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Source: Journal of Wildlife Diseases, 43(4) : 700-710

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-43.4.700

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Journal of Wildlife Diseases, 43(4), 2007, pp. 700–710
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Variability in assays used for detection of lentiviral infection in bobcats (*Lynx rufus*), pumas (*Puma concolor*), and ocelots (*Leopardus pardalis*)

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ABSTRACT: Although lentiviruses similar to feline immunodeficiency virus (FIV) are known to infect numerous felid species, the relative utility of assays used for detecting lentiviral infection has not been compared for many of these hosts. We tested bobcats (*Lynx rufus*), pumas (*Felis concolor*), and ocelots (*Leopardus pardalis*) for exposure to lentivirus using five different assays: puma lentivirus (PLV), African lion lentivirus (LLV), and domestic cat FIV-based immunoblots, a commercially available enzyme-linked immunosorbent assay (ELISA) kit, and nested polymerase chain reaction (PCR). Puma lentivirus immunoblots identified more seropositive individuals than the other antibody-detection assays. The commercial ELISA provided a fair ability to recognize seropositive samples when compared with PLV immunoblot for screening bobcats and ocelots, but not pumas. Polymerase chain reaction identified fewer positive samples than PLV immunoblot for all three species. Immunoblot results were equivalent whether the sample tested was serum, plasma, or whole blood. The results from this study and previous investigations suggest that the PLV immunoblot has the greatest ability to detect reactive samples when screening wild felids of North America and is unlikely to produce false positive results. However, the commercial ELISA kit may provide an adequate alternative for screening of some species and is more easily adapted to field conditions.

Key words: Bobcat, ELISA, FIV, immunoblot, lentivirus, ocelot, PCR, puma.

INTRODUCTION

Diseases pose health risks to individuals and may threaten the persistence of wildlife populations (Deem et al., 2001). Collection of baseline prevalence data and surveillance of wildlife populations, therefore, are needed to recognize incipient outbreaks so that intervention can be instigated expeditiously. The results of diagnostic assays, and any management actions dependent on those test results, need to be interpreted with consideration of the sensitivity and specificity of the assay. This is of particular concern when assays developed for domestic animal diseases are used to test nondomestic species; in most cases, those tests have not been validated for use in wildlife (Gardner et al., 1996).

Host-specific lentiviruses have been isolated from numerous nondomesticated felids, including three American species: puma (*Puma concolor*; Carpenter et al., 1996), bobcat (*Lynx rufus*; Franklin et al., 2007), and ocelot (*Leopardus pardalis*; Troyer et al., 2005). Unlike domestic cat feline immunodeficiency virus (FIV), which can cause severe immunosuppression and human immunodeficiency virus (HIV)-like symptoms in felids, lentiviruses of wild felids have not historically been associated with significant pathogenicity (VandeWoude and Apetrei, 2006). However, several captive African lions (*Panthera leo*) have manifested clinical

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signs or hematologic abnormalities associated with lentiviral infection (Poli et al., 1995; Spencer et al., 1995; Bull et al., 2003). Although few studies have investigated the clinical consequences of lentiviral infection in wild felids, a recent study has demonstrated that infection in wild African lions (*P. leo*) and pumas causes CD4+ lymphocyte depression (Miller et al., 2006; Roelke et al., 2006). Further, feline leukemia virus (FeLV) infection in feral domestic cats (*Felis catus*) has been associated with FIV infection (Luria et al., 2004), and FeLV has recently been reported in the endangered Florida panther (*Puma concolor coryi*) population (Cunningham et al., 2004), posing an additional threat to the persistence of this subspecies. Finally, just as FeLV was likely transmitted from domestic cats to Florida panthers, domestic cat FIV spillover into populations of wild felids could have potentially devastating consequences. For all these reasons, screening wild felids for lentiviral infection has become an important component of population health assessments.

Numerous serologic and molecular techniques, such as immunoblot, antigen enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), and immunofluorescent antibody (IFA) have been used to diagnose lentiviral infection in domestic and wild cats with variable sensitivity, specificity, and ease of use (Barr et al., 1991; Reid et al., 1992; Bienzle et al., 2004). Study of lentivirus in wild African felids and Florida panthers suggests that assays using antigen from puma lentivirus (PLV) are more sensitive and specific than FIV-based assays, including immunoblot, IFA, and ELISA (Osofsky et al., 1996; Van Vuuren et al., 2003; Miller et al., 2006). These assays are likely to vary in sensitivity in wild species because of the substantial genetic variability among the lentiviruses infecting the different species (Troyer et al., 2005). For example, domestic cat FIV, the basis of most commercially available feline lentiviral assays, genetically varies from lion and puma viruses by 25% in its most conserved regions (Brown et al., 1994; Carpenter et al., 1996) and has only 55% amino acid identity to PLV strain 14 in the Gag domain (Langley et al., 1994), the antigenic portion of the virus most often targeted by host antibodies. Further, anti-feline secondary antibodies may have variable ability to bind to wild felid immunoglobulins. For these reasons, results from disease surveys are difficult to interpret unless the diagnostic assay has been evaluated for use in that particular felid species.

In this study, serum, plasma, or whole blood obtained from ocelots, bobcats, and pumas was screened for exposure to lentivirus with five different assays to determine the relative utility of each assay and the effects of different sample use. The assays included immunoblots prepared with 1) PLV, 2) lion lentivirus (LLV), or 3) domestic cat FIV antigens, 4) a commercially available ELISA kit, and 5) PCR using degenerate primers for the reverse-transcriptase region of the FIV polymerase gene (*pol*). Because the true disease status of our samples remains unknown, we compared the number of positive results generated using the same samples with different assays to determine which assay maximized detection of reactive samples. To assess the likelihood of false positive results, we evaluated the consistency of results among different assays, the uniqueness of amplified nucleotide sequences, and data from experimental studies using these same assays.

MATERIALS AND METHODS

Sample collection and processing

Blood samples were collected from wild bobcats and pumas in Ventura, Orange, Riverside, and San Diego counties in Southern California and from wild ocelots on Barro Colorado Island, Panama (9°9′N, 79°51′W). Samples from each individual included some combination of 1) whole blood, 2) plasma samples processed by centrifugation for

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10 min at 25 × G after collection in ethylenediaminetetraacetic acid (EDTA) treated tubes, or 3) serum and coagulated red blood cell (RBC) pellet from blood collected in untreated tubes. Not all sample types were available for all individuals. Blood products were stored at either −70 C or −20 C for 6 mo to 2 yr, with the exception of samples from two individuals that were stored for 6 yr before shipment to Colorado State University (CSU) where analyses were performed. Some samples were stored in less than optimal conditions and had been thawed and refrozen before testing. For the 16 individuals from whom whole blood was used in serologic assays, the blood had been collected in an EDTA tube and frozen before centrifugation. These samples were thawed and then centrifuged at 4 × G for 8 min before use with immunoblots or the ELISA kits.

Immunoblot analysis

Three-antigen immunoblot screening was performed for 32 animals (Table 1), whereas a PLV immunoblot was performed to screen the majority (n=92) of animals. Antigens were prepared from viral cultures of domestic cat FIV (FIV-B2546), puma lentivirus (PLV-1695), and lion lentivirus (LLV-438). Stocks were grown in domestic cat origin cell line Mya-1 (Miyazawa et al., 1989), and viral proteins were isolated as previously described (VandeWoude et al., 1997b; TerWee et al., 2005). Reverse-transcriptase positive tissue culture supernatant was centrifuged at 200 × G (GPR centrifuge, Beckman Instruments, Inc., Fullerton, California, USA) for 10 min at 5 C to remove cellular material. The supernatant was then centrifuged at 130,000 × G at 4 C for 2 hr in a Beckman L-70 ultracentrifuge using an SW28 rotor. The supernatant was discarded, and the pellet was resuspended in a volume of one-twentieth of the original culture supernatant. The protein content was assayed using a Bio-Rad protein assay (Bio Rad Laboratories, Hercules, California, USA).

For single-antigen immunoblot analysis, PLV protein was run on a 12% polyacrylamide gel with a single well containing 150 μg of viral antigen. The antigen was subsequently transferred to a Immun-Blot™ polyvinylidene difluoride membrane (Bio Rad Laboratories) in a Mini-PROTEAN® 3 cell unit as suggested by manufacturer (Bio Rad Laboratories). Following electrophoresis and electrophoretic transfer to a nitrocellulose membrane, 15 strips were cut from the 7-cm-wide membrane. For the three-antigen immunoblot, 0.5 cm wide wells were loaded with 5–10 μg of antigen from one of the three viruses. Media was used as a reference for non-specific antibody binding to fetal bovine serum albumin.

TABLE 1. Results of serosurvey with triple chemiluminescent immunoblots using feline immunodeficiency virus (FIV), lion lentivirus (LLV), or puma lentivirus (PLV) antigens. The specificity of FIV and LLV immunoblot relative to PLV immunoblot was 100% for all three species; all FIV and LLV immunoblot positive samples were positive by PLV immunoblot.

		Immunoblot		
	n	FIV+	LLV+	PLV+
Bobcat	18	5	9	10
Ocelot	12	3	6	6
Puma	2	1	1	1

Samples of serum, plasma, or whole blood were diluted 1:25 in phosphate buffered saline (PBS). Positive control sera (cat sera from an experimentally FIV-infected animal) were used at a 1:100 dilution. Negative cat sera were used as a negative control at a 1:25 dilution. Samples were incubated for 1 hr at room temperature with immobilon strips. Three 5-min washes with PBS Tween (999.5 ml of 1× PBS with 500 μL of Tween® 20 SigmaUltra) were performed followed by incubation with goat anti-cat alkaline phosphatase-conjugated antibody (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Maryland, USA) at a 1:2,000 dilution in PBS for approximately 1 hr. Three additional 5-min washes were performed in PBS before the strips were incubated with alkaline phosphatase for approximately 15 min. Strips were rinsed in deionized water to stop the reaction. Strips were assessed visually and given one of four scores depending on the affinity of antibody for the p24 Gag protein: 0 = negative, 1 = equivocal, 2 = positive, 3 = strong positive (Fig. 1).

Commercial ELISA kit

Feline immunodeficiency virus/feline leukemia virus (FeLV) Combo SNAP™ tests (Idexx Laboratories, Westbrook, Maine, USA) were performed on 67 serum, 16 whole blood, and 2 plasma samples from 85 animals (31 pumas, 43 bobcats, 11 ocelots) according to the manufacturer instructions (Table 2). Three drops of sample were mixed with four drops of anti-FeLV/FIV Ag:HRPO conjugate

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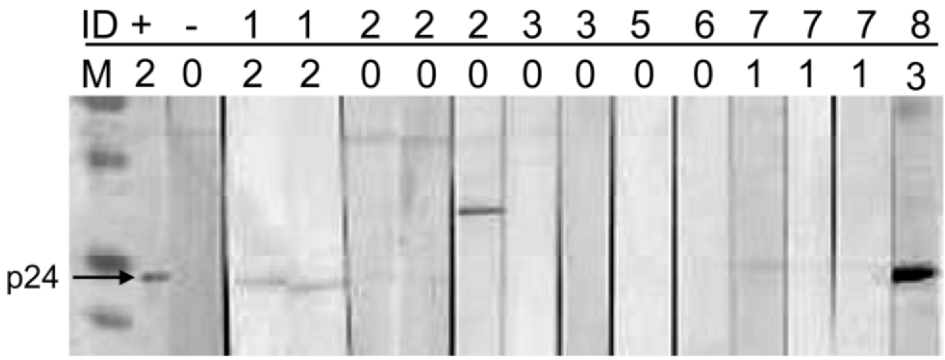


FIGURE 1. Scoring reaction intensity on puma lentivirus (PLV) antigen–based immunoblot strips. PLV-1695 viral antigens were prepared as described in the text and transferred to nitrocellulose strips. Puma sera from six animals are shown above (identification [ID] shown in top row). The sera were reacted at 1:25 dilutions (in single, duplicate, or triplicate samples), and antibody detection was detected by anti-cat immunoglobulin G alkaline phosphatase (IgG ALP) as described in the text. M = molecular weight markers; arrow indicates p24; + = positive control; − indicates negative control. Comparisons are shown in bottom row for scores of 0 (animals 2, 3, 5, and 6), 1 (animal 7), 2 (animal 1) or 3 (animal 8).

reagent, placed in the sample well, and the device activated once sample diffused into the activation window. Devices were assessed visually between 10 min and 15 min after activation and graded, relative to the positive control, using the same scale as used for immunoblots (0 = negative, 1 = equivocal, 2 = positive, 3 = strong positive).

PCR amplification

Deoxyribonucleic acid was extracted by two different techniques depending on the type and quantity of sample available. If only serum and coagulated RBCs were available, standard phenol chloroform extraction was performed (Sambrook and Russell, 2001). If the sample was whole blood collected in an EDTA tube that had not been centrifuged, DNA was recovered from 200 µl of blood using a QIAamp® DNA Blood Mini Kit (Qiagen Inc., Valencia, California, USA) following manufacturer's instructions. Deoxyribonucleic acid recovered from this technique was analyzed by visualization following electrophoresis and ethidium bromide staining on a 1.2% SeaKem agarose gel (Cambrex Bio Science Baltimore, Inc., Baltimore, Maryland, USA). If DNA quantity was inadequate for PCR amplification, or if initial PCR amplification attempts were unsuccessful on individuals identified as antibody positive by immunoblot, phenol chloroform extraction was performed using a larger quantity of blood. The amount of sample used for phenol chloroform extraction varied between 200 µl and 3 ml depending on the volume of sample available.

Nested PCR was performed on 103 different animals using degenerate primers (Table 3). First-round primers were designed from the conserved reverse-transcriptase region of *pol*. GenBank sequences of FIV (accession M25381 and U11820), PLV (accession U03982), and FIV-Oma (*Otocolobus manul*; accession U56928) were used to design both the internal and external primers (Troyer et al., 2005). Polymerase chain reactions were performed using 5 µl of DNA (generally corresponding to 100–500 ng of genomic DNA) with 500 mM sodium chloride (KCl); 100 mM Tris-hydrochloride (HCl; pH 8.3); 25 mM magnesium chloride ($MgCl_2$); 0.025 mM (each) of deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP), and deoxythymidine triphosphate (dTTP); 2 mM of each primer, and 1 unit of Taq

TABLE 2. Comparison of feline immunodeficiency virus (FIV)/feline leukemia virus (FeLV) Combo SNAP™ tests (Idexx Laboratories) and puma lentivirus (PLV) immunoblot. The specificity of the enzyme-linked immunosorbent assay (ELISA) relative to PLV immunoblot was 100% for all three species; all ELISA positive samples were also positive by PLV immunoblot.

	n	ELISA+	PLV+
Bobcat	43	20	27
Puma	31	5	16
Ocelot	11	4	5

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TABLE 3. Two-by-two tables comparing results from polymerase chain reaction (PCR) and puma lentivirus (PLV) immunoblot.

		Pumas		Bobcats		Ocelots	
		PLV immunoblot		PLV immunoblot		PLV immunoblot	
		+	−	+	−	+	−
PCR	+	7	2	15	3	0	0
	−	9	13	9	33	6	6

DNA polymerase (Sigma-Aldrich Corporation, Saint Louis, Missouri, USA), resulting in a total volume of 50 µl. Polymerase chain reaction cycling conditions were as follows: 4 min at 94 C; 45 cycles of 30 sec at 94 C, 45 sec at 52 C, and 90 sec at 72 C; and a final extension of 7 min at 72 C. The second-round of amplification was performed under the same conditions using 5 µl of first-round reaction product. Amplification reactions were performed on either a PerkinElmer (Waltham, Massachusetts, USA) 9700 or BioRad (Hercules, California, USA) iCycler. Five or 10 µl of sample was electrophoresed on 1 or 1.2% SeaKem (FMC Corporation, Philadelphia, Pennsylvania, USA) agarose gels at 100 V and inspected visually following ethidium bromide staining for the presence of a ~550-bp amplicon. Positive and negative controls were run concurrently. Some individuals were amplified multiple times to acquire product for DNA sequencing or when initial attempts at amplification did not yield product despite a positive serologic test result.

Data analysis

Two-by-two tables were created to compare the consistency of classifying samples as reactive or nonreactive using the different assays. Five comparisons were made: 1) immunoblot results using FIV, LLV, and PLV antigens for bobcat, puma, and ocelot samples (n=32, Table 1); 2) PLV immunoblot vs. ELISA for bobcat, puma, and ocelot samples (n=85, Table 2); 3) PLV immunoblot vs. PCR for bobcat, puma, and ocelot samples (n=103, Table 3); 4) PLV immunoblot results using serum vs. plasma for puma samples (n=13); 5) PLV immunoblot results when testing was performed with whole blood vs. PCR (n=16 bobcat samples).

Equivocal results for any immunoblot or the ELISA (i.e., score of 1) were categorized as negative for purposes of creating the 2×2 tables. If PCR amplification was performed multiple times for an individual, only the result of the first attempt was used in construction of the 2×2 table. PCR reactions in which the positive control was not amplified were excluded.

RESULTS

We performed immunoblots using three different antigens on 32 samples (18 bobcats, 12 ocelots, and 2 pumas). Puma lentivirus immunoblot identified approximately twice as many positive reactors as FIV immunoblot and one more positive reactor than LLV immunoblot for bobcats and ocelots (Table 1). Too few puma samples were tested (n=2) to detect a difference among the three immunoblot types for this species. Visual inspection of immunoblots using all three antigens revealed that bobcat and ocelot antilentiviral antibodies had qualitatively greater affinity for PLV antigen than for LLV antigen (Fig. 2). Of the 16 individuals that were positive on both PLV and LLV immunoblots, 14 were scored as 3 by PLV immunoblot but only eight were scored as 3 by LLV immunoblot. No samples that were negative on PLV immunoblot were positive on the LLV or FIV immunoblots. The commercial ELISA detected fewer positive samples than PLV immunoblot because only 31, 74, and 80% of the PLV immunoblot–positive samples were positive on ELISA for pumas, bobcats, and ocelots, respectively (Table 2). None of the samples that tested negative on PLV immunoblot tested positive on ELISA.

Polymerase chain reaction detected fewer positive individuals than PLV immunoblot with successful amplification of only 0, 44, and 63% of the PLV immunoblot–positive samples for ocelots, pumas, and bobcats, respectively (Table 3). Polymerase chain reaction identified five positive samples that were either negative (one puma) or equivocal (one puma, three bobcats) on PLV immunoblot. Genetic sequencing demonstrated that the single puma sample that was negative on PLV

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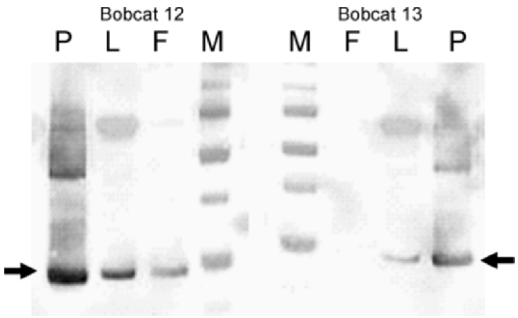


FIGURE 2. Representative comparison of feline immunodeficiency virus (FIV), lion lentivirus (LLV), or puma lentivirus (PLV) immunoblot sensitivity. Bobcat 12 reacted to all antigens, whereas Bobcat 13 did not react to FIV antigens. In each case, binding to PLV antigens was most robust. P=PLV-1695; L=LLV-438; F=FIV-C-PG antigen; M=molecular weight markers; arrows indicate Gag p24 binding.

immunoblot was a laboratory FIV-strain contaminant. All four samples that were equivocal on PLV immunoblot were sequenced and determined to be unique isolates of PLV. All other sequenced PCR products, except for the aforementioned contaminant, were homogeneous with previously described PLV sequences (Carpenter et al., 1996; Franklin et al., 2007).

The type of samples used (serum, plasma, or whole blood) did not affect assay results. Serum and plasma tested from the same pumas produced identical results on PLV immunoblot for 13 individuals. Of the 16 bobcats that were screened for infection using whole blood, 10 were positive by PLV immunoblot, four were negative, and two were equivocal. The 10 positive animals were successfully amplified by PCR on the first attempt, whereas the four negative and two equivocal samples were not.

DISCUSSION

When samples from the same animal were tested by multiple assays, PLV immunoblot was almost twice as likely to detect antilentiviral antibodies in bobcats and ocelots as FIV antigen immunoblots (Table 1). We hypothesize that a PLV immunoblot would also be more able to identify PLV-infected pumas than FIV immunoblot. Although we did not screen enough pumas with both types of immunoblots to test this hypothesis, a previous study that demonstrated that immunoblots prepared with species-specific viral antigens were 10% more sensitive than cross-species viral antigen supports this prediction (Troyer et al., 2005).

Puma lentivirus immunoblot detected only one more positive reactor than LLV immunoblot, but qualitative scoring of reaction intensity revealed that bobcat and ocelot antibodies had greater affinity for PLV antigens compared with LLV antigens. These results suggest that PLV and LLV immunoblot likely have similar ability to detect positive samples. However, because our data provide some evidence that PLV immunoblot is more sensitive than LLV immunoblot and because there is no difference in cost or time investment between these assays, we conclude that if an immunoblot is going to be used for lentiviral screening of North American felids, the PLV immunoblot should be used to maximize the detection of reactive samples.

The PLV immunoblot used in this study was also more likely to detect positive reactors than the commercial ELISA assay. The classification of less than one third of PLV immunoblot positive puma samples as positive by ELISA suggests that this assay is not a suitable method for testing samples from this species (Table 2). We speculate that the predominant antibody produced by PLV-infected pumas does not have high affinity for the antigen used in the ELISA, or that the secondary detection antibody does not efficiently bind puma immunoglobulin. Low sensitivity of FIV-based ELISAs has also been documented in screening of wild African felids (Osofsky et al., 1996), indicating that the commercial ELISA test results should be interpreted with caution in species where the test has not been evaluated.

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Although inadequate for puma samples, the ELISA may provide sufficient sensitivity for screening of bobcats and ocelot populations depending on the goals of the particular study. The incentive to use the ELISA is that it is the least expensive assay and can readily be performed under field conditions. The disadvantage is that the ELISA identifies approximately 25% fewer positive samples than the PLV immunoblot (Table 2), a deficiency that may be inadequate in many situations. Further, this estimate needs to be considered conservative in ocelots because there were only five reactive ocelot samples in our data, limiting the confidence that can be placed in the ability of this assay to detect reactive samples from this species.

Polymerase chain reaction also identified fewer positive animals than PLV immunoblot (Table 3). Sixty-three percent of bobcat, 44% of puma, and 0% of ocelot PLV immunoblot positive individuals were PCR positive, indicating that nested PCR with degenerate primers is a poor assay choice for population screening. Previous studies support this conclusion, as even species-specific (nondegenerate) primers can have low sensitivity (Brown et al., 1994; Carpenter et al., 1996; Troyer et al., 2005). Deoxyribonucleic acid PCR may have low sensitivity for detecting lentiviral strains because of low proviral load in blood cells (Blake et al., 2006; Brennan et al., 2006). Further, although use of degenerate primers enhances the ability to detect uncharacterized viral isolates, it simultaneously limits efficiency of PCR reactions by limiting the amount of primer in the reaction that complements the DNA sample. Polymerase chain reaction is also restricted in its utility because it is highly dependent on the quantity and quality of DNA that can be extracted from samples, requires the most technical skill, and is subject to a higher rate of false positive results as evidenced by amplification of one laboratory contaminant in this study.

Although nested PCR with degenerate primers is a poor choice for single-assay screening of populations, its use in conjunction with PLV immunoblot provided corroboration for samples that were weakly positive with antibody-detection assays. Four samples equivocal on PLV immunoblot were PCR amplified and confirmed to be unique PLV isolates (Table 3). Further, sequencing of PCR products allows confirmation of viral characterization and assists in determining the true positive nature of the results.

Although the type of lentiviral antigen used in preparation of the immunoblot greatly affected the results of the test, the type of sample tested was of little importance. All pumas tested for antibodies with both plasma and serum ($n=3$) produced identical results on PLV immunoblot. Similarly, every individual that was positive by PLV immunoblot using whole blood ($n=10$) was successfully PCR-amplified and subsequent sequencing indicated none were contaminants. Use of whole blood did not appear to compromise sensitivity of the immunoblot because all four bobcats negative on PLV immunoblot using whole blood were also negative by LLV and FIV immunoblots, ELISA, and PCR.

As with the PLV immunoblot, use of whole blood did not appear to decrease accuracy of the ELISA, a finding consistent with the manufacturer's instructions that whole blood, serum, or plasma from domestic cats are adequate with its use. The ELISA failed to produce any reactors from samples that were PLV immunoblot negative, contrary to hypotheses that use of whole blood with ELISAs may increase the number of false positives (Lutz and Pedersen, 1986; Barr, 1996). Likewise, because nine of the 10 individuals that were positive on PLV immunoblot were also positive using whole blood with the ELISA, it appears that sensitivity of the ELISA was not greatly impaired using whole blood.

Because the true infection status of our study subjects was unknown, the veritable

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sensitivity and specificity of each of the assays cannot be quantified, and it is possible that the higher estimates of antibody prevalence generated by the PLV immunoblot could be a consequence of false positive results. However, several observations suggest that false positive or negative results were unlikely to have occurred with use of the PLV immunoblot. Negative control sera were negative in every assay, and the majority of sera of unknown lentiviral status were negative (75 of 124 animals), inconsistent with a high false-positive rate. All positive samples, including positive controls, had similar banding patterns characterized by greater affinity for the p24 antigen than any other antigen, suggesting that all positive results were true positives and not unspecific antibody binding. Further, during experimental infection of domestic cats with PLV, PLV immunoblots were 100% specific for PLV infection because none of the 19 sham-inoculated animals used in controlled studies were positive by PLV immunoblot, coculture, or PCR (VandeWoude et al., 1997a; VandeWoude et al., 2003; Sondgeroth et al., 2005; TerWee et al., 2005).

The apparent specificity of the PLV immunoblot used in our laboratory comes despite consideration of just a single band to the p24 protein as indicative of a positive result, in contrast to other studies that have required two virus-specific bands to consider the sample to be antibody positive (Barr et al., 1989, 1991; Reid et al., 1992; Osofsky et al., 1996; Van Vuuren et al., 2003). Barr, et al. (1989) considered a single band on immunoblot to be equivocal with a high probability that the sample was positive. The four aforementioned experimental studies in domestic cats (VandeWoude et al., 1997a, 2003; Sondgeroth et al., 2005; TerWee et al., 2005) had 100% specificity using one band as a positive indicator. Further, the sensitivity was high because 25 of 27 domestic cats that were inoculated with PLV were positive by PLV immunoblot, viral coculture, and PCR. The two individuals that were not positive on PLV immunoblot after exposure were also coculture and DNA PCR negative. Our findings suggest that considering a single band to the p24 antigen in our assay system increased sensitivity without sacrificing specificity. The need to maximize sensitivity of the immunoblot is underscored by the finding that some equivocal results in this study were truly positive as determined by PCR amplification and nucleotide sequencing.

In summary, the results of this study strongly suggest that use of a PLV immunoblot, regardless of whether the sample used is serum, plasma, or whole blood, will maximize identification of reactive samples from populations of bobcats, pumas, or ocelots, when compared with the other assays examined. Further, although the true infection status of wild individuals is difficult to determine conclusively, it appears unlikely that PLV immunoblot produces false positive results. These findings are consistent with those of previous studies that have demonstrated that immunoblot is superior to other techniques (e.g., ELISA, IFA, PCR) for detecting lentiviral infection in primates and felids (VandeWoude and Apetrei, 2006), and that wild felid lentivirus-based assays are more sensitive for detecting lentiviral infection in wild felids than domestic cat FIV assays (Osofsky et al., 1996; Van Vuuren et al., 2003; Miller et al., 2006). We recommend that a PLV immunoblot be used for screening of bobcat, puma, or ocelot populations should only one assay be used for screening. A commercially available ELISA kit may be a convenient, though less sensitive, alternative to PLV immunoblot for screening bobcats and ocelots when a moderately decreased ability to identify positive samples is acceptable. Although PCR amplification with degenerate primers may not detect infections that can be identified by antibody assays, its concurrent use with PLV immunoblot can maximize the accu-

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racy of serosurveys and provide sequence data for more sophisticated genotypic and strain analysis (Biek et al., 2003; Troyer et al., 2004; Troyer et al., 2005).

ACKNOWLEDGMENTS

Capture of animals for collection of samples was approved by institutional animal care and use committees at the University of Wisconsin-Madison, Colorado State University, the University of California at Davis, the Smithsonian Tropical Research Institute, and the National Park Service. Funding was provided by the Geraldine R. Dodge Foundation Frontiers in Veterinary Medicine Fellowship, a Merck Merial summer research fellowship, the U.S. Geological Survey, the University of Wisconsin-Madison, Colorado State University, The Nature Conservancy, the National Park Service, National Geographic Society, National Science Foundation, The Peninsula Foundation, California State Parks, California Department of Fish and Game, and the Anza Borrego Foundation. We thank Idexx Inc for donation of some ELISA test kits. We thank G. Ryan for assistance with PCR protocols; C. Fiorello, R. Mares, R. Moreno, D. Bogan, and M. Wikelski for help with ocelot captures; E. Ambat, G. Geye, C. Haas, T. Smith, G. Turschak, and S. Yamamoto for help with bobcat captures; and J. Bauer, K. Logan, M. Puzzo, L. Sweanor, W. Vickers, and E. York for help with puma captures and sampling.

This research was supported in part by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research, under contract NOI-CO-12400, The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government. This work was funded in part by NSF grant EF-0723676 as part of the Joint NSF-NIH Ecology of Infectious Disease Program.

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Received for publication 24 September 2006.

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