not report losses so that specimens could be obtained. By collecting tonsil biopsies from 20 to 30 pigs in suspect herds, it was possible to confirm HC infection and justify depopulation.

Fluorescent Antibody Cell Culture Test (FACCT) (Carbrey et al., 1971)

Proficiency in the growth of cell cultures is necessary if a laboratory is to isolate and identify HC virus. For the laboratory that does not have this competence, it is a major and sometimes difficult step. The availability of commercially prepared cell culture media, plastic utensils, and disposable glassware makes it much easier although more expensive. Obtaining a supply of pure water suitable for cell cultures and good serum supplements without viral or mycoplasmal contamination remains a serious problem.

The serum used as a supplement in the propagation of pig kidney cell cultures intended for HC virus detection must be free of antibodies against bovine viral diarrhea (BVD) virus. Such antibodies will neutralize HC virus and interfere with its ability to infect the cells. If bovine fetal serum is used, there is an additional problem of BVD virus contamination arising from intrauterine infection of the donor calf. The presence of BVD virus in the supplement serum will result in a false positive confirmation since the HC conjugate will readily
stain the BVD infected cells. A practical solution is to obtain a calf as soon after birth as possible, deprive it of colostrum, and raise it in isolation. Its blood should be tested for BVD virus and antibody. A high quality nutrient serum can be obtained from the calf using biweekly bleedings for up to one year of age.

A suspension of splenic, tonsil, or lymph node tissue prepared in cell culture medium is inoculated into Leighton tubes with coverslip cell cultures. The PK-15 (pig kidney) cell line has been used extensively. After incubation at 37°C for one hour, the cultures are washed, the medium is replaced and the cultures are maintained at 37°C. After incubation overnight the coverslips are removed, fixed with acetone, and stained with conjugate. Using fluorescence microscopy HC-infected cells are readily detected by their bright green color in contrast to the darker green or brown color of the uninfected cells. Negative and questionable results should be confirmed by repeating the procedure with the remaining inoculated cell cultures incubated for an additional 24 hours.

Although the FACCT is much more difficult than the FATST, it provides additional confirmation of positive cases and is more convenient than pig inoculation. Distinguishing the specific viral fluorescence in coverslip cell cultures is much easier than in tissue sections. The few fluorescing cells observed in doubtful cases will become clusters of positive cells after the additional incubation of replicate cultures.

Neutralization Test (Nt)

Since many of the indigenous strains of HC virus are of reduced virulence and produce chronic infections, the detection of serum antibodies with the Nt can provide a diagnostic confirmation of HC infection (Carbrey et al, 1969). This test was an extremely useful tool in the later stages of the eradication program in the United States. Using PK-15 cell cultures and the fluorescent antibody technique, coverslip cultures are exposed to fourfold dilutions of serum mixed with virus. Cell cultures grown in four-chambered Lab-Tek slides are a convenient substitute for Leighton tubes. Positive titers of 1:4 to 1:16 are detected in pigs as early as 21 days after infection, and peak titers of 1:64 to 1:1024 are found in five to six weeks. However, vaccination, even with highly attenuated live virus vaccine, will produce significant neutralizing titers. Also, the previous administration of hyperimmune antiserum or the ingestion of colostrum by baby pigs nursing an immune sow will cause passive antibody titers. Another problem in interpreting low HC antibody titers is the frequent, inapparent infection of pigs with BVD virus. This often occurs when pigs are maintained in close contact with cattle. Pigs with titers of 1:256 to 1:1024 against BVD may have cross neutralizing titers against HC as high as
1:4 to 1:16. When using the Nt for HC, the laboratory must routinely test all positive serums against BVD virus to make certain the HC titer is specific and not due to cross-neutralization by BVD antibodies.

Since the eradication of HC in the U.S., serologic surveys have been conducted continuously in high risk areas. These have included herds in the northeastern U.S. that use garbage as a food source, pigs located near the Mexico-Texas border, and pigs in Florida, Puerto Rico, and the Virgin Islands. Blood samples were collected at slaughter, and an effective identification system permitted traceback to the farm of origin. Serums were screened at the 1:16 dilution for HC antibodies, and all positive serums were titered against HC and BVD viruses.

From July 1, 1980, through June 30, 1981, 27,580 serums were tested for HC from the above listed sources: 26,764 (97.0%) were negative, 332 (1.2%) were positive and 484 (1.8%) were unsuitable for testing (Table 1). The HC positive serums were retested to determine the endpoint titers against HC and BVD viruses. All but 9 out of 319 positive serums had BVD antibody titers equal to or greater than the HC titers (Table 2). The 9 pigs from which these serums were obtained were traced back to the source herd and an investigation was made that included the collection of tonsil biopsies and additional serums. In all cases a diagnosis of HC was not established. Of the total serums tested in the survey only 0.03% were in this highly critical category. The remainder of the positive serums all had equal or higher titers against BVD than HC: 31 had BVD titers equal to HC titers, 98 BVD four times higher, 141 16 times higher, and 40 64 times higher. There were 13 serums for which there was an insufficient volume to perform the BVD test. The 31 serums with the same titers against HC and BVD were only 0.11% of the total.

In countries where the use of vaccine is quite limited, testing of serums collected at slaughter combined with a swine identification system would be an efficient method for detecting the location of HC infected herds. In many instances the traceback will lead to a herd where the virus is no longer present. However, additional traceouts may be made from this herd to others where the virus might still be actively causing disease.

SUMMARY

In some countries there is interest in the eradication of HC with the expectation that ASF may then be more easily diagnosed if it is introduced. Certainly there are better reasons for controlling HC than the difficulty of its differential diagnosis from ASF. The natural source of HC virus is the pig. The virus is usually introduced by movement of pigs. Many herdsmen will attempt to reduce their losses by sending the remaining healthy pigs to the salebarn after sickness appears in the herd. A competent diagnostic laboratory is
only one fundamental part of a control program. The swine owners must be informed and willing to support a program. The campaign should be administered by veterinarians whose activities are coordinated by a progressive government agency. Vaccination alone will not eliminate the disease. In fact, the routine semi-annual cost of vaccination is one of the best arguments for eradication.

The diagnostic tests are available to detect HC infection. It only remains for those concerned to apply their best efforts to the control of the disease.

REFERENCES


TABLE 1
Serologic Surveillance for Hog Cholera in Selected Areas of the United States from 7/1/80 through 6/30/81 - Initial Screening Test at the 1:16 Dilution

<table>
<thead>
<tr>
<th>Total</th>
<th>Negative</th>
<th>Hog Cholera</th>
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<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>(toxic)</td>
</tr>
<tr>
<td>Number of Serums</td>
<td>27,580</td>
<td>26,764</td>
<td>332</td>
</tr>
<tr>
<td>Percent</td>
<td>97.0</td>
<td>1.2</td>
<td>1.8</td>
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TABLE 2
Bovine Viral Diarrhea Antibody Titers of Serums Positive for Hog Cholera at 1:16 and Higher

<table>
<thead>
<tr>
<th>Hog Cholera</th>
<th>BVD &lt;16 BVD</th>
<th>BVD = 4X</th>
<th>BVD 16X</th>
<th>BVD 64X</th>
<th>Insuf. Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos.</td>
<td>BVD HC +</td>
<td>BVD HC &gt;</td>
<td>BVD HC &gt;</td>
<td>BVD HC &gt;</td>
<td>for</td>
</tr>
<tr>
<td>HC</td>
<td>BVD HC HC HC BVD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Serums</td>
<td>332</td>
<td>9</td>
<td>31</td>
<td>98</td>
<td>141</td>
</tr>
<tr>
<td>% of HC Positive</td>
<td>2.7</td>
<td>9.3</td>
<td>29.5</td>
<td>42.5</td>
<td>12.0</td>
</tr>
<tr>
<td>% of Total Tested</td>
<td>1.2</td>
<td>0.03</td>
<td>0.11</td>
<td></td>
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