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References


Simplified characterization of pseudorabies viruses using monoclonal antibody and selective cell culture analyses

Jonathan Bruce Katz, John George Landgraf

All modified-live pseudorabies virus (PRV) vaccines (V-PRV) used in the United States today contain glycoprotein gI (gI) gene deletions and/or deletions in the thymidine kinase (TK) gene (Table 1). Typically, wild type pseudorabies viruses (WT-PRV) are both gI and TK positive. Companion diagnostic glycoprotein-specific enzyme-linked immunosorbent assay (ELISA) systems are available; these allow serologic differentiation between swine vaccinated with V-PRV and those exposed to WT-PRV. Determination of TK phenotype has been previously described. A sample of 10⁴-10⁵ plaque forming units (PFU) of each isolate was serially passaged 4 times at 2-3-day intervals in a TK mouse connective tissue cell line (LMTK, American Type Culture Collection Certified Cell Line 1.3) in the presence of methotrexate. TK⁻ viruses propagated normally in these cells, but TK⁺ viruses were selectively unable to do so. Reference TK⁺ and TK⁻ PRVs were used as procedural controls. Following serial passage, culture supernatants were titrated for the presence of PRV in Madin Darby bovine kidney (MDBK) cells. TK⁺ viruses maintained viral titer, but TK⁻ viruses were completely eliminated (0 PFU/0.1 ml). TK⁺ results were always reconfirmed. Viral gI status was routinely assessed by examining 50-200 viral plaques in infected MDBK cell monolayers using 2 gI-specific but epitopically noncompetitive monoclonal antibodies (MAbs) in an indirect fluorescent antibody test (IFAT) format. Both of these MAbs (7-3 and 8-2) were used separately on each isolate to improve the probability of detecting gI expression.
Table 1. Phenotypic characterization of US-licensed modified-live pseudorabies virus vaccines.

<table>
<thead>
<tr>
<th>Name</th>
<th>Glycoprotein phenotype</th>
<th>Thymidine kinase phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR Vaccine, Ingelvac</td>
<td>gI</td>
<td>TK+</td>
</tr>
<tr>
<td>PR-Vac</td>
<td>gI</td>
<td>TK+</td>
</tr>
<tr>
<td>Tolvid</td>
<td>gI, gX</td>
<td>TK+</td>
</tr>
<tr>
<td>PRV-Marker</td>
<td>gI, gX</td>
<td>TK+</td>
</tr>
<tr>
<td>PRV-Marker Gold</td>
<td>gI, gX</td>
<td>TK+</td>
</tr>
<tr>
<td>Omnivac</td>
<td>gI</td>
<td>TK+</td>
</tr>
<tr>
<td>Omnimmak</td>
<td>gI, gIII</td>
<td>TK+</td>
</tr>
</tbody>
</table>

Other gI-specific MAbs were also found acceptable for assessment of gI status.

Ninety-five diagnostic isolates were examined using the TK and gI tests (81 porcine, 8 bovine, 4 ovine, 2 canine). Three reference WT-PRV isolates and all US licensed V-PRVs were also evaluated (Table 2). Among the diagnostic isolates, 88 were TK+ and gI+, 5 were TK+ and gI-, 1 was TK- and gI+, and 1 was TK- and gI+ (MAb 7-3+, MAb 8-2-). Isolate Nos. 1-88 were classified as typical WT-PRV. Isolate Nos. 90-94 (TK+, gI-) were determined by REA as different from either of the 2 TK+, gI+ V-PRV vaccine strains, indicating that they were either of atypical WT-PRV or altered V-PRV origin. The 2 atypical porcine isolates (Nos. 93, 94) differed by REA from each other and from the 3 atypical bovine isolates (Nos. 90-92). Isolate Nos. 90, 91, and 92 originated from the same Iowa cattle herd and had REA patterns indistinguishable from each other and from another atypical bovine isolate, No. 89 (TK-, gI-), which originated from Illinois (Fig. 1).

Isolate Nos. 90-92 were not eliminated by serial propagation in LMTK- cells but were reduced in titer to approximately 10^2 PFU/0.1 ml. In contrast, reference TK+ viruses and isolate Nos. 1-88 titered 10^4-10^6 PFU/0.1 ml. Isolate Nos. 90-92 were doubly plaque purified, each in quadruplicate, and the resulting 12 viruses reconfirmed phenotypically with uniform results.

Isolate No. 95 was TK+ and repeatedly IFAT positive with MAb 7-3 but negative with MAb 8-2. No V-PRV shares this profile (Tables 1, 2). A 27-kg PRV-seronegative pig inoculated intranasally with 10^7.5 PFU of isolate No. 95 developed severe clinical signs of PRV infection 5 days postinoculation (PI). Using a commercial gI-specific diagnostic ELISA test, gI+ seroconversion was detected 9 days PI and confirmed 19 days PI, indicating that isolate No. 95 was a virulent but atypical WT-PRV and that its degree of gI alteration did not preclude correct gI-specific serologic diagnosis.

The TK-selective cell culture/gI IFAT analytic system correctly classified all US-licensed PRV vaccines as V-PRV. The great majority of current field isolates were TK+ and gI+. The minority with atypical TK and/or gI phenotypes must be studied further to differentiate them into unaltered V-PRV, potentially altered V-PRV, and atypical WT-PRV categories. The possibility of multiple strains in an isolate must be considered, and multiple analyses of plaque-purified isolates may be necessary if strain heterogeneity is suspected. No unaltered V-PRV was found in this study.

Bovine herpesvirus 1 modified-live vaccines have under-
gone REA pattern alterations following only 1 passage through the host species.11 Some of the atypical WT-PRV identified in this study may have arisen in a similar manner. The similarities between the REA pattern of No. 89 and those of Nos. 90-92 suggests a possible iatrogenic V-PRV origin for these viruses. There is 1 report from Germany of a bovine origin PRV isolate with a pattern of split MAb reactivity similar to that found with isolate No. 95.8 In that study, the molecular basis of the unusual MAb reactivity was associated with a truncated form of gI.8 The simple phenotypic screening system described here will help identify those unusual isolates requiring further study to determine their possible origins and veterinary diagnostic significance.

Sources and manufacturers
b. SmithKline Beecham Animal Health, Lincoln, NE.
c. The Upjohn Co., Kalamazoo, MI.
d. Syntrovet, Lenexa, KS.
e. Fermenta Animal Health Co., Kansas City, MO.
f. American Type Culture Collection, Rockville, MD.
g. Lederle Parenterals, Carolina, PR.
h. Dr. N. Pfeiffer, SmithKline Beecham Animal Health, Inc., Lincoln, NE.
i. Dr. Q. Tonelli, IDEXX Corp., Westbrook, ME.
j. Dr. Tamar Ben-Porat, Vanderbilt University, Nashville, TN.

The use of a particular manufacturer’s product does not constitute an endorsement on behalf of the USDA.

References


Isolation of bovine herpesvirus 1 from preputial swabs and semen of bulls with balanoposthitis

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Balanoposthitis is a disease of the reproductive tract and may be caused by bovine herpesvirus-1 (BHV-1).7 BHV-1-infected animals, including those with nonapparent infection, become lifelong carriers.7 Latent infection may be sporadically reactivated by natural mechanisms or by corticosteroid therapy.8 In bulls used for semen production, the potential for viral reactivation presents a special problem because semen can be contaminated with large quantities of virus.8

Contaminated semen presents a potential threat to the cattle population because BHV-1 can cause infectious pustular vulvovaginitis, endometritis, salpingitis, shortened estrous cycles, and abortions in susceptible female cattle8 and balanoposthitis in susceptible bulls.7

Because of the potential for bovine herpesvirus dissemination by semen, only seronegative bulls should be used as donors at artificial insemination stations.7 However, seronegativity provides no assurance that semen will be free of BHV-1 virus. Semen may have been contaminated by a primary preputial infection before production of antibodies; seronegative animals carrying latent infection have been reported.8,9 Cell culture inoculation test has been generally used...