Seroprevalence of bovine viral diarrhea virus in alpacas in the United States and assessment of risk factors for exposure, 2006–2007

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Abstract

Objective—To estimate seroprevalence of antibodies against bovine viral diarrhea virus (BVDV) and incidence of seroconversion in alpacas in the United States during 2006 to 2007 and to evaluate associations between BVDV seropositive status and potential risk factors for exposure to BVDV.

Design—Cross-sectional and longitudinal cohort study.

Sample—Blood samples from 192 alpacas > 6 months old in 39 herds from 20 states; 40 owners who completed questionnaires.

Procedures—550 US alpaca owners, stratified by state and randomly selected from a list of approximately 4,300 owners, were mailed a study description, voluntary participation request, and questionnaire. Thirty-nine owners submitted blood samples from up to 6 alpacas > 6 months old; 27 of 39 owners submitted another blood sample from the same alpacas > 1 month later. Samples were tested for serum virus-neutralizing antibodies against BVDV. Seropositive status was used to indicate BVDV exposure. Associations between seropositive status and potential risk factors for BVDV exposure described in questionnaires were evaluated by use of a Fisher exact test.

Results—8 of 192 (4.2%) alpacas in 3 of 39 (7.7%) herds were seropositive. Larger herds had a greater percentage of seropositive alpacas than did smaller herds. No alpaca from which a second blood sample was obtained seroconverted during 292 to 1,460 alpaca-days (mean, 740 alpaca-days) of potential exposure.

Conclusions and Clinical Relevance—Results contributed to information on assessment of BVDV prevalence, risk factors for exposure, and alpaca industry practices in 2006 to 2007 during the emergence of BVDV as a major disease in alpacas.

Disciplines
Large or Food Animal and Equine Medicine | Veterinary Medicine | Veterinary Pathology and Pathobiology | Veterinary Preventive Medicine, Epidemiology, and Public Health

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Julie Ann C. Jarvinen, PhD, DVM, and Annette M. O’Connor, DVMc

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Prior to 2005, the seroprevalence of BVDV in South American camelids was low, clinical BVDV infections were uncommon in South American camelids, and BVDV was considered a minor health problem associated with exposure of South American camelids to BVDV-infected cattle.1–13 In 2005, the first alpaca PI with BVDV was documented, and it was associated with multiple cases of ill-thrift and reproductive loss in an alpaca herd.14,15 Subsequently, additional PI cria were detected in multiple alpaca herds throughout the United States and United Kingdom, and BVDV transmission from PI alpacas to naive alpacas was confirmed for naturally acquired and experimentally induced infections.16–28,29

The likelihood that alpacas PI with BVDV would increase the transmission and prevalence of BVDV within this species coupled with increasing numbers of clinical BVDV infections among camels in North America led to growing concerns in the alpaca industry.13,16,18,19,27 To address these concerns, a study of the national alpaca population was initiated in 2005, with the goal of determining the prevalence of BVDV in US alpaca herds and identifying potential risk factors for exposure to BVDV among alpacas < 6 months old.

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<table>
<thead>
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<th>Abbreviations</th>
<th>Definition</th>
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<tr>
<td>AOB</td>
<td>Alpaca Owners and Breeders Association</td>
</tr>
<tr>
<td>BVDV</td>
<td>Bovine viral diarrhea virus</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>PI</td>
<td>Persistently infected</td>
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<tr>
<td>SVN</td>
<td>Serum virus neutralization</td>
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</table>

The purpose of the study reported here was to determine the prevalence of BVDV in alpaca herds and identify potential risk factors for exposure to BVDV among alpacas > 6 months old, independent of that previous study.15 The first objective was to determine the seroprevalence for BVDV in US alpaca herds during 2006 on the basis of the SVN antibodies against BVDV in alpacas > 6 months old, which indicated exposure to BVD. On the basis of prior studies of South American camelids, we hypothesized that alpaca herds in the United States would have a seroprevalence of 5% to 10% and an incidence of infection < 5%. The second objective was to evaluate associations between seropositivity for anti-BVDV antibodies and potential risk factors for exposure to BVDV with the intent of identifying risk factors most likely to have a large impact for control of BVDV exposure among alpacas. Results of the study reported here would complement those of the previous study15 which would provide a comprehensive assessment of BVDV in alpacas in the United States in 2006 during its emergence as a major disease of alpacas.
Materials and Methods

Sample—The seroprevalence of antibodies against BVDV in US alpaca herds was estimated in a combined cross-sectional and longitudinal cohort study. The protocol for the study was approved by the Iowa State University Institutional Animal Care and Use Committee. On the basis of seroprevalences reported in earlier studies of BVDV in South American camelids, it was determined that a precision-based sample size of approximately 200 herds was needed to characterize a population with an expected prevalence of 10% and error of 2.5%. On the basis of simulated power calculations for determining strength of associations, a more feasible target sample size of 150 herds and blood samples from 5 animals within each herd was established.

A list of approximately 4,300 alpaca owners in the United States, sorted alphabetically by state, was compiled from multiple print and Internet sources for the South American camelid industry, including the 2005 AOBBA membership directories, an online marketplace, alpaca breeder organizations, and other state and regional alpaca associations. From this list, 550 owners (13% of the owners from each state) were selected by use of a random numbers table. On April 7, 2006, the owners were mailed a letter that described the study and solicited their voluntary confidential participation. Participants were asked to submit a blood sample (5 to 10 mL of blood collected into tubes with no anticoagulant) from each of five 6-month-old alpacas in their herd during April 1 to July 31, 2006 (period 1), and a second sample from those same alpacas collected at least 1 month later during August 1 to November 30, 2006 (period 2); provide descriptive information for each alpaca from which a blood sample was collected and submitted; and complete a questionnaire at the time of each sample collection. Included in the mailing were instructions for the collection, identification, packaging, and shipping of blood samples as well as the questionnaire for period 1. Each herd was assigned a code number, and all blood samples and questionnaires received were processed by code number. Owners selected the alpacas from which blood samples were collected.

The incidence of BVDV exposure was determined on the basis of seroconversion. Results of SVN assays for the initial blood samples were emailed to each participant; that email also included a reminder to submit the second set of blood samples and the questionnaire for period 2.

SVN assay—Blood samples were shipped to the investigators, who harvested the serum and submitted the serum samples to the Iowa State University Veterinary Diagnostic Laboratory. Samples were tested for neutralizing antibodies against BVDV type 1 (Singer 1a strain) and type 2 (125 strain) with a standard SVN antibody assay assumed to have a sensitivity of 90% to 99.6% and specificity of 100%. Sera were heat inactivated at 56°C for 30 minutes, and 2-fold serial dilutions from 1:2 to 1:4,096 were prepared in 96-well microtiter plates. Plates with an optimal TCID removal of BVDV were incubated at room temperature (approx 25°C) for 60 minutes. Bovine turbinate cells were then added to each well, and the plates were incubated at 37°C in 5% CO for 5 days. During incubation, viral cytopathic effects were monitored by microscopic inspection, and the reciprocal of the highest serum dilution that prevented cytopathic effects for each alpaca was recorded as the titer. A titer > 4 was considered a positive result, a titer < 2 was considered a negative result, and a titer of 2 to 4 was considered inconclusive. A herd was considered seronegative if all alpacas from which samples were collected had titers < 2 and seropositive if at least 1 alpaca had a titer > 4. For the longitudinal portion of the study, seroconversion of at least 1 alpaca in a previously seronegative herd was considered indicative of a new exposure.

Questionnaires—Owners completed a questionnaire at the time of each sample collection. Data included date of birth, breed, sex, and origin (imported, purchased, or home-raised) for each alpaca from which samples were obtained and the pregnancy status of females from which samples were collected. Owners indicated the number of alpacas in their herd by selecting 1 of 6 herd sizes (< 10, 11 to 25, 26 to 50, 51 to 100, 101 to 500, and > 500 alpacas). Questionnaire 1 covered the 5 to 6 years preceding period 1 and comprised 35 closed (2 choices or a checklist of choices) questions under headings of general information, herd management, herd health status, and current herd BVDV status. Questionnaire 2 covered the interval between collection of the samples in periods 1 and 2. It comprised 1 open-ended (narrative answer) and 49 closed (2 choices or a checklist of choices) questions under the headings of risk factors for BVDV exposure, current herd health, current BVDV status, and biosecurity practices.

Data analysis—A herd was the unit of analysis. The proportions of farms and of animals with a positive response (ie, yes) on the questionnaires were calculated, and 95% CIs were determined by use of an exact binomial approach. For each interval, the total population was based on respondents only (ie, blank responses were excluded). Associations between being seropositive for BVDV and potential risk factors described in questionnaire 1 were assessed by use of a Fisher exact test, and prevalence ratios were reported as the summary statistic. Associations between being seropositive for BVDV and potential risk factors described in questionnaire 2 were not assessed because only 1 of 27 herds in this subset was seropositive and none of the herds seroconverted. Follow-up historical information regarding BVDV exposure was obtained from owners of seropositive herds via telephone interviews or email.

On the basis of the distribution of seropositive results among herds of various sizes, we hypothesized that the risk of a herd or alpaca being seropositive would increase as herd size increased. This hypothesis was tested by calculating and comparing prevalence ratios for several ranges of herd size by use of a Fisher exact test. We compared various herd sizes (ie, ≤ 25 alpacas vs ≥ 26 alpacas, ≤ 50 alpacas vs ≥ 51 alpacas, and ≤ 100 alpacas vs ≥ 101 alpacas). Values of P < 0.05 were considered significant.

Results

Study participants—Of 550 letters mailed to alpaca owners to solicit participation in the study, 7 mailed to
owners in Oregon, New Jersey, Texas, and Virginia were returned because of incorrect addresses. Of the remaining 543 owners, 481 did not respond. Of the 62 owners who responded, 40 participated and 22 declined. These 22 owners declined to participate because it was too expensive (n = 1), they no longer owned alpacas (2), or they had a closed herd seronegative for BVDV (1); 18 gave no reason for not participating.

In period 1, 192 blood samples were received from 39 of the 550 (7.1%) solicited herds (Table 1). This included 5 alpacas from each of 36 herds and 6, 4, and 2 alpacas from 1 herd each (mean, 4.9 alpacas/herd). Participating herds were located in 20 states (5 herds each in New York and Wisconsin; 4 in Michigan; 3 in Ohio; 2 each in Georgia, Kansas, Minnesota, New Jersey, North Carolina, and Oregon; and 1 each in Colorado, Florida, Idaho, Indiana, Iowa, Kentucky, Maine, Nebraska, New Mexico, and Virginia). Fortiety owners, one of whom did not submit blood samples, completed questionnaire 1.

Of the 39 owners who submitted blood samples in period 1, 28 (71.8%) continued to participate in period 2 (1 declined further participation because of the expense involved, and 10 did not respond to the request to continue to participate). The 28 herds contained 132 alpacas from which blood samples were obtained during period 1; however, 10 alpacas were no longer available during period 2, and blood samples obtained from 5 alpacas of 1 herd during period 2 were unsuitable for testing because they were collected in tubes containing EDTA. Thus, usable samples for period 2 were received from 117 alpacas of 27 herds (Table 2). This included 5 alpacas each from 15 herds, 4 each from 8 herds, 3 each from 3 herds, and 1 alpaca from 1 herd (mean, 4.3 alpacas/herd). Participating herds were located in 16 states (3 each in New York and Wisconsin; 2 each in Georgia, Minnesota, Kansas, New Jersey, North Carolina, Ohio, and Oregon; and 1 each in Colorado, Florida, Iowa, Kentucky, Michigan, Nebraska, and New Mexico). Of the 28 herd owners participating in period 2, 2 submitted blood samples but did not complete questionnaire 2, and 1 completed questionnaire 2 but submitted unusable blood samples.

Median herd sizes of the sample population were 11 to 25 alpacas (Table 1). Huacaya female alpacas comprised most of the sample population in both sample collection periods (Table 3).

Sample collection periods—The actual sample collection periods exceeded the planned periods. Period 1 comprised April 26 to August 9, 2006 (105 days), and period 2 comprised October 9, 2006, to April 9, 2007 (182 days). For individual herds, the interval between collection dates for samples in periods 1 and 2 ranged from 75 to 292 days (mean, 176 days; median, 174 days), and the potential exposure to BVDV (calculated as the number of alpacas from which a second blood sample was collected X the interval in days between the samples for periods 1 and 2) ranged from 292 to 1,460 alpaca-days (mean, 740 alpaca-days; median, 695 alpaca-days).

BVDV SVN assay results—In period 1, 3 (designated as A, B, and C) of 39 (7.7%) herds from which blood samples were obtained and 8 of 192 (4.2%) alpacas were seropositive (Table 1), including 3 of 5 alpacas in herd A, 4 of 5 alpacas in herd B, and 1 of 5 alpacas in herd C. Two additional herds (D and E) each had 2 alpacas with inconclusive titers and 3 seronegative alpacas. All seropositive alpacas had higher titers against BVDV type 1 (geometric mean, 181; range, 32 to 1,024) than against BVDV type 2 (geometric mean, 195).

Table 1—Herd alpaca tested and positive SVN assay results during period 1 (April 26 to August 9, 2006) for herds and alpacas on the basis of herd size for 192 alpacas > 6 months old from 39 herds.

<table>
<thead>
<tr>
<th>Herd size</th>
<th>No. (%) of herds tested</th>
<th>No. (%) of seropositive herds</th>
<th>No. (%) of alpacas tested</th>
<th>No. (%) of seropositive alpacas</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10</td>
<td>9 (22.1%)</td>
<td>0 (0)</td>
<td>44 (22.9%)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>11–25</td>
<td>14 (35.9%)</td>
<td>1 (7.1%)</td>
<td>68 (35.4%)</td>
<td>1 (1.5%)</td>
</tr>
<tr>
<td>26–50</td>
<td>6 (19.4%)</td>
<td>0 (0)</td>
<td>30 (15.6%)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>51–100</td>
<td>7 (17.9%)</td>
<td>1 (14.3%)</td>
<td>33 (18.2%)</td>
<td>3 (8.6%)</td>
</tr>
<tr>
<td>101–500</td>
<td>3 (7.7%)</td>
<td>1 (33.3%)</td>
<td>15 (7.8%)</td>
<td>4 (26.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>39 (100%)</td>
<td>3 (7.7%)</td>
<td>192 (99.5%)</td>
<td>8 (4.2%)</td>
</tr>
</tbody>
</table>

* A titer > 4 was considered a positive result, a titer < 2 was considered a negative result, and a titer of 2 to 4 was considered inconclusive. A herd was considered seronegative if all alpacas from which samples were collected had titers < 2 and seropositive if at least 1 alpaca had a titer > 4.
* Excludes 1 herd for which a completed questionnaire was submitted but no blood samples were submitted. Percentages do not sum to 100% because of rounding.

Table 2—Results (number [%]) of a BVDV SVN assay for blood samples obtained for herds and alpacas during periods 1 and 2 (October 9, 2006, to April 9, 2007).

<table>
<thead>
<tr>
<th>Period 1</th>
<th>Period 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>SVN result</td>
<td>Alpacas</td>
</tr>
<tr>
<td>Positive</td>
<td>8 (4.2)</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>5 (2.6)</td>
</tr>
<tr>
<td>Negative</td>
<td>179 (93.2)</td>
</tr>
<tr>
<td>Total tested</td>
<td>192 (100)</td>
</tr>
</tbody>
</table>

*All seropositive herds had individual alpacas that were seronegative. Includes 1 blood sample with blood hemolysis for which no titer was reported. Includes 1 blood sample that was reported as a titer < 2 against BVDV type 1 and an insufficient quantity for testing against BVDV type 2. See Table 1 for remainder of key.

Table 3—Values (number [%]) for characteristics of alpacas in the sample population during periods 1 and 2.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Period 1 (n = 192)</th>
<th>Period 2 (n = 117)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breed Huacaya</td>
<td>154 (80.2)</td>
<td>83 (70.9)</td>
</tr>
<tr>
<td>Suri</td>
<td>36 (18.8)</td>
<td>32 (27.4)</td>
</tr>
<tr>
<td>Suri X Huacaya crossbred</td>
<td>2 (1.0)</td>
<td>2 (1.7)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sexually intact male</td>
<td>53 (27.6)</td>
<td>40 (34.2)</td>
</tr>
<tr>
<td>Gelded male</td>
<td>4 (2.1)</td>
<td>3 (2.6)</td>
</tr>
<tr>
<td>Female</td>
<td>135 (70.3)</td>
<td>74 (63.2)</td>
</tr>
<tr>
<td>Currently pregnant</td>
<td>—</td>
<td>71 (57.7)</td>
</tr>
<tr>
<td>Origin*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Born on farm</td>
<td>—</td>
<td>49 (39.8)</td>
</tr>
<tr>
<td>Purchased†</td>
<td>—</td>
<td>74 (60.2)</td>
</tr>
<tr>
<td>Status between blood samples 1 and 2*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Off-farm</td>
<td>—</td>
<td>47 (38.2)</td>
</tr>
<tr>
<td>Clinical illness</td>
<td>—</td>
<td>3 (2.4)</td>
</tr>
</tbody>
</table>

*Results are based on information provided for 123 alpacas, which included 5 alpacas from 1 herd that submitted unusable blood samples and 1 alpaca for which an inconclusive titer was reported. Includes 1 alpaca imported from South America. |
| — = Data not collected. |
Seropositive alpacas ranged from 0.67 to 8.83 years of age (mean, 5.3 years).

During period 2, 1 of 117 alpacas in 1 (A) of 27 herds from which a second blood sample was obtained were seropositive (Table 2). The remaining 116 alpacas in 26 herds that were sampled twice were seronegative in both periods, and because none of these alpacas had seroconverted, the incidence of BVDV exposure was 0%. The seropositive alpaca in herd A had anti-BVDV type 1 titers of 1,024 during periods 1 and 2 and anti-BVDV type 2 titers of 128 in period 1 and 256 in period 2. A second blood sample was not collected from any alpaca in herds B or C that was seropositive in period 1. Of 5 alpacas each in herds D and E, 3 were seronegative and 2 had inconclusive titers in period 1, and 5 of 5 were seronegative in period 2.

Medical history of BVDV exposure for seropositive herds—Follow-up information on medical histories was obtained from owners of seropositive herds A, B, and C. In 2003, a pregnant female recently introduced into herd A gave birth to a cria that died at 5 months of age. The cria was retrospectively presumed to be PI with BVDV when numerous reproductive losses and birth of a cria confirmed to be PI with BVDV were reported among females that had been exposed during gestation to the suspected PI cria. Approximately 2.5 years after birth of the suspected PI cria, 26 of 61 (42.6%) alpacas tested in herd A were BVDV seropositive.

A pregnant female was introduced into herd B in 2004 and subsequently gave birth to a cria that was confirmed as PI with BVDV before it died at 3.5 months of age. By 4 months after the PI cria was born, 39 of 69 (56.5%) alpacas in herd B had seroconverted and 7 more crias developed acute BVDV infections (as verified by PCR assay for BVDV) as a result of exposure to the PI cria.

The single seropositive alpaca in herd C was a female with a history suggestive of BVDV infection prior to introduction into this herd. The alpaca was purchased at an auction in another state and transported directly to a farm to be bred; it reportedly conceived at that farm but lost the pregnancy and was rebred before being introduced into herd C, where it gave birth to a premature cria that died of undetermined causes. Circumstantial evidence (data not shown) linked herds A, B, and C to several other herds (none participated in the present study) in which ≥ 1 gestating females exposed to suspected or confirmed PI crias subsequently seroconverted, had reproductive losses, and gave birth to PI crias.

Reproductive losses—Thirty-eight of 40 (95%; 95% CI, 83% to 99%) respondents who completed questionnaire 1 indicated that they routinely tested bred females in their herds by use of ultrasonographic examination or progesterone assay to determine pregnancy status, and 19 of 40 (48%; 95% CI, 31% to 64%) had at least 1 alpaca abort or lose a confirmed pregnancy during the year prior to the present study. During the interval between periods 1 and 2, 7 of 26 (27%; 95% CI, 11% to 48%) respondents had a female alpaca lose a confirmed pregnancy, 6 (23%; 95% CI, 9% to 44%) had a female that aborted, and 8 (31%; 95% CI, 14% to 52%) had crias that were weak and underweight at birth.

Commingling of alpacas—During the 5 years prior to period 1, 35 of 40 (88%; 95% CI, 67% to 93%) respondents had shipped female alpacas to other farms for breeding, and 20 of 35 (57%; 95% CI, 39% to 74%) respondents indicated a cria accompanied the female. Also, 26 of 40 (65%; 95% CI, 48% to 79%) respondents had female alpacas from other farms shipped to their farm for breeding, and 19 of 25 (76%; 95% CI, 55% to 90%) females were accompanied by their cria.

During the interval between periods 1 and 2, 22 of 26 (85%; 95% CI, 85% to 95%) respondents had alpacas leave the premises and return home after off-premises commingling with other alpacas. Reasons for commingling most frequently cited included shipping female alpacas to another farm for breeding (9/22 [41%]; 95% CI, 21% to 63%), shipping male alpacas to another farm to breed females (5/22 [23%]; 95% CI, 7% to 45%), and participating in a show, exhibition, or fair (5/22 [23%]; 95% CI, 8% to 45%). Also, during the interval between periods 1 and 2, 25 of 26 (96%; 95% CI, 80% to 100%) respondents indicated alpacas from other farms had been shipped to reside at their farm. Reasons most frequently cited included housing of females to be bred by a resident male (15/25 [60%]; 95% CI, 30% to 79%), addition of newly purchased alpacas to their herd (12/25 [48%]; 95% CI, 29% to 71%), and boarding alpacas owned by others (9/25 [36%]; 95% CI, 19% to 59%).

Exposure to other domestic animals—Thirty-five of 40 (88%) respondents indicated other domestic animals, including dogs, cats, horses, swine, llamas, sheep, goats, cattle, farmed elk, or poultry, also resided on their farms. Four of 40 (10%; 95% CI, 3% to 24%) respondents had cattle, and 3 of these had never had BVDV diagnosed in their cattle (1 did not enter a response). Two of these 4 respondents indicated that they vaccinated their cattle against BVDV.

BVDV testing—Responses to questionnaire 1 revealed that 30 of 40 (75%; 95% CI, 59% to 87%) owners had tested their alpacas for BVDV prior to period 1. Four of 40 (10%; 95% CI, 3% to 24%) respondents indicated 1 or more alpacas on their farm had positive results when tested for anti-BVDV antibodies. One of 40 (3%; 95% CI, 0.06% to 13%) respondents indicated that they had laboratory confirmation of persistent BVDV infection in their herd.

During the interval between periods 1 and 2, 22 of 26 (85%; 95% CI, 65% to 96%) owners tested other alpacas on their farm (ie, alpacas other than the ones from which they collected blood samples specifically for the present study); 19 of 22 owners indicated that testing was by PCR assay. Of 26 respondents, 17 (65%; 95% CI, 51% to 90%) had conducted BVDV testing because it was required for show participation and 14 (54%; 95% CI, 45% to 88%) had conducted testing to determine the BVDV status of their herd for knowledge and herd management purposes. Testing for BVDV was also conducted to satisfy requirements promulgated by auctions (7/19 [37%]), breeding facilities (7/20 [35%]), private sales (6/18 [33%]), or trans-
porters (4/18 [22%]). Finally, testing for BVDV was conducted because it was required for boarding their alpacas at another farm (3/18 [17%]), because it was recommended by their veterinarian (5/18 [28%]), or because alpacas in their herd had clinical signs suggestive of BVDV infection (1/18 [6%]).

Biosecurity—Of 40 respondents who completed questionnaire 1, 17 (43%: 95% CI, 27% to 59%) indicated that they routinely isolated sick alpacas to prevent nose-to-nose contact with healthy alpacas on their farm. Thirty-six of 40 (90%: 95% CI, 76% to 97%) respondents routinely vaccinated their herd against infectious diseases such as rabies, tetanus, clostralid infections, leptoepiosis, or West Nile virus infection, but none vaccinated their alpacas against BVDV before or during the present study.

Twenty-four of 40 (60%; 95% CI, 43% to 75%) respondents had administered colostrum or plasma obtained from animals outside their herd to alpacas in their herd. Twenty-three of 26 (92%: 95% CI, 74% to 99%) respondents required that any alpaca originating from other farms be tested for BVDV before being shipped to their farm, and 22 specifically required a BVDV-negative PCR assay result. Seven of 26 (27%: 95% CI, 11% to 47%) respondents quarantined incoming alpacas originating from other farms for at least 3 weeks, 4 of 26 (15%: 95% CI, 4% to 33%) respondents quarantined their own alpacas for at least 3 weeks or tested them for BVDV after return to their farms, and 13 of 25 (52%: 95% CI, 31% to 72%) respondents placed alpacas back with their herdmates immediately after return home from breeding farms or shows. Of 25 respondents, 24 (96%: 95% CI, 79% to 100%) considered their herd to be free of BVDV infection, and 1 of 25 (4%; 95% CI, <1% to 20%) respondents maintained a closed herd. Ten of 25 (40%; 95% CI, 21% to 61%) respondents had implemented or improved biosecurity practices because of concerns about BVDV. However, 17 (68%; 95% CI, 46% to 85%) respondents considered the risk of introducing BVDV into their herd too low to warrant making additional changes in their biosecurity practices.

Risk factor analysis—No significant association was found between BVDV seropositive status for herds and any risk factors described in questionnaire 1. Larger herds had a greater percentage of seropositive alpacas than did smaller herds at both the herd and alpaca level (Table 1). Seropositive status for individual alpacas was significantly associated with herd size, but seropositive status for herds was not (Table 4).

Discussion

In the cross-sectional study of alpaca herds in the United States reported here, 8 of 192 (4.2%) non-BVDV-vaccinated alpacas > 6 months old from 3 of 39 (7.7%) herds had SVN antibodies against BVDV, which confirmed our hypothesis that the seroprevalence would be 5% to 10%. Additionally, we hypothesized a BVDV incidence of <5%. An incidence of 0% was determined because none of 26 seronegative herds from which samples were obtained twice during a mean 740 alpaca-days of potential exposure seroconverted. Follow-up information obtained from owners of seropositive herds provided medical or circumstantial evidence that linked BVDV seropositive herds to exposure of gestating females to suspected or confirmed PI cria. Questionnaire responses identified common alpaca industry practices that were potential risk factors for BVDV exposure. Finally, herd size was significantly associated with seropositive status for individual alpacas but not for herds, and there were no significant associations between herd BVDV seropositive status and potential risk factors.

Prior to 2003, little information specifically addressed the prevalence of BVDV in alpacas in the United States. Seroprevalences of 0.9% in llamas and alpacas in Georgia, Alabama, and Tennessee and 4.4% in llamas in Oregon had been reported. Given the major developments regarding BVDV in alpacas since that time, more current data on the prevalence of BVDV in the national alpaca population and identification of risk factors for exposure to BVDV were needed. Results of the present study, in combination with those in another study, provide a comprehensive picture of BVDV in alpacas in the United States in 2006 to 2007. Although different approaches were used in the present study and that other study, authors arrived at extremely similar conclusions for both studies. The prevalence of 6.3% for BVDV-infected alpaca herds as determined by investigation of alpacas <6 months old was comparable to the herd seroprevalence of 7.7% determined in the present study by evaluation of alpacas > 6 months old. In both the present study and that other study, transmission of BVDV infection into a new herd often resulted when naive females and females with a PI cria cohabited at a breeding facility.

In North and South America, seroprevalences of BVDV reported for alpacas or llamas range from 0.9% to 25.4%. Accordingly, this is a wide range, and the estimate for the present study was toward the lower end. However, it can be difficult to compare prevalence

Table 4—Prevalence ratio (95% CI) for associations between seropositive status of herds and alpacas on the basis of various herd sizes.

<table>
<thead>
<tr>
<th>Herd size comparison (No. of alpacas)</th>
<th>Herd</th>
<th>Prevalence ratio (95% CI)</th>
<th>P value*</th>
<th>Alpaca</th>
<th>Prevalence ratio (95% CI)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 25 vs ≥ 26</td>
<td>2.88</td>
<td>0.28-29.08</td>
<td>0.55</td>
<td>9.8</td>
<td>1.23-78.1</td>
<td>0.009</td>
</tr>
<tr>
<td>≤ 50 vs ≥ 51</td>
<td>5.90</td>
<td>0.59-57.29</td>
<td>0.15</td>
<td>19.7</td>
<td>2.51-157.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≤ 100 vs &gt; 101</td>
<td>6.00</td>
<td>0.74-48.59</td>
<td>0.22</td>
<td>11.8</td>
<td>3.28-42.52</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Fisher exact test; values were significant at P < 0.05. See Table 1 for remainder of key.
estimates because the original purpose of a study and the method of selection for participation in a study can introduce bias, and it is not clear that all studies were conducted with the intent of estimating prevalence. For example, studies conducted to assess risk factors may not have been designed to provide a generalizable estimate of prevalence. In such studies, a convenience sample is likely to be used, and as such, the source population is not clear. Alternatively, for studies with the primary purpose of determining seroprevalence, the use of a random method for selection of individuals invited to participate does not prevent selection bias because participation is voluntary after random selection. It is difficult to predict the direction of selection bias. For example, in 1 study, samples were obtained from 296 alpacas of 24 herds, but alpacas from only 2 herds comprised 189 (63.9%) of the sample population. The presence or absence of PI alpacas in one or both of these herds would have resulted in an overestimate or underestimate of seroprevalence for the sample population. In another study, the sample population was randomly selected from among 702 members listed in the 2005 AOBAl membership directory who each had at least 12 female alpacas in their herd. The impact of this eligibility requirement is unclear. For example, the requirement for at least 12 females could result in an overestimate of the seroprevalence and incidence of BVDV because there are more likely to be PI alpacas in herds with ≥12 females than in herds with fewer females or herds comprising predominantly or exclusively males that would be excluded from the sample population. It has also been suggested that perhaps AOBAl members enter their animals in shows more frequently than do nonmembers, and thus, commingling of alpacas is higher for AOBAl members. Both entering animals in shows and commingling of alpacas at shows or other venues could favor BVDV transmission. Alternatively, AOBAl members may have higher standards of management that reduce BVDV seroprevalence. Despite the many differences in design among studies of BVDV prevalence in South American camels, there is a relatively low level of BVDV exposure in South American camels, compared with the level of BVDV exposure in cattle.

Other factors such as BVDV vaccination status, animal age, and presence of passively acquired antibodies could result in overestimation of true seroprevalence (ie, endogenous antibodies resulting from BVDV infection), but these factors often have not been addressed in BVDV seroprevalence studies of South American camels. Vaccination of alpacas against BVDV was not a source of misclassification in the present study or 2 contemporaneous studies of BVDV seroprevalence in alpacas in the United States, and none of the participants in the present study reported use of BVDV vaccines in their herd. Although 25.4% of alpacas <6 months old were BVDV seropositive in another study, this likely was an overestimate because BVDV seropositive status was significantly associated with feeding bovine colostrum to cria. By limiting our sample population to alpacas ≥6 months old, we minimized the presence of passively acquired antibodies against BVDV as a potential source of misclassification. Consequently, the herd seroprevalence of 7.7% in older alpacas likely was a more accurate estimate of the true seroprevalence of BVDV in alpacas in the United States in 2006 to 2007.

Evidence suggests that PI alpacas may maintain transmission of BVDV within the alpaca population in the absence of exposure to infected cattle, and exposure of alpaca females to PI cria accompanying their dams at breeding facilities has been implicated as a source of BVDV infection. Because of immunotolerance, PI alpacas typically do not produce antibodies against the infecting BVDV type, and in the absence of passively acquired antibodies, infection is not likely to be detected by the SVN test. Thus, if PI alpacas were among those included in the sample population, results of the present study could have underestimated the animal-level seroprevalence of BVDV. However, this would be unlikely to affect herd-level prevalence estimates because exposure to PI alpacas would result in seroconversion of other herd members within a short period. We expected to minimize this potential source of misclassification by collecting samples from 5 alpacas/herd and reporting both alpaca-level and herd-level seroprevalences.

Seropositive alpacas in the present study had SVN antibodies against both BVDV types 1 and 2, but titers were consistently higher against type 1. Similar results have been reported in naturally exposed alpacas and reflect both the cross-reactivity between types 1 and 2 and the predominance of type 1b isolates obtained from naturally infected alpacas.

Because of the heightened awareness and concern regarding BVDV infection that prevailed within the alpaca industry in North America when the present study was undertaken, we hypothesized that 25% of contacted owners would participate; on the basis of a target sample size of >150 herds, we expected to be able to detect factors strongly associated with BVDV seroprevalence and incidence (ie, prevalence ratio >8). Although no risk factors were associated with BVDV seropositive status at the herd level in the present study, this result should not be interpreted to mean there are no associations between exposure to BVDV and the various risk factors evaluated. It was more likely that the sample size of 39 herds did not provide the statistical power necessary to detect associations between BVDV seropositive status and potential risk factors and reflected a high probability of type II error (not detecting a significant difference when one truly exists). Analysis of the results of the present study suggested that herd size was a possible risk factor for exposure to BVDV, and exploration of this possibility yielded the fact that larger herd size was significantly associated with BVDV seropositive status for individual alpacas but not for herds. Because herd size has been identified as a risk factor for BVDV infection in cattle, further evaluation of alpaca herd size as a risk factor should be considered.

The low amount of participation in the present study likely reflected a response bias potentially influenced by several factors. For example, low participation in the present study may have resulted from inclusion of non-OBAl members in the sample population because they might have been less likely than OBAl members to participate in research.
members to participate in industry activities or be less informed about BVDV. Other factors such as competition with contemporaneous BVDV seroprevalence studies for participants, relative costs and benefits of participation, and prior testing for BVDV could also have reduced participation in the present study. Owners bore the entire cost of collecting and submitting blood samples in the present study and received only BVDV SVN test results in return. Investigators of another contemporary study partially subsidized the costs to participate in their study and provided owners with results from SVN, virus isolation, or PCR tests. Effective June 1, 2006, the AOBA required a requirement that any owner bringing an alpaca to an AOBA-sponsored show must provide proof the animal is not PI with BVDV. Currently, only negative results for a PCR test are acceptable as proof (prior to January 1, 2010, negative results for virus isolation were also acceptable); results of testing by any other method, including SVN, are not acceptable. Most respondents had already tested alpacas in their herds for BVDV before the onset of the present study and cited the show requirement as a reason for the testing. In future studies of risk factors associated with BVDV exposure, a low response from members of the alpaca industry should be anticipated and incentives provided for participation to ensure an adequate sample size.

Questionnaire responses in the present study provided insights into the practices and attitudes prevalent in the alpaca industry of the United States in 2006 to 2007. Responses with a wide 95% CI reflected the small sample size and, at best, might be interpreted as industry trends, whereas responses with a narrow 95% CI can be considered as established characteristics of the alpaca industry in the United States. Several practices were used by a high percentage of respondents. For example, 36 of 40 (90%) respondents routinely vaccinated their alpacas against infectious diseases, and 38 of 40 (95%) routinely tested bred females to confirm pregnancy. Testing to detect BVDV in alpacas coming onto their premises from other farms for breeding or boarding was required by 23 of 25 (92%) respondents. Testing of alpacas to detect BVDV was conducted by 22 of 26 (85%) respondents on their farms, and 17 of 23 (74%) indicated that BVDV testing was conducted to satisfy AOBA show requirements. Although the impact of this self-imposed industry requirement is unknown, it has likely helped control BVDV transmission by minimizing exposure to PI alpacas at show venues.

Questionnaire responses also revealed practices that were not widely adopted by study participants. For example, none of the study participants vaccinated their alpacas against BVDV, only 1 of 25 (4%) maintained a closed herd, 17 of 40 (43%) isolated sick alpacas to prevent nose-to-nose contact, 7 of 26 (27%) required a 3-week quarantine of alpacas visiting from other farms, 4 of 26 (15%) quarantined or tested their own alpacas for BVDV after return from off-farm sites, and 13 of 25 (52%) placed alpacas back with their herdmates immediately after return from off-farm sites. Respondents likely had not considered that alpacas from their own herd that had spent time off the farm, especially in conditions whereby they were stressed and exposed to other alpacas or livestock, essentially had the same potential to introduce pathogens (acquired while away from the home farm) as would newly acquired animals.

The extensive movement of alpacas within the United States and frequent commingling of alpacas from various herds, which was clearly documented by the questionnaire responses, provided ample opportunity for between-herd transmission of BVDV. For example, 25 of 26 (96%) respondents had alpacas from other farms that resided briefly for boarding or breeding at their farm. Given the evidence linking new BVDV infections for herds with exposure to PI crias accompanying their dams to breeding facilities, it is noteworthy that 26 of 40 (65%) study participants had females from other herds shipped to their farm for breeding, and 19 of 25 (76%) indicated that crias had accompanied the dams. Similarly, 35 of 40 (88%) respondents had shipped their own females to other herds for breeding, and 20 of those 35 (57%) indicated a cria had accompanied the female. In the present study, 24 of 25 (96%) respondents considered their herd to be free of BVDV infection, but 17 of 25 (68%) considered the risk of introducing BVDV into their herd to be too low to warrant better biosecurity practices and only 10 of 25 (40%) had implemented or improved their biosecurity practices because of BVDV. Recommendations provided by veterinarians regarding herd health, biosecurity, and BVDV control are of limited value unless they are adopted by the alpaca industry. Clearly, veterinary practitioners could play a greater role in control of BVDV in alpacas by helping clients understand and implement better biosecurity practices.

References