Natural bovine lentiviral type 1 infection in Holstein dairy cattle. I. Clinical, serological, and pathological observations

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Abstract

Clinical, serological, and pathological abnormalities observed in Holstein cows naturally infected with bovine lentivirus 1 bovine immunodeficiency virus (BIV) and other infections were progressive and most commonly associated with weight loss, lymphoid system deficiency, and behavioral changes. Clinical evidence of meningoencephalitis was dullness, stupor, and occasional head or nose pressing postures. The polymerase chain reactions associated the BIV provirus with the lesions in the central nervous system and lymphoid tissues. Multiple concurrent infections developed in retrovirally infected cows undergoing normal stresses associated with parturition and lactation. A major functional correlate of the lymphoreticular alterations was the development of multiple secondary infections which failed to resolve after appropriate antibacterial therapy. The chronic disease syndrome in dairy cows associated with BIV may be useful as a model system for investigation of the pathogenesis of

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Abbreviations: BIV, bovine immunodeficiency virus; PCR, polymerase chain reaction; LN, lymph node; HLN, hemal lymph node; BLV, bovine leukosis virus; HIV, human immunodeficiency virus.

the nervous system lesions and lymphoid organ changes that occur in humans with lentiviral infection.

Keywords: Bovine immunodeficiency virus; Bovine lentivirus type 1; Lentiviral disease; Meningo-encephalitis; Hemal lymph node; Secondary infection; Weight loss; Cattle

Résumé

Les anomalies cliniques, sérologiques et pathologiques observées chez les vaches Holstein infectées naturellement par le lentivirus bovin de type 1 (virus de l'imunodéficience bovine; VIB) et par d'autres infections sont généralement progressives, associées à une perte de poids, une déficience du système lymphoïde et à des changements du comportement. Les évidences cliniques de méningo-encéphalite sont la torpeur, la stupeur et des postures occasionnelles avec pression de la tête ou du nez contre un objet. Le provirus VIB est associé aux amplifications de séquences nucléotidiques (PCR) généralement trouvées lors de lésions du système nerveux central et du tissu lymphoïde. Des infections simultanées multiples apparaissent chez les vaches infectées par le rétrovirus sous l'effet du stress survenant durant la parturition et la lactation. Des altérations lymphoréticulaires sont associées au développement d'infections secondaires multiples qui ne peuvent généralement être traitées par les antibiotiques traditionnels. Le syndrome de la maladie chronique associée au VIB chez les vaches laitières représente probablement un modèle utile pour l'étude du développement des lésions du système nerveux et des changements de l'organe lymphoïde chez l'homme infecté par les lentivirus. © 2002 Elsevier Science Ltd. All rights reserved.

Mots-clé: Virus de l'imunodéficience bovine; Lentivirus Type 1 bovin; Infections secondaires; Perte de poids; Méningo-encéphalite; Ganglion hématique; Amplification des séquences nucléotidiques

1. Introduction

In 1969, a Louisiana dairy cow (R-29) was found to be infected with a virus that was identified as a retrovirus. It was subsequently named bovine immunodeficiency virus, also known as bovine lentivirus-1 (BIV) [1–4]. The cow had persistent lymphocytosis, became severely weak and emaciated following parturition and was euthanized. The lymph nodes (LN) were enlarged; although, evidence of lymphosarcoma was not detected. The LN had generalized hyperplasia and the brain had mild lymphocytic perivascular cuffing [1]. Subsequent experimental infection of cattle with this isolated agent (BIV isolate R-29) produced lesions in the lymphoid system, ranging from lymphocytosis to lymphoid hyperplasia of the LN and hemal lymph nodes (HLN). Studies of BIV were discontinued until the value of investigating the comparative features of this agent with the human immunodeficiency virus (HIV) was recognized [5–8].

Serological studies in Louisiana have demonstrated substantial prevalence (11%) of BIV infection in dairy cattle [9]. Endemic BIV infection of 48–77% has been documented [10]. During an adverse winter and spring of 1991, more than 30% of the cows in a production herd died or were culled for multiple primary and secondary disease processes [10]. The cows had multiple disease conditions including abscesses (non-responsive to treatment), laminitis and infectious pododermatitis, and mastitis. A cosmopolitan distribution of BIV has been demonstrated by detection of immunologic and molecular methods [3,4,11].

Until recently, studies of cattle maintained under non-stressful experimental conditions and inoculated with BIV isolates have demonstrated subclinical effects in lymphoid tissues and relatively minor alterations of in vivo immunological function [3,12]. Experimental BIV infection of cattle has been associated with immune dysfunction which included a decreased CD4/CD8 ratio with delayed and lower antibody responses to bovine viral diarrhea virus (BVDV) and bovine herpesvirus 1 [13]. Viral replication was identified by detection of BIV provirus or by virus isolation in a variety of tissues, including brain [14–17]. Lymphadenopathy and non-suppurative meningoencephalitis have been identified in calves experimentally infected with BIV strain FL112 isolated from a dairy cow in Florida, USA [18–21]. Two of six calves inoculated with BIV had clinical ataxia [21], suggestive of the bovine paraplegic syndrome (BPS) observed in Venezuelan cattle [22].

During a 17 month period, 16 cows were removed from a dairy herd with endemic BIV infection and euthanized due to one or more disease conditions. Efforts were made to: (1) identify all the infectious disease processes and the etiologic agents in each cow, (2) document the presence of BIV provirus in the brain and lymphoid organs, and (3) characterize the lymphoid tissue and brain lesions. This report documents clinical, serological, and pathological observations in Holstein dairy cattle associated with natural infections of bovine lentivirus-1 (BIV).

2. Materials and methods

2.1. Animals and analyses

Fifteen adult Holstein cows and one juvenile adult, ages 14 months to 6 years (mean = 2.9 years) were obtained from a dairy herd ($n = 90$), with endemic retroviral infection, humanely euthanized and necropsied during a 17 month period (Table 1). The dairy herd was a well managed, producing herd used for teaching and research activities at the Baton Rouge, Louisiana unit of the Louisiana Agricultural Experiment Station. Cows were fed a total mixed ration and had rolling herd average milk of 8816 kg (19,395 lb) per year. Veterinary examinations and treatments were provided, as needed, through the Large Animal Clinical Service of the LSU Veterinary Teaching Hospital and Clinics. There were no unusual environmental stresses such as exposure to inclement weather. Clinical disease problems, weights, and body condition scores [23] were recorded, at the time of parturition, before the onset of clinical disease, and at euthanasia (Tables 1 and 2). Immediately prior to euthanasia, whole blood was collected for hematological and serological analyses. A prompt necropsy was conducted beginning with the collection of the brain and completed for all tissues within 2 h following death.

Tissues were submitted to the Louisiana Veterinary Medical Diagnostic Laboratory for immunofluorescence examination and/or culture for the following pathogens: BVDV, infectious bovine rhinotracheitis virus (IBRV), parainfluenza virus-3 (PI3), bovine respiratory syncytial virus (BRSV), bovine parvovirus (BPV), bovine rotavirus (BRV), bovine coronavirus (BCV), and *Chlamydia psittaci*. Serum samples were tested for antibody to bovine leukosis virus (BLV) (ELISA kit, IDEXX Herd Chek BLV Antibody

Table 1
Age, body weights, duration of clinical illness, and weight loss for 16 Holstein cows infected with natural bovine immunodeficiency virus infection

Animal number	Age (years)	Last weight[a]		Necropsy day weight		Weeks of illness	Weight loss (kg)	% loss
		Weight (kg)	Body score	Weight (kg)	Body score			
1	4	591	3.0	364	1.8	4	227	38.4
2	6	636	3.1	591	2.5	40	45	7.1
3	3	636	3.1	409	2.3	8	227	35.7
4	4	591	3.5	591	3.5	40	0	0
5	4	591	3.0	500	2.5	3	91	15.4
6	4.5	636	2.8	386	1.5	8	250	39.3
7	3	591	2.5	580	2.5	20	11	1.9
8	2	591	2.8	409	2.0	8	182	30.8
9	2	545	2.8	455	2.2	4	90	16.5
10	2	545	2.8	386	1.8	8	159	29.2
11	2	500	2.3	545	2.4	4	G[b]	–
12	3	591	3.0	580	3.0	4	11	1.9
13	1[c]	364	2.8	309	2.3	12	55	15.1
14	2	591	3.1	470	2.8	8	121	20.5
15	3	591	3.3	591	3.3	8	0	0
16	2	500	3.0	409	2.5	12	91	18.2

[a] Last weight recorded prior to onset of clinical disease effects.
[b] G: Cow gained 55 kg.
[c] Young adult, age 14 months.

Table 2
Retroviral detection, presence of encephalitis and clincal disease conditions in 16 Holstein cows from a herd with endemic bovine immunodeficiency virus infection

Animal number	BIV		BLV	Encephalitis[a] (viral-type)	Disease problems (primary and opportunistic)
	T[b]	E[c]	E		
1	+	+	+	+	Postpartum calcium therapy, metritis, mastitis, subcutaneous abscesses (*E. coli*)
2	−	+	+	+	Abscess—1 foot; abscessed mammary lymph node (*S. bovis*)
3	−	+	+	+	Teat laceration, mastitis with abscessation (*S. bovis)*
4	−	+	+	+	Left displaced abomasum, chronic mastitis with abscessation and fibrosis (*S. uberis*)
5	−	−	+	+	Nocardial mastitis (2 quarters), mammary LN (*Corynebacterium* spp.), focal interdigital ulceration
6	+	−	+	+	Prepartum LDA surgery, mastitis, myositis and multiple subcutaneous abscesses, abscess caudal to stifle (*Actinomyces pyogenes*), hepatic distomiasis, NC-BVDV[d]
7	+	+	+	+	Myositis and abscess, arthritis and tendonitis (*S. Agalactiae*), NC-BVDV
8	−	+	−	+	Uterine rupture and peritonitis (*S. agalactiae*), hepatic abscesses, subcutaneous seroma on lateral abdomen, hepatic distomiasis, NC-BVDV
9	+	−	−	+	Metritis, mastitis with abscessation (*S. agalactiae*), hepatic distomiasis, NC-BVDV
10	+	+	+	+	Mastitis with abscessation (beta-hemolytic *Streptococcus* spp), septicemia, hepatic distomiasis
11	+	−	−[e]	(+/−)	Mastitis (no bacterial isolate), ovarian cyst, erosions of soles, liver (*Salmonella* sp. Isolated)
12	+	+	+	+	Agalactia, erosions of sole and pododermatitis—4 feet, hepatic distomiasis, C-BVDV
13	+	NT	−[e]	+	Periarticular cellulitis, tendonitis and abscesses deep at right stifle, hepatic distomiasis
14	+	+	−[e]	+	Myositis and stifle abscess (beta-hemolytic *Streptococcus* sp.), generalized Demodex infestation, hepatic distomiasis
15	+	+	+	+	Lymphosarcoma, multiple organs, (uterus), interdigital dermatitis and separation of hoof wall, 1 foot, abortion, positive Chlamydia antibody
16	+	+	+	+	Hygroma, myositis, mastitis, erosions of soles with abscess—2 feet, rumenitis (*E. coli* isolated from liver), hepatic distomiasis

[a] Histologic evaluation.
[b] Detection of BIV provirus in tissue.
[c] ELISA serum antibody detection.
[d] NC-BVDV, C-BVDV: Non-cytopathic, cytopathic bovine viral diarrhea virus; NT: not tested.
[e] Previously tested positive for BLV.

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Test Kit). Detection of BIV antibody by ELISA was performed as previously described [10]. Bacteriologic cultures were performed on all gross tissue lesions suspected to have a bacterial etiology. Intestine and liver were submitted for *Salmonella* culture. Colon contents and bile were collected for parasitological examination. Routine sections of all major organs were collected, fixed in 10% zinc formalin, and processed by routine methods for staining and histology.

2.2. Detection of BIV in tissues

The polymerase chain reaction (PCR) to amplify BIV proviral sequences was completed on DNA recovered from various brain regions and from selected lymphoid organs, using standard methods [24–27]. The left hemisphere of the brain was sectioned for the collection of regions identified as cerebral cortex, hippocampus, medial geniculate body, brainstem, cerebellar peduncles, cerebellar folia, and medulla oblongata at the level of the cerebellar vermis. Tissues were frozen at $-20\,°C$ and stored at $-70\,°C$ for 1–52 weeks prior to shipment to Mississippi State for BIV-PCR analysis. Brain tissues from 14 of 16 cows, and lymphoid organs from 15 of 16 cows, were tested for the presence of BIV proviral DNA (Table 3).

Purification of DNA from tissue was accomplished with the Puregene kit (Gentra Systems). The DNA from BIV-R29-infected, and uninfected, fetal bovine lung (FBL) cells was purified by the same method and served as positive and negative controls, respectively. The purified DNA was probed for BIV proviral DNA sequences by targeting a 495 bp region of the reverse transcriptase domain of the BIV *pol* gene [27]. The positive sense primer sequence was 5′ATGCTAATGGATTTTAGGGA3′ and the negative primer sequence was 5′AACGCCATTTCTTGGGTGTG3′. The PCR reaction mixture consisted of $0.1\,\mu M$ of each primer, 2.5 mM $MgCl_2$, PCR reaction buffer, and 5–10 μg template DNA. The reaction mixture was heated from 93 to $100\,°C$ for 10 min. The mixture was cooled on ice for 5 min, then 200 μM each of dATP, dTTP, dCTP, dGTP, and 1 μl of 0.5 U Taq DNA polymerase (Promega) was added. The final reaction volume was $100\,\mu l$. The reaction mixture was overlaid with mineral oil, then subjected to 33 cycles of the following cycling scheme, using the Delta Cycler II thermocycler (Ericomp): $94\,°C$ for 45 s, $45\,°C$ for 30 s, and $72\,°C$ for 2 min. The PCR reaction products were electrophoresed in 7.5% polyacrylamide gels; the gels were stained with ethidium bromide, and the DNA products were visualized using a UV transilluminator. Tissues that yielded indeterminate PCR reactions had product of inappropriate size, compared to the positive controls.

3. Results

3.1. Clinical observations

Eleven of the 16 cows had between 7.1 and 39.3% weight loss after the onset of clinical problems and the majority had more than one active disease process at the time of euthanasia (Tables 1 and 2). Body condition scores fell from a preclinical disease level of 2.5–3.5 (mean 2.9) to 1.5–3.5 (mean 2.4). Twelve of 15 postpartum cows were in the first

Table 3
Tissues tested by polymerase chain reaction for bovine immunodeficiency virus proviral DNA for 16 Holstein cows with endemic infections

Animal number	Brain—anatomic regions			Lymphoid organs		
		Positive	Negative		Positive	Negative
1	(0/0)[a]			+(2/3)	Spleen, Hemal LN	Mesenteric LN
2	−(0/2)		Brainstem, cerebellar peduncle	−(0/3)		Spleen, mesenteric LN, hemal LN
3	(0/0)			−(0/2)		Spleen, hemal LN
4	−(0/2)		Brainstem, cerebellar peduncle	−(0/2)		Spleen, hemal LN
5	−(0/2)		Brainstem, cerebellar peduncle	(0/0)		
6	+(1/2)	Medial gen. body	Hippocampus, brain (unspecified site)	+(2/3)	Thymus, pre-scapular LN	Spleen
7	+(2/3)	Brainstem, hippocampus		−(0/2)		Spleen, mesenteric LN
8	−(0/4)		Brainstem, cerebral cortex, hippocampus medial gen. body	−(0/1)		Spleen
9	+(3/3)	Brainstem, cerebral cortex, hippocampus		−(0/2)		Spleen, mesenteric LN
10	+(1/1)	Cerebral cortex		−(0/2)		Mesenteric LN, hemal LN
11	−(0/3)		Brainstem, cerebral cortex, medial gen. body	+(2/5)	Thymus, hemal LN	Spleen, mesenteric LN, pre-scapular LN
12	+(2/4)	Brainstem, hippocampus[b]	Cerebral cortex, medial gen. body	+(2/2)	Spleen, mesenteric LN	
13	+(1/2)	Medial gen. body[b]	Brainstem	+(2/4)	Spleen, mesenteric LN[b]	Pre-scapular LN, hemal LN
14	+(1/3)	Brainstem[b]	Cerebral cortex, hippocampus	−(0/4)		Spleen, mesenteric LN, pre-scapular LN, hemal LN
15	+(2/3)	Medial gen. body, brain (unspecified site)	Brainstem	−(0/3)		Spleen, mesenteric LN, pre-scapular LN
16	+(3/3)	Cerebral cortex, hippocampus, medial gen. body		+(4/4)	Thymus, spleen, mesenteric LN, hemal LN	

[a] ( ) = Positive/total brain regions or lymphoid organs tested.
[b] Indeterminate.

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stage of lactation and the 11 cows that lost weight during the terminal disease process leading to the decision for euthanasia had a decrease in mean body condition score from 2.94 to 2.20 (Table 1). Clinical disease duration ranged from 3 to 40 weeks. One juvenile adult, animal 13, age 14 months, was approximately 55 kg (15.1%) below its previous weight. No consistent hematological changes were detected in any of the cows, except for a lymphocytosis in 6 of 15 cows, including cow 15 which had grossly detectable lymphosarcoma in the uterus and multiple LN.

The secondary disease processes, and their incidence included: metritis (12.5%), subcutaneous abscesses (18.75%), purulent arthritis (18.75%), laminitis and infectious pododermatitis (37.5%), distomiasis (50%), and mastitis (56.25%) (Table 2). In addition to weight loss, clinical signs included reduced vitality, torpidity, dullness, and stupor.

3.2. Microbiologic and serologic findings

Eleven of 15 cows had positive BIV ELISA reactions, four were negative and the juvenile adult, animal 13, was not tested. Antibody to BLV was demonstrated in 11 of 16 cows; however, three of the BLV negative cows had previously tested positive for BLV, yielding 14 of 16 (87.5%) serologically positive animals (Table 2). Four cows (animals 6, 7, 8, and 9) tested positive by tissue culture for non-cytopathic BVDV (NC-BVDV), although the tissues had initially tested negative using the fluorescent antibody method. Cow 12 yielded cytopathic BVDV (C-BVDV) from the buffy coat cells of a fresh blood sample collected prior to euthanasia. No cow had clinical or morphologic changes diagnostic for acute or chronic BVDV infection. Tests for IBRV, PI3, BRSV, BPV, BRV, and BCV were negative.

Tissue from the brain and small intestine of each cow tested negative for *C. psittaci* using the fluorescent antibody method and cell culture inoculation. Cow 15 aborted 2 months prior to euthanasia and had uterine lymphosarcoma as well as a positive serological response for *C. psittaci*.

Bacteriologic culture of abscesses, mastitic glands, and regional LN yielded the following pathogens (Table 2): *Streptococcus bovis*, *Streptococcus agalactiae*, *Streptococcus uberis*, *E. coli*, *Nocardia* sp., and *Corynebacterium* sp. Normal liver tissue from cow 11 yielded a *Salmonella* sp. although no signs or lesions of salmonellosis were detected histologically.

Generalized *Demodex folliculorum* infestation was present in cow 14. Hepatic distomiasis due to *Fasciola hepatica* was identified in 8 of 16 cows by the presence of ova in the bile or feces or enlarged fibrotic bile ducts (Table 2). Occasional cows had low numbers of Trichostrongylid ova in the feces; however, no parasite-induced abomasal or intestinal lesions were observed.

3.3. Lesions observed

Thirteen cows had moderate to marked enlargement of the HLN. These were evident as slightly movable subcutaneous nodules, most commonly in the neck and flank. Enlarged HLN were also noted in the sublumbar regions and in association with LN. Morphology of LN varied from hyperplastic to normal to atrophic with changes in architecture which were

correlated with immunologic deficiencies [28]. All 16 cows had morphologic evidence of central nervous system (CNS) disease with variable lesion development. Encephalitis with mononuclear cell infiltration was present in 15 of 16 cows. The pituitary of some cows had multifocal and perivascular accumulations of lymphocytes similar to the infiltration pattern observed in the brain.

3.4. PCR analysis

Brain tissues from 6 of 14 cows and lymphoid tissues from 5 of 15 cows were positive for BIV provirus using PCR to amplify a region of the BIV *pol* gene (Table 3). Brain tissue of 3 of 14 cows and lymphoid tissue of 1 of 15 cows yielded indeterminate PCR reactions. Only 5 of 16 cows were negative for BIV provirus in all the tissues examined. In the brain tissue, BIV provirus was detected in the medial geniculate body ($n = 3$), brainstem ($n = 2$), hippocampus ($n = 3$), and cerebral cortex ($n = 5$) (Table 3). In the lymphoid tissues, BIV provirus was detected in the thymus ($n = 3$), mesenteric LN ($n = 2$), prescapular LN ($n = 1$), HLN ($n = 2$), and spleen ($n = 3$) (Table 3). Brainstem ($n = 2$), hippocampus, medial geniculate body, spleen, and mesenteric lymph node of three cows yielded indeterminate PCR product. Four of the five PCR negative cows were positive by ELISA for serum BIV antibody, and all five had histologic encephalitis.

4. Discussion

Detection of BIV provirus in lymphoid and brain tissue was consistent with the endemic occurrence of BIV in the herd. Storage of tissues for prolonged periods (8–12 months) may have resulted in a higher incidence of negative results. The frequent development of concurrent infections in BIV-infected animals, especially those associated with abscesses, mastitis, and laminitis, suggested that persistent BIV infection had a role in reducing functional immune competence. Lentiviral infection of cells of the reticuloendothelial system, such as the follicular dendritic cells (FDC) can interfere with immunological recognition at several levels and may be implicated in the generation of abnormal immunological reactions [29]. In the animals infected with the bovine lentivirus 1, FDC infection and functional alteration of these cells and/or the lymphoid tissue microenvironment [30] may allow the perpetuation of a chronic infection independent of on-going viral effects on the lymphocytes of the immune system. Under some circumstances the presence of parasitic and other antigenic sources has produced an accelerated onset and clinical course of HIV disease and may be a factor for precipitation of the BIV-associated clinical disease [31,32]. Concurrent endemic BLV and co-infection with BIV may have influenced disease manifestation. In the United States, BLV herd infection rates were 89% for all US dairy operations and 99% for those in the southeast region which included Louisiana [33].

The recovery of four isolates of NC-BVDV and one C-BVDV isolate suggests that BVDV may be a primary or secondary disease agent in BIV-infected cows. The standard of veterinary care for the dairy included calfhood vaccination for BVD using killed BVDV vaccine. A reduced level of antiviral protection may have been related to the BIV infection

as a lower antibody titer for BVDV has been demonstrated following experimental BIV infection [13]. There were no mucosal lesions of BVDV recognized in the cows.

The failure to associate Chlamydial infection with the lesions suggests two possibilities. The initial reports of Chlamydial-associated sporadic bovine encephalo-myelitis (SBE) may have been instances where *Chlamydia* sp. occurred as a concurrent pathogen and the BIV retroviral infection was not recognized. Another possibility is that the nervous system lesions of SBE and BIV are closely similar or identical at the light microscopic level [34]. Months after completion of this investigation, a cow aborted with a pigeon strain of *C. psittaci* which was isolated from the placenta and fetus [35]. Several weeks later this cow (which was serologically positive for BIV) died with metritis and brain lesions of non-suppurative lymphocytic encephalitis. At the time of death the cow was negative for Chlamydia antibody [35], suggestive of anergy, possibly similar to that reported for BIV as a loss of BIV Gag-specific antibody during the course of infection [36]. Another potential explanation for the encephalitis, other than primary lentiviral infection, is activation of latent vaccinal IBRV. The animals; however, had received calfhood vaccinations and no evidence of IBRV infection or recrudescence was found.

In most of the cows with debilitation and weight loss, the LN were normal or reduced in size with reduction or absence of follicular hyperplasia. In cows that had weight loss of approximately 40%, the HLN were not enlarged. Muscle wasting, which occurred while the cows had free-choice access to feed, raised the possibility of a central depression in appetite, suggestive of a clinical neurogenic anorexia which was observed in BIV-infected calves [31]. Weight loss is a component of the recognized progressive changes of initial symptomatic disease in humans with AIDS [37,38].

Localization of BIV provirus in brain and lymphoid tissues was consistent with the observations of others [14–16,39]. Torpidity, stupor, dullness and reduced vitality were observed and clinically attributed to the systemic effect of the multiple subcutaneous abscesses and laminitis, but were possibly the result of lentiviral-induced CNS lesions [10,40]. Long-term multiyear investigations of dairy cows under modern production stresses are needed to elucidate the mechanisms of cellular infection, viral proliferation, and transmission of BIV.

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