

# Controversies and clarifications regarding bovine lentivirus infections

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**B**ovine lentiviruses have been recognized since 1972.<sup>1</sup> The virus was initially referred to as bovine visna-like virus because of structural and biological similarities to maedi-visna virus of sheep.<sup>2</sup> The virus acquired the name **bovine immunodeficiency-like virus (BIV)** when common sequences with **human immunodeficiency virus (HIV)** were detected in 1987.<sup>3</sup> Since that time, numerous studies have explored the molecular biological and immunologic features and, to a lesser extent, the epidemiologic features of the virus.<sup>4-7</sup> For the purposes of this report, the virus will be regarded as a bovine lentivirus and referred to as BIV, because that name is most commonly used in the scientific literature.<sup>3-7</sup>

Questions regarding BIV center around 2 primary points: its role as a sole pathogen and its role as a primary immunodeficiency virus that causes secondary infections that lead to substantial illness and economic loss in cattle.<sup>8-9</sup> Despite extensive experimental studies and focused epidemiologic reports, the true pathogenic nature of BIV remains controversial.<sup>10-14</sup> Because of the lack of a gold standard diagnostic assay for detection of BIV infection, determining the true prevalence of BIV infection in the United States and other countries has been problematic. Results of serologic assays have indicated prevalence rates ranging from 4% in the United States and France to 5.5% in Canada.<sup>7,14</sup> In a recent review,<sup>8</sup> BIV and a related bovine lentivirus, Jembrana disease virus, were classified in a group that causes brief mild clinical signs. The authors concluded that lentivirus infection in cattle does not cause substantial immunodeficiency, comparable with lentivirus infections of sheep and goats. **Ovine progressive pneumonia (OPP)** and **caprine arthritis-encephalitis (CAE)** virus infections may cause pronounced lymphoproliferative response in a low percentage of infected animals.<sup>15</sup> It was recommended that BIV be referred to as

bovine lentivirus type 1 and Jembrana disease virus as bovine lentivirus type 2.<sup>8</sup>

Miller et al<sup>16</sup> recently reviewed the comparative aspects of HIV with multiple animal lentiviruses, including BIV. Their conclusions indicated that BIV, although lymphotropic, did not cause immune deficiency or disease. They further recommended that the study of natural host animal-lentivirus systems such as BIV may enhance the understanding of asymptomatic phases of infection.<sup>16</sup>

The purpose of this report was to present current information regarding BIV infection and propose action that will help resolve the controversies and clarify the true nature of the infection in cattle.

## Comparative and Molecular Biological Features of BIV

The BIV genome is organized similarly to other retroviruses, with *gag*, *pol*, and *env* genes flanked by long terminal repeats and a central region between the *pol* and *env* genes that contains as many as 6 regulatory genes. The BIV *gag* gene products are translated as a polyprotein that is further processed into the matrix (p16), capsid (p26), and nucleocapsid (p13) proteins.<sup>17</sup> These are important structural proteins that are involved in virion production and represent major antigens for antibody production, especially the capsid protein. The capsid protein has elements that are highly conserved between lentiviruses and some immunologic cross-reactivity with HIV and **equine infectious anemia (EIA)** virus.<sup>3,17</sup> The polymerase proteins are also produced as a polyprotein and contain at least 3 proteins: the reverse transcriptase (p72), endonuclease/integrase (p32), and protease (p11) enzymes.<sup>3</sup> The 2 surface proteins are also translated as a polyprotein, including the surface envelope protein (gp110) and the transmembrane protein (gp42). These proteins are believed to be some of the most variable proteins in BIV, because they are under host antibody immune pressure.

Of the 6 BIV genes that code for regulatory proteins, *tat* and *rev* have been the most extensively studied and are considered to have important roles in controlling viral replication. The *rev*-coded protein positively regulates *gag*- and *env*-coded protein expression and virion production,<sup>18</sup> presumably by a mechanism similar to that of other lentiviruses in which it facilitates transport of unspliced and singly spliced transcripts out of the nucleus.<sup>18</sup>

The *tat*-coded protein also regulates the production of the full-length viral genome, which is necessary for virus replication.<sup>19</sup> The BIV *tat*-coded protein inter-

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acts with a specific RNA sequence, the transactivating response (*tar*) gene region, and is located immediately downstream from the transcription start site. The *tar* region is thought to form a RNA hairpin. In the presence of the *tat*-coded protein, the RNA polymerase complex will more effectively transcribe through the RNA hairpin and produce full-length transcripts, increasing viral production. In the absence of the *tat*-coded protein, the RNA polymerase complex stalls at the RNA hairpin and produces mostly aborted short transcripts and little virus.<sup>20</sup> Although the *tat*-coded protein performs a similar role for all lentiviruses, the binding of BIV *tat*-coded protein to the RNA *tar* gene region appears to be unique from other lentiviruses.<sup>20</sup>

Retroviruses, in general, have a high mutation rate because of the relative poor fidelity of the reverse transcriptase enzyme, and BIV is not an exception.<sup>14,21</sup> The reverse transcriptase (RT) gene and the surface envelope (SU) gene have the most sequence available for comparison. The RT gene is more conserved, with 89% nucleotide (89% amino acid) or greater sequence similarity between isolates in pairwise comparisons.<sup>22</sup> The SU gene is more variable, with 45% nucleotide (51% amino acid) or greater sequence similarity among BIV isolates in pairwise comparisons. The SU gene may vary greatly in size among different viral isolates and even within viruses isolated from the same animal.<sup>23</sup> The size variation occurs primarily in hypervariable region 2, and passage of BIV in cell culture may select for the smaller SU genes.<sup>23</sup> The closely related bovine lentivirus 2 (Jembrana disease virus) has also been sequenced and compared with BIV. The RT genes of Jembrana disease and BIV (R29 strain) have 68% amino acid sequence similarity.<sup>24</sup> The BIV, as judged by sequence similarity of the RT gene to other lentiviruses, appears to be equally distantly related to the small ruminant lentiviruses maedi-visna, OPP, and CAE viruses as well as EIA, feline immunodeficiency virus, and HIV.<sup>7,8,15,21</sup>

### Host Range and Transmission

The natural host range of BIV is thought to be restricted to cattle.<sup>2,14,25</sup> The virus is strongly cell-associated and may be transmitted in infected blood, colostrum, and milk that contains intact lymphoreticular cells.<sup>8,13,26</sup> Nash et al<sup>27</sup> reported the detection of BIV in stud bull semen from randomly selected repository samples. However, a recent study of 3 experimentally infected bulls detected BIV in blood by use of virus isolation and polymerase chain reaction (PCR), but the virus was not detected in semen.<sup>28</sup> In a survey of 30 BIV-seronegative bulls at 3 artificial insemination centers, there were no BIV-positive bulls detected by use of PCR analysis of semen leukocytes, spermatozoa, or blood leukocytes.<sup>29</sup> There is limited evidence to support vertical transmission. It was reported that 40% (14/35) of naturally infected BIV-seropositive cows gave birth to BIV-seropositive calves,<sup>30</sup> and calf BIV antibody status before ingestion of colostrum was not associated with development or frequency of clinical signs. Cattle seroconvert to BIV within 2 to 4 weeks after infection and may remain seropositive for extended periods (> 2 years).<sup>5,6,26</sup> Although alterations of the

immune response have been reported, they are generally regarded as transitory and not life-threatening.<sup>10,31</sup> Once infected with BIV, cattle are presumed to be infected for life.<sup>2,8</sup> Results of long-term studies of virus persistence in experimentally infected cattle indicate that the virus may remain infectious for as long as 4 years without progression to clinical disease.<sup>12,22</sup> Results of comparative studies with related lentiviruses (OPP and CAE viruses) support the premise of life-long infections.<sup>8,13,32</sup> Goats and sheep are susceptible to experimental infections with BIV-infected cells, but there is limited evidence to support natural BIV infection in these species.<sup>6,8</sup> These small ruminants also seroconvert to the virus, but infectious virus cannot be reisolated.<sup>6,8</sup> This supports the contention that the lentiviruses are species specific, probably because of viral receptors on susceptible host cells.<sup>15,26</sup>

Cofactors that may predispose BIV-infected cattle to disease may be concurrent bovine herpesvirus type-1 infection (infectious bovine rhinotracheitis), bovine viral diarrhea (BVD) virus infection, and genetic predisposition.<sup>2,25,26</sup> As with other lentivirus infections, these cofactors may prove to be of importance in the overall assessment of the pathogenesis of BIV.<sup>8,25</sup>

Similar to other lentiviruses of domestic animals, BIV persists in blood leukocytes, and BIV provirus has been detected in monocytes and lymphocytes from blood of infected cattle.<sup>22</sup> Persistence of infection has been substantiated by the ability to continuously detect specific antibodies.<sup>5,6</sup> Distribution of BIV in organs of persistently infected cattle includes blood mononuclear cells, liver, lung, and spleen.<sup>33</sup> The mechanism of BIV persistence in vivo appears to be similar to that of other lentiviruses and involves regulation of provirus gene expression and antigenic variation of envelope glycoprotein.<sup>14,15</sup>

### Effects on Lymphoreticular Tissues

Infection of cattle with BIV has been associated with lymphoproliferation, lymphadenopathy, immunosuppression, neuropathy, and progressive emaciation.<sup>9,25,34</sup> In initial BIV experimental infections of colostrum-deprived calves, infected calves developed leukocytosis and peripheral lymphadenopathy characterized histologically by lymphoid follicular hyperplasia.<sup>9</sup> The calves in that experiment were studied for < 1 year. Follow-up experimental inoculations of cattle confirmed lymphoid follicular hyperplasia in lymph nodes, hemal nodes, spleen, tonsils, Peyer's patches, and gut-associated lymphoid tissue.<sup>34</sup> Overall evidence derived from long-term studies suggests that BIV infection progresses sequentially from blood to lymphoid organs.<sup>25</sup> Some authors conclude that the lymphoid changes seen in BIV infection are similar to those found in early infection with HIV.<sup>25,35</sup> Results of studies of monocytes from experimentally infected cattle indicate decreased function, including reduction in superoxide anion release, phagocytic activity, and chemotactic responsiveness. However, results of additional studies indicate either mild or no immunosuppression judged on the basis of lymphocyte blastogenesis, neutrophil function, or mononuclear cell subset analyses.<sup>10,12,31</sup> Results of a more recent study indicate a

decrease in the CD4 to CD8 ratio during the period from 2 to 7 weeks after inoculation.<sup>36</sup> This decreased ratio was a result of increased numbers of CD8 cells concurrent with a smaller increase in number of CD4 cells. Sequential infection of BIV-inoculated calves with bovine herpesvirus type 1 followed by immunization with BVD virus vaccine elicited lower BVD and bovine herpes virus type-1 neutralizing antibodies than in calves not infected with BIV.<sup>36</sup> These results were in contrast to those reported by Whetstone et al<sup>37</sup> who demonstrated that BIV (FL112) caused a B-cell lymphocytosis with no consistent substantial changes in other mononuclear cell populations, including CD4 and CD8 cells.

Results of a recent study<sup>34</sup> confirm that lymphadenopathy is associated with experimental BIV infection. Under certain environmental stresses, numerous secondary infections may be associated with BIV infection of cattle<sup>35</sup>; encephalitis, lymphoid tissue depletion, and secondary infections developed during a 7-year period in dairy cattle infected with BIV. Although results of these studies suggest the potential contributions of bovine leukemia virus (BLV), other studies did not detect association between BIV infection and BLV infection.<sup>7,38</sup>

### Evidence Linking BIV to Clinical Abnormalities

In 1 herd with high BIV seroprevalence (55.6%), numerous clinical and pathologic abnormalities were detected in adult dairy cows.<sup>25,35</sup> Pathologic findings included lymphoid depletion affecting lymph nodes and spleen, meningoencephalitis, and multiple secondary diseases (predominantly bacterial infections).<sup>25</sup> The lymphoid tissue response was similar to that of other lentivirus infections of humans and other animals.<sup>8,14</sup> Additional lymphoid tissue lesions observed in affected cattle include edema, neutrophil infiltration, necrosis, and hemorrhage.<sup>35</sup> The DNA sequences of BIV have been identified in various tissues of animals with these pathologic changes by use of conventional and in situ PCR methods.<sup>33</sup>

Primary brain lesions include nonsuppurative meningoencephalitis characterized by mononuclear cell perivascular infiltrates and astrocytic gliosis.<sup>25,35</sup> Microscopic lesions with these characteristics were observed in 42 of 66 (63.6%) cows from the herd with endemic BIV infection.<sup>35</sup> Provirus DNA of BIV was identified in affected brains.<sup>25,35</sup> Meningoencephalitis has been observed as early as 1 year after experimental BIV infection of BLV-free calves.<sup>34</sup> There were no distinctive clinical signs of nervous system disease observed in 1 herd with naturally occurring BIV infection, although ataxia or paraplegia may be evident in some BIV-infected cattle.<sup>39</sup>

### Evidence that Suggests Low Pathogenicity

Inoculation of BIV into colostrum-deprived isolation-reared calves resulted in nonpersistent lymphocytosis and hyperplasia of small subcutaneous lymphatic nodules (hemal nodes).<sup>1,2</sup> At necropsy several months later, the calves had lymphoid hyperplasia of external

and internal lymph nodes and hemal nodes. These results were interpreted as an immunologic response to an antigenic stimulation and were regarded as mild.<sup>40</sup>

Whetstone et al<sup>5</sup> inoculated cell culture-passaged BIV (R29) virus into 2 calves followed by subpassage of blood to a third calf. Serial inoculation of blood was then carried through 3 more in vivo passages at approximately 2-week intervals. All calves were colostrum-deprived and seronegative for BIV. One calf died 3 days after inoculation, but death was not attributed to BIV infection. The other calves developed BIV antibodies, but signs of clinical disease were not reported.<sup>5</sup> Inoculation of goats induced infection and seroconversion without inducing signs of clinical disease.<sup>6</sup>

Carpenter et al<sup>9</sup> reported experimental inoculation of calves with BIV (R29) virus grown in bovine embryonic spleen cells, fetal bovine lung cells, or a clone of cell-free BIV (BIV 106) derived from BIV (R29). Serial in vivo passage was used in an effort to enhance viral virulence. Calves were necropsied 5 to 6 weeks after inoculation. Episodes of fever and hematologic changes ranging from neutropenia to lymphocytosis were observed in some calves. Clinical illness attributable to BIV did not develop. At necropsy, slight to moderate swelling of lymph nodes and hemal nodes (bulging of the parenchyma) was detected when the nodes were incised. Microscopically, follicular hyperplasia of lymph nodes, hemal nodes, spleen, tonsil, and other gut-associated lymphoid tissue was detected. Other calves were inoculated with the same preparation and followed for as long as 27 months after inoculation.<sup>10,31,40</sup> Increased lymphocyte blastogenesis and decreased neutrophil function were detected, but no substantial effects on chemotactic or random migration of monocytes or on antibody-dependent cell-mediated cytotoxicity were found.<sup>31</sup> Phagocytosis was reduced in early infection but returned to normal 8 months after inoculation. One calf did develop thymic lymphosarcoma at 11 months of age, but association with BIV infection was not proven.<sup>41</sup> The other calves did not develop signs of ill health.

New wild-type isolates of BIV (BIV [FL112] and BIV [FL491]) were reported by Suarez et al<sup>14,42</sup> in 1993. Inoculation of susceptible calves induced leukocytosis, but the calves did not develop signs of clinical illness during 4 months of observation.<sup>14,36,42</sup> Munro et al<sup>34</sup> recently reported on calves inoculated with BIV (FL112). Clinical signs included slightly enlarged lymph nodes, hemal node enlargements, and subtle shifting lameness. Lymphoid hyperplasia as well as meningoencephalitis was confirmed at necropsy.<sup>34</sup> Experimental inoculation of BIV into cattle and rabbits in Japan<sup>43</sup> and cattle in Costa Rica<sup>11</sup> and the United States<sup>3,9</sup> caused infection, but signs of clinical illness were not described. Similar results in cattle and sheep were reported by workers in Canada.<sup>44</sup> Also noteworthy was the inoculation of BIV (FL112) into Bali cattle (*Bos javanicus*).<sup>45</sup> Infection was established, but signs of clinical illness were not observed during the 8-week study. This is in sharp contrast to findings in Bali cattle experimentally infected with Jembrana disease virus, which may develop an acute febrile illness that may be fatal.<sup>46</sup>

Most experimental studies of BIV have been conducted with BIV (R29) or clones derived from the original isolates. Inoculated animals usually develop persistent infections without signs of clinical illness.<sup>5,9,47</sup> More recently, wild-type BIV isolates have been available for study, and, to date, experimental infections in cattle have caused only subtle signs of illness, or the cattle have remained healthy.<sup>17,23,37</sup>

### Detection Methods Used to Follow Viral Infection

Various assays have been developed for BIV detection, including agar-gel immunodiffusion (AGID), indirect fluorescent antibody (IFA) tests, western blot, ELISA, and PCR.<sup>5,26,28,48-50</sup> The IFA assay is simple to perform and may be used to rapidly screen small numbers of animals. However, the assay lacks sensitivity in BIV-infected animals that are followed long term. Whetstone et al<sup>6</sup> found that after 2 years, IFA titers were undetectable.

The AGID assays are simple to perform and have been the mainstay of BLV and EIA testing for years. For BIV, the production of sufficient antigen for the assay has been problematic, but this would have been overcome if reliability of the assay were better. Not all sera from experimentally infected cattle yield a positive response in the AGID assay, and positive responses are detected only during the first several months after infection.<sup>6</sup> Western blot analysis is 40 times more sensitive than AGID<sup>6</sup> but has limitations for use in detection of BIV infection in cattle. Higher costs and limited supplies of specific BIV antigens restrict the use of western blot analysis for routine herd testing. Some investigators have used concentrated whole virus, infected cell extracts, and BIV antigen expressed in bacteria or insect cells.<sup>5,49</sup> An ideal BIV western blot would incorporate multiple BIV antigens and minimal extraneous proteins. Zhang et al<sup>49</sup> reported a strong correlation between results of a western blot assay, using a recombinant gag-coded protein and a PCR assay for detection of BIV-infected cattle during a short period.

Serologic assays, using ELISA, are ideally suited for screening large numbers of animals. The use of whole-virus or infected cell extracts as the source of antigen can lead to high background values that may obscure weak responses. For BIV, this is of concern, because antibody responses do not remain constant with time. Isaacson et al<sup>48</sup> monitored antibody responses to gag- and env-coded proteins after experimental infections. Anti-gag antibodies declined to undetectable concentrations by 40 weeks after inoculation in some cattle. The ELISA and western blot assays that use only gag antigens could be vulnerable to this declining reactivity. Recently, ELISA have been designed to detect BIV transmembrane protein antibodies and offer promise for herd screening.<sup>50</sup>

Nucleic acid detection by use of PCR could become the gold standard in defining BIV infection status of cattle.<sup>22,28,32,51</sup> As indicated, there are only a few isolates of BIV, and not all have been sequenced. In order to establish the usefulness of a PCR test, it is necessary to ensure that the primer pairs span a region of

the genome that is conserved from one BIV strain to another. Even with few nucleotide sequences, a valid assay could be developed if numerous isolates were available to validate the universal reactivity of the primer pairs. At this time, there are limited sequences from a limited number of BIV isolates. A variation of the PCR was reported in which PCR was used in an in situ hybridization assay to define the presence of BIV in various tissues of experimentally infected cattle.<sup>33</sup> This type of assay essentially replaces immunohistochemistry for defining the presence of virus in specific tissues and has worked well in an experimental setting. Because a diagnostic assay that is considered the gold standard for BIV infection is not available, a combination of assays that includes a reliable serologic assay for screening cattle for BIV infection and a specific PCR for definitive identification of carrier animals is important for understanding the epidemiologic features of BIV infection.<sup>51</sup>

### Risk Assessment

Assessment of the risk posed by BIV to the US cattle industry is at an early stage. The information presently available regarding BIV leads the committee to conclude that the knowledge that would enable evaluation of the probability of introduction of more pathogenic biotypes of BIV than those encountered to date and the consequences of such introduction simply does not exist at this time. Reviews of the BIV literature by others often return to the same themes: the plasticity of the BIV genome and the need for better diagnostic tests and long-term pathogenesis studies.<sup>8,14</sup>

A fundamental unanswered question for veterinarians and cattle producers regarding BIV is the extent to which BIV infection can be implicated in multifactorial production diseases of cattle. Numerous factors such as host genotype, BIV strain, environmental factors, concurrent infections, nutrition, housing, and climate have been suggested.<sup>8,14,25,34,52</sup> With the exception of the well-characterized prototype BIV (R29), studies of the few herds in which associations have been made between BIV infection and disease have not resulted in extensive reports characterizing additional viral isolates or their pathogenicity.<sup>1,5,25,35</sup> Thus far, only 3 BIV isolates have been fully or partially characterized in pathogenesis studies.<sup>10,33,37</sup>

### Committee Recommendations

**Research considerations**—In an era of scarce public resources and many competing animal agricultural research priorities, it is incumbent upon scientists active in BIV research to focus their efforts and prioritize objectives. In this context, little more can be gained from descriptions of clinical or pathologic findings from herd-centered disease attributed to BIV infection that are based primarily on concurrent positive results of serologic tests.<sup>31,35,39</sup> Koch's postulates continue to be a valid standard of proof in animal disease research, and results of BIV pathogenesis research that is consistent with these postulates has so far established evidence only for transient mild perturbations of immune response, persistent lymphadenopathy, and clinically inapparent meningoencephalitis.<sup>10,12,31,48</sup>

Herd investigations in which BIV-related disease is suspected may be a useful guide for well-focused pathogenesis research but cannot replace a complete production-medicine approach. Such herd studies would be particularly useful in establishing whether infection by BIV is a sufficient prerequisite for development of opportunistic infections or whether observed clinical disease is associated with other common management errors. In particular, diagnostic virologic investigation of such herds should investigate the possibility of persistent coinfections with agents such as BVD virus, a prevalent virus in the cattle population that has recognized immunosuppressive effects.<sup>14</sup> Attempts to isolate and characterize BIV from diseased cattle will be necessary to assess concerns about variations in BIV pathogenicity among isolates. One can only conclude from the published literature that expressions of clinical disease among BIV-infected cattle are usually subtle or rare.<sup>7,37,52</sup> With respect to the potential for increased susceptibility of BIV-infected cattle to secondary infections, a comment of McNab et al<sup>7</sup> is insightful: "More investigation is needed to confirm these findings, to investigate such mechanisms (of secondary infections) and to place the potential negative impact of BIV into perspective, relative to the other challenges that face the dairy industry. The latter may provide greater marginal returns on investment in their correction, than might the control of BIV."

Researchers face a fundamental choice in efforts to determine whether this virus is associated with specific disease or a disease syndrome that is of economic importance to the cattle industry. One alternative is pursuit of multi-herd population studies, similar to those performed by McNab et al<sup>7</sup> and Jacobs et al,<sup>52</sup> that attempt to identify important associations between BIV infection and industry management practices as well as consequences of BIV infection for production, reproduction, culling, calf viability, or other economically important measures of herd performance. Such population studies would require sensitive and specific diagnostic assays with the capacity to identify strains of BIV other than those presently characterized. A second alternative is to conduct long-term pathogenesis studies, using several BIV strains and possibly incorporating coinfection experiments with common bovine viruses, especially BVD virus.

The identification of Jembrana disease virus, a bovine lentivirus with some similarities to BIV, illustrates the pathogenic potential of this genus in bovine species.<sup>24</sup> Indications that viral load (replication efficiency) *in vivo* may in part determine pathogenic potential and could provide a means for selecting candidate isolates for long-term pathogenesis studies.<sup>16</sup> In addition, consideration should be given to selection of lactating dairy cows as experimental animals, reflecting the natural history of BIV-associated disease.<sup>25,35,42</sup>

**Environmental considerations**—The common association of BIV-associated disease and high seroprevalence with herds located in tropical or subtropical locations is striking.<sup>5,35,38,42</sup> Of particular interest are different rates of BIV seroprevalence between 2 Mississippi institutional dairy herds that share a com-

mon heifer-raising facility.<sup>38</sup> Clinical disease was not described in either herd. Assuming these results were not confounded by differing age distributions, this suggests that different management practices or environmental entities are involved in BIV transmission. Although it is tempting to speculate about the possible role of arthropod-borne transmission of BIV or concurrent arboviral infections, evidence for such is lacking. It may be more informative to investigate such management practices as use of blood-contaminated needles or instruments, use of blood-based vaccines, or the increased risk of introducing multiple bovine viruses into herds in nutritionally deficient areas when dairy replacements are acquired from many sources.

**Trade considerations**—Considering the present state of knowledge regarding BIV, including serologic evidence for global distribution, any consideration of regulatory constraints on trade must be approached with great caution. As a signatory to the World Trade Organization, the United States is committed to science-based trade regulations, transparency in adoption of such regulations, and equivalent treatment of all international and domestic traders. Simply put, any trade regulations that attempt to exclude foreign cattle on the basis of alleged BIV status or between-country differences in BIV strains would have to meet a high standard of justification. Likely standards would include validation of diagnostic tests, demonstrable differences in pathogenicity and prevalence of BIV biotypes between trading partners, and existence of a domestic BIV control program for such regulations not to be in violation of the zoosanitary standards of the World Trade Organization.

As a practical matter, almost all important international trade of live US cattle takes place with Canada and Mexico. This trade has existed for many years, so there is every reason to believe the BIV status of these 3 countries to be similar. Cattle from western Europe are currently forbidden entry into the United States because of bovine spongiform encephalopathy. No cattle are imported from Indonesia. Accordingly, the risk of introducing new strains of BIV through live cattle trade appears to be quite low.

With respect to semen and embryos, there is limited information regarding the potential for transmission of BIV through embryo transfer. Recent studies on the retention of BIV infectivity of mock-infected preimplantation-stage bovine embryos with BIV (R29 strain) after cryopreservation in liquid nitrogen did not reveal infectious virus.<sup>53</sup> Experimental evidence and that from actual practice indicates that transmission of another bovine retrovirus, BLV, is completely controlled by commonly employed embryo transfer techniques.<sup>54</sup> Similar control measures should serve to reduce the potential spread of BIV among susceptible cattle.

The risk of spreading BIV through artificial insemination has been controversial. Detection of BIV by use of PCR in processed frozen-thawed semen raised the possibility of transmission through contaminated semen.<sup>14,27</sup> However, this work did not provide any evidence of viability of BIV in the processed semen nor provide convincing epidemiologic evidence for semi-

nal transmission.<sup>27</sup> Although 1 study indicated that bulls were twice as likely (12.6%) to be BIV-seropositive than the general dairy population in Ontario, Canada (5.5%), neither data on semen quality nor BIV infectivity in semen were reported.<sup>55</sup> Results of subsequent studies indicate that experimentally infected bulls have negative results of PCR for BIV in their semen.<sup>28</sup> Again, experiences from BLV control may be instructive. After several decades of study, there is no credible direct or epidemiologic evidence for transmission of BLV by frozen semen processed for commercial artificial insemination.<sup>56</sup> Clearly, regulations restricting semen trade that are justified by purported BIV control would bear a heavy burden of scientific justification and proof of compliance with World Trade Organization standards.

**Public health considerations**—Several reviews of the scientific literature have revealed no evidence of zoonotic potential for BIV.<sup>16,57</sup> The virus is routinely inactivated by pasteurization of milk.<sup>58</sup>

With regard to animal health policy development and funding priorities, the authors suggest that prioritization of support for BIV research will be driven by cattle industry perceptions of its economic losses from BIV relative to other important infectious agents and disease complexes. In this context, multierd studies of economic impacts of BIV will be a research priority inseparable from those discussed in this report.

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