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## SEROPREVALENCE TO AND INFLUENCE OF EXPOSURE TO BOVINE REPRODUCTIVE DISEASE CAUSATIVE AGENTS ON PREGNANCY AND PREWEANING CALF SURVIVAL OF ELK (ARTIODACTYLA: CERVIDAE)

## SEROPREVALENCIA E INFLUENCIA DE LA EXPOSICIÓN A LOS AGENTES CAUSANTES DE LA ENFERMEDAD REPRODUCTIVA BOVINA EN LA PREÑEZ Y SUPERVIVENCIA DE LA CRÍA DE WAPITÍ EN EL PREDESTETE (ARTIODACTYLA: CERVIDAE)

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**ABSTRACT.** Success of recent introductions of elk (*Cervus elaphus* Linnaeus, 1758) in Mexico partly depends upon elk-livestock interactions and conflicts. Disease can impact reproduction of elk and cattle, but is seldom considered in wild ruminants when reproductive output declines. We surveyed serological exposure of elk to causative agents in a bovine abortion profile (i.e., agents of brucellosis, leptospirosis, infectious bovine rhinotracheitis (IBR), bovine viral diarrhea, and neosporosis), as these diseases can negatively affect reproduction of cattle and elk, which frequently co-occur. We determined seroprevalence of exposure to these agents and used hierarchical logistic regression to model both pregnancy and lactation status (a surrogate for calf survival to weaning) as a function of population and exposure to disease causative agents. Tested elk populations were exposed to 2-4 of the agents except for *Brucella abortus*, which was not present. Pregnancy varied by population ( $P < 0.016$ ) but not by exposure to any agent ( $P > 0.213$ ). Proportion of females lactating in autumn did not vary among populations ( $P > 0.247$ ) nor by exposure to any agent ( $P > 0.281$ ). Exposure did not affect productivity of elk, despite exposure levels reflective of previous surveys throughout North America and low pregnancy and calf survival in some populations. Because all surveyed elk populations showed exposure to bovine herpesvirus-1 (BHV-1), IBR would be the most likely disease to be introduced with elk, although risk is low given high seroprevalence to BHV-1 among cattle in Mexico. Conversely, brucellosis is endemic in Mexico and can significantly impact productivity of elk. Thus, contracting brucellosis from cattle is the highest disease-related threat to elk introductions or translocation in Mexico.

**Key words:** Elk, cattle, causative agents, reproductive disease, serology.

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**RESUMEN.** Una parte del éxito de las reintroducciones de ciervo wapití (*C. elaphus*) en México depende de las interacciones y posibles conflictos con el ganado doméstico. Algunos agentes patógenos pueden impactar la reproducción del wapití y al ganado, pero es raro que se considere para los rumiantes silvestres cuando existe un declive en la productividad de la población. Se investigó la exposición a enfermedades de los wapití utilizando un perfil de aborto bovino (e.g. Brucelosis, leptospirosis, rinotraqueitis infecciosa bovina, (RIB), diarrea viral bovina, e infección por *Neospora* sp.), ya que estas enfermedades pueden afectar negativamente a la reproducción de los bovinos y rumiantes silvestres, y el wapití y el ganado frecuentemente co-ocurren. Utilizamos una regresión logística jerárquica para modelar la proporción de preñez de las hembras en periodo de lactancia (un sustituto para la supervivencia de la cría al destete) como una función de la población y la exposición de las enfermedades reproductivas. Se hicieron pruebas a poblaciones de wapití y fueron expuestos a 2-4 de los agentes patógenos evaluados, excepto para la brucelosis, que no estaba presente. La preñez de las hembras osciló entre 0.73-0.96 y la proporción de supervivencia al pre-destete desde 0.40-0.67 entre poblaciones. La época de preñez varió por población en todos los contrastes ( $P < 0,016$ ), pero no por la exposición a cualquier enfermedad ( $P > 0,213$ ). La proporción de hembras lactantes en otoño no varió entre las poblaciones ( $P > 0,247$ ), ni por la exposición a cualquier patógeno ( $P > 0,281$ ). La exposición a patógenos no afectó la productividad del wapití, a pesar de los niveles de exposición que reflejan previos estudios en Estados Unidos de Norteamérica y la baja preñez y la sobrevivencia de crías entre algunas poblaciones. Debido a su presencia en todas las poblaciones de wapití

## INTRODUCTION

Recent introductions of elk (*Cervus elaphus* Linnaeus 1758) in Mexico (e.g., McKinney & Villalobos, 2014) necessitate better understanding of elk-livestock disease interactions, both to increase likelihood of successful introductions and to minimize conflicts with Mexico's cattle industry. Productivity (i.e., production and survival of calves) of elk populations is declining in several areas of the USA (Noyes *et al.*, 2002; Rearden, 2005; Piassecke, 2006). Disease is a potential contributing factor to decreased elk productivity, particularly where they are sympatric with cattle, but aside from malnutrition (Cook *et al.*, 2004; Bender & Cook, 2005) and brucellosis (Cheville *et al.*, 1998; Thorne, 2001) has received little evaluation. Elk are sympatric with cattle throughout most of their range, and are potential hosts for a variety of diseases that may affect elk and cattle (Thorne *et al.*, 2002). Of these, the most important are diseases that affect the reproductive output of each species, as these have the greatest potential to impact recreational or economic returns from either population.

Several diseases can affect pregnancy, cause abortion, and influence calf survival in elk and cattle, particularly brucellosis, leptospirosis, infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), and neosporosis (Thorne *et al.*, 2002), and these diseases are included in most bovine abortion profiles (i.e., exposure assessments). Each of these can interfere with reproductive function, primarily by causing abortions (Kahrs, 1981; Van Campen *et al.*, 2001; Thorne *et al.*, 2002; Baszler, 2003; Cook *et al.*, 2004), although some can also cause fetal malformation, stillbirth, and nonviable neonates, among many other manifestations (e.g., Van Campen *et al.*, 2001). Through these impacts, these diseases can potentially compromise individual reproduction, and thus decrease reproductive output of the population. Even diseases that are usually rare in wild elk populations and that occur in only specific local areas, such as brucellosis and leptospirosis, can be a significant concern because of potential impacts to cattle (Bender & Hall, 1996; Thorne *et al.*, 2002; Peel *et*

en Estados Unidos de Norteamérica, si los productores de ganado adyacentes a los sitios de introducción de wapití en México están viendo menos crías que las esperadas, es posible que quieran vigilar su ganado para IBR para ver si BHV-1 puede ser un posible factor contribuyente, ya que el patógeno está relacionado con la reproducción y es probable que esté presente en los wapití.

**Palabras clave:** Wapití, patógenos, reproducción, serología.

*al.*, 2010; Milián-Suazo *et al.*, 2016). However, the cattle industry is advantaged in that vaccines are available for most reproductive diseases (Castro, 2001; Thorne, 2001; Leighton & Kuiken, 2001; Van Campen *et al.*, 2001; Segura-Correa *et al.*, 2016), although vaccines may always be effective (Xue *et al.*, 2011). In contrast, vaccination of free-ranging wildlife is largely impossible, so shared diseases are likely to disproportionately affect elk. Increasing public demand for “natural” (i.e., unvaccinated) beef, however, is increasing the number of vaccine-free cattle operations worldwide.

Our goal was to test whether exposure as indicated by positive serology to causative agents of diseases associated with reproductive failure in cattle affected productivity of elk, specifically pregnancy and survival of calves to weaning. Positive serology indicates presence of antibodies to an agent, which includes previous exposure or past infection, not necessarily active infection (Calisher & Taylor, 1993). However, high seroprevalence or longitudinal persistence in positive serology can indicate disease presence (Calisher & Taylor, 1993; Bender *et al.*, 2003), and thus serological surveys are commonly used to evaluate the potential presence of, and risk factors associated with, disease in populations (e.g., Bender *et al.*, 2003; Milián-Suazo *et al.*, 2016; Segura-Correa *et al.*, 2016). Therefore, we assessed seroprevalence to causative agents of bovine reproductive diseases in multiple elk populations throughout the western USA. We compared serological prevalence with previously published data, and modeled exposure effects on pregnancy and preweaning calf survival of elk. We also identify disease risks for both elk and cattle associated with introductions or translocations of elk in Mexico.

## MATERIALS AND METHODS

**Study populations.** Our study populations covered a variety of locations throughout the United States (Table 1). Chaco Culture National Historic Park (CC) is located in northwestern New Mexico (approximately 36° 00' N,

**Table 1.** Mean high temperature in July (°C; July high), mean January low temperature (°C; Jan low), mean annual precipitation (cm; Precip), elk population density (elk/km<sup>2</sup>), proportion pregnant (Pregnant), proportion lactating (Lactation), and number of population-years for each study population of elk.

Population <sup>1</sup>	July high	Jan low	Precip	Elk/km <sup>2</sup>	Pregnancy	Lactation	Pop-years
CC	32.2	-10.6	23	0.10	0.73	0.43	3
Ft. Riley	32.2	-9.4	87	2.7	0.96	0.67	2
RMNP <sup>1</sup>	26.1	-7.8	35	1.3	0.77	---	1
LNF	21.7	-8.3	67	0.7	0.94	0.50	3
Forks	22.4	1.8	304	4.0	0.76	0.40	1
VC	31.6	-6.9	605	> 6.9	0.91	---	1

<sup>1</sup>CC = Chaco Culture National Historic Park; RMNP = Rocky Mountain National Park; LNF = Lincoln National Forest; VC = Valles Caldera National Preserve.

108° 00' W). This site is a desert grass and shrubland with scattered pinyon (*Pinus edulis*)-juniper (*Juniperus* spp.) woodlands. Fort Riley is a 403 km<sup>2</sup> military training facility located in the Flint Hills of northeastern Kansas (approximately 39° 06' N, 96° 48' W). The area is primarily rolling tallgrass prairie of big bluestem (*Andropogon gerardii*) and other tallgrass natives with scattered wooded areas along riparian corridors and lowlands, interspersed with agricultural fields and wildlife plantings. Rocky Mountain National Park (RMNP) covers 1,076 km<sup>2</sup> in the Rocky Mountain Front Range of northcentral Colorado (approximately 40° 23' N, 105° 38' W). The site consists primarily of montane forest interspersed with grassland, shrublands, and open tundra occur at higher elevations. Lincoln National Forest (LNF) is located in the Sacramento Mountains of southcentral New Mexico (approximately 32° 51' N, 105° 44' W). This study area was primarily semiarid woodland and montane forest interspersed with small grassy meadows at high elevations. The Forks study site was located in the coastal hills of western Washington state (approximately 47° 54' N, 124° 35' W). Land-use in this area is primarily industrial tree farms of Douglas-fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla*). The Valles Caldera National Preserve was located in the Jemez Mountains of northcentral New Mexico (approximately 35° 55' N, 106° 31' W). This study area consists of high elevation mesic montane grasslands and mixed conifer forest.

**Capture.** We captured cow elk  $\geq 1.5$  years old in autumn (November) and late-winter (March–April). Elk were darted from a Bell 206B Jet Ranger helicopter (or from vehicles along roads in RMNP) using carfentanil citrate and xylazine hydrochloride (3.6 mg carfentanil + 100 mg xylazine/elk) as sedatives, and blindfolded to reduce stress and prevent eye injury (Kreeger, 1996; Bender, 2015). We

also treated each elk with penicillin, vitamin E/selenium, vitamin B, and an 8-way *Clostridium* bacterin to reduce physiological stress and trauma of capture. Captured elk were aged to yearling or adult using presence or absence of deciduous teeth (Quimby & Gaab, 1957). Immobilants were antagonized with 300 mg naltrexone (half intravenous and half subcutaneous) and 800 mg tolazoline (delivered intravenously) (Kreeger, 1996; Bender, 2015).

**Disease screening.** We obtained whole blood samples for the bovine abortion profile and pregnancy testing from immobilized elk through jugular venal puncture. Whole blood samples were transferred to serology tubes, which were spun (4,500 rpm; 8–10 min) to separate serum shortly after collection. Serum samples were then frozen until analysis.

We determined pregnancy status from pregnancy-specific placental protein B (PSPB) (BioTracking, Moscow, Idaho, USA). Elk from which autumn PSPB results were uncertain were corroborated using serum progesterone (Colorado State University Endocrinology Lab, Fort Collins, Colorado, USA). Progesterone levels of  $\geq 1.0$  ng/ml and  $\leq 93\%$  binding of elk antiserum to PSPB (Noyes *et al.*, 1997; Bender *et al.*, 2002) indicated pregnancy. We determined lactation status for cows by checking the udder for milk, which indicated survival of a calf to within  $\leq 3$ –11 days (Bender *et al.*, 2002). We could not determine lactation status from spring captures because most calves are weaned by this time (Johnson, 1951).

A bovine abortion profile was performed on serum samples from individual elk to detect exposure to causative agents of profiled diseases (New Mexico Department of Agriculture Veterinary Diagnostic Laboratory, Albuquerque, New Mexico, USA; Washington Animal Disease Diagnostic Lab, Pullman, Washington, USA). Serology included the card test for brucellosis (*Brucella abortus*;

Alton *et al.*, 1988), virus neutralization for BVD (bovine viral diarrhea virus [BVDV]) and IBR (bovine herpesvirus 1 [BHV-1] (Carbrey *et al.* 1971), the microscopic agglutination test for leptospirosis (including *Leptospira interrogans* serovars *pomona*, *hardjo*, *grippotyphosa*, *ictero-hemorrhagiae*, *bratislava*, *canicola*) (Gouchenour *et al.*, 1958), and an enzyme-linked immunosorbent assay (ELISA) for neosporosis (*Neospora caninum*; Shares *et al.*, 2001). Serology was considered negative at <1:4 for BVDV and BHV-1, <1:100 for *Leptospira* serovars, no agglutination for *B. abortus*, and ELISA values <30% for *N. caninum*.

**Data analysis.** We compared seroprevalence to causative agents among populations using Fisher’s exact tests (Zar, 1996). We used hierarchical logistic regression to model the dichotomous outcomes of pregnancy and lactation (i.e., pregnant/not pregnant, lactating/not lactating) at the individual level as a function of population and whether each cow elk was exposed to a particular agent or not (Hosmer & Lemeshow, 1989; Kuss, 2004). If the serological result from either autumn or spring during pregnancy was positive, we classed these as positive exposure for pregnancy modeling. For lactation modeling, we used

only serological results from the autumn after the calf was born, i.e., when the cow was lactating. For analyses of lactation, we excluded yearling elk because they are never lactating (Raedeke *et al.*, 2002).

## RESULTS

Among populations, we tested 177–194 cow elk for pregnancy and exposure to disease causative agents, and 107–122 cow elk for lactation status and exposure to causative agents. Seroprevalence to causative agents varied among populations (Fisher’s exact  $P < 0.01$ ) with the exception of *B. abortus*, for which we did not detect exposure in any population (Table 2).

Pregnancy averaged 0.84 (SE = 0.04; range 0.73–0.96) and lactation averaged 0.50 (SE = 0.06; range = 0.40–0.67) among populations. Pregnancy varied by population in all contrasts ( $P < 0.016$ ) but not by exposure to any agent ( $P > 0.213$ ) (Table 3). Proportion of cow elk lactating in autumn did not vary among populations ( $P > 0.247$ ) nor by exposure to any agent ( $P > 0.281$ ) (Table 3). One cow that was definitively pregnant when tested in autumn was

**Table 2.** Results from various studies, showing proportion of elk testing positive for exposure to various disease processes, sample sizes, location of study, and reference.

Disease <sup>1</sup>	Proportion Positive	N	Location <sup>2</sup>	Source
<i>Brucella</i>	0.00–0.37	1–909	Greater Yellowstone Area	Ferrari & Garrott, 2002 Etter & Drew, 2006 Barber-Meyer <i>et al.</i> , 2007 Proffitt <i>et al.</i> , 2015
	0.00	28–2338	Colorado	Adrian & Keiss, 1977
	0.00	57	Idaho	Ferrari & Garrott, 2002
	0.06	47	Utah	Merrell & Wright, 1978
	0.00	403	Nebraska	Cover <i>et al.</i> , 2011
	0.00	170	Arkansas	Corn <i>et al.</i> , 2010
	0.00	54	Idaho	Vaughn <i>et al.</i> , 1973
	<b>0.00</b>	<b>52</b>	<b>New Mexico (CC)</b>	<b>This study</b>
	<b>0.00</b>	<b>47</b>	<b>New Mexico (LNF)</b>	<b>This study</b>
	0.00	45	Washington	Hein <i>et al.</i> , 1991
	0.00	31	Kentucky	Corn <i>et al.</i> , 2010
	<b>0.00</b>	<b>30</b>	<b>Colorado (RMNP)</b>	<b>This study</b>
	<b>0.00</b>	<b>26</b>	<b>Ft. Riley</b>	<b>This study</b>
	0.00	23	Alberta	Kingscote <i>et al.</i> , 1987
	<b>0.00</b>	<b>22</b>	<b>VCNM</b>	<b>This study</b>

Disease <sup>1</sup>	Proportion Positive	N	Location <sup>2</sup>	Source
BVD	0.52	23	Alberta	Kingscote <i>et al.</i> , 1987
	<b>0.22</b>	<b>22</b>	<b>New Mexico (VC)</b>	<b>This study</b>
	0.05	346	Nebraska	Cover <i>et al.</i> , 2011
	0.04	170	Arkansas	Corn <i>et al.</i> , 2010
	0.04	25	New Mexico	Wolfe <i>et al.</i> , 1982
	<b>0.02</b>	<b>47</b>	<b>New Mexico (LNF)</b>	<b>This study</b>
	0.02	45	Washington	Hein <i>et al.</i> , 1991
	<b>0.00</b>	<b>52</b>	<b>New Mexico (CC)</b>	<b>This study</b>
	0.00	50	Idaho	Vaughn <i>et al.</i> , 1973
	0.00	31	Kentucky	Corn <i>et al.</i> , 2010
	<b>0.00</b>	<b>30</b>	<b>Colorado (RMNP)</b>	<b>This study</b>
	<b>0.00</b>	<b>26</b>	<b>Kansas (Ft. Riley)</b>	<b>This study</b>
	IBR	0.45	22	Alberta
<b>0.43</b>		<b>30</b>	<b>Colorado (RMNP)</b>	<b>This study</b>
0.38		45	Washington	Hein <i>et al.</i> , 1991
<b>0.30</b>		<b>47</b>	<b>New Mexico (LNF)</b>	<b>This study</b>
<b>0.23</b>		<b>22</b>	<b>New Mexico (VC)</b>	<b>This study</b>
0.19		31	Kentucky	Corn <i>et al.</i> , 2010
<b>0.13</b>		<b>52</b>	<b>New Mexico (CC)</b>	<b>This study</b>
0.04		170	Arkansas	Corn <i>et al.</i> , 2010
<b>0.04</b>		<b>26</b>	<b>Kansas (Ft. Riley)</b>	<b>This study</b>
0.00		50	Idaho	Vaughn <i>et al.</i> , 1973
Leptospirosis	0.82	11	Washington	Bender & Hall, 1996
	0.38	24	Alberta	Kingscote <i>et al.</i> , 1987
	0.34	38	Oregon	Weber, 1973
	<b>0.29</b>	<b>17</b>	<b>Washington (Forks)</b>	<b>This study</b>
	< 0.26	31	Kentucky	Corn <i>et al.</i> , 2010
	< 10	170	Arkansas	Corn <i>et al.</i> , 2010
	<b>0.10</b>	<b>30</b>	<b>Colorado (RMNP)</b>	<b>This study</b>
	<b>0.09</b>	<b>22</b>	<b>New Mexico (VC)</b>	<b>This study</b>
	0.07	289	Nebraska	Cover <i>et al.</i> , 2011
	0.00	331	Canada	Canadian Wildlife Service, 1966
	0.00	163	Colorado	Denney, 1965
	0.00	109	Oregon	Trainer, 1971
	<b>0.00</b>	<b>52</b>	<b>New Mexico (CC)</b>	<b>This study</b>
	0.00	39–50	Idaho	Vaughn <i>et al.</i> , 1973
	<b>0.00</b>	<b>47</b>	<b>New Mexico (LNF)</b>	<b>This study</b>
	0.00	45	Washington	Hein <i>et al.</i> , 1991
<b>0.00</b>	<b>26</b>	<b>Ft. Riley</b>	<b>This study</b>	

Disease <sup>1</sup>	Proportion Positive	N	Location <sup>2</sup>	Source
<i>Neospora</i> spp.	0.00–0.20	8–71	Alberta	Pruvot <i>et al.</i> , 2014
	0.15	47	New Mexico (LNF)	This study
	0.12	26	Kansas (Ft. Riley)	This study
	0.05	22	New Mexico (VC)	This study
	0.00	52	New Mexico (CC)	This study
	0.00	30	Colorado (RMNP)	This study

<sup>1</sup> BVD = bovine viral diarrhea; IBR = infectious bovine rhinotracheitis.

<sup>2</sup> GYA = greater Yellowstone area; CC = Chaco Culture National Historic Park, New Mexico; LNF = Lincoln National Forest, New Mexico; VC = Valles Caldera National Preserve, New Mexico.

found to be not pregnant when subsequently recaptured and retested again in late winter. She was negative for all screened causative agents.

## DISCUSSION

The primary risk factors associated with transmission of most bovine reproductive diseases among elk include high elk densities and co-occurrence of elk and cattle (Thorne, 2001; Thorne *et al.*, 2002). Seroprevalence of screened agents in our populations was reflective of the range of exposure seen in elk throughout North America (Table 2), highlighting the potential for exposure of elk to reproductive diseases of cattle (and vice versa) wherever elk and cattle co-occur. Despite high seroprevalence to certain agents, however, exposure was not related to pregnancy or preweaning calf survival in our study popula-

tions (Table 3), even though several of these populations showed relatively low pregnancy rates and calf survival (Table 1; Piasecke, 2006). Although our index of calf survival (lactation status) assessed only preweaning survival (Bender *et al.*, 2002) and not survival of a calf to recruitment, once a calf has survived to weaning it has passed the peak of juvenile mortality and will most likely survive to reproductive age (Guinness *et al.*, 1978; Taber *et al.*, 1982; Clutton-Brock *et al.*, 1988). These results, as well as the negative exposure result for the 1 cow that lost its fetus, indicates that past or current exposure to common reproductive diseases of cattle likely has negligible effects on population productivity of elk. The exception to this is brucellosis, which can cause significant declines in elk productivity where endemic in North America (Cheville *et al.*, 1998; Thorne, 2001).

Exposure to cattle reproductive disease causative agents is relatively widespread in Mexico, both in dairy

**Table 3.** Results of hierarchical logistic regression modeling of probability of pregnancy and lactation in elk lactation as related to population and exposure to bovine reproductive diseases, including results of likelihood ratio  $\chi^2$  test and odds ratio and 95% CI of odds ratio associated with successful pregnancy or successfully raising a calf to weaning if exposed to disease.

Test	Disease <sup>1</sup>	Population			Exposure				n
		$\chi^2$	P	N	$\chi^2$	P	Odds	95% CI	
Pregnancy	Neospora	10.9	0.028	4	0.7	0.409	0.4	0.04–3.8	177
	IBR	11.7	0.020	4	1.2	0.268	4.9	0.8–21.8	177
	BVD	12.2	0.016	4	1.6	0.213	0.5	0.1–2.9	177
	Leptospirosis	11.1	0.050	5	0.7	0.408	0.5	0.1–2.5	194
*Lactation	Neospora	4.1	0.247	3	0.03	0.872	1.2	0.2–7.9	107
	IBR	3.4	0.330	3	0.08	0.784	1.2	0.4–3.6	107
	BVD	3.3	0.343	3	<0.01	0.987	10.0	0.1–99	107
	Leptospirosis	3.8	0.431	4	1.2	0.281	0.3	0.02–3.1	122

<sup>1</sup>BVD = bovine viral diarrhea; IBR = infectious bovine rhinotracheitis; Leptospirosis includes *Leptospira pomona*, *L. hardjo*, *L. grippo-typhosa*, *L. ictero-hemorrhagiae*, *L. bratislava*, and *L. canicola*).

(Milián-Suazo *et al.*, 2016) and beef (Segura-Correa *et al.*, 2016) cattle. For example, Milián-Suazo *et al.* (2016) recently surveyed multiple dairy operations throughout Mexico and found seroprevalence of 4–15% (depending upon test used), 37%, 79%, and 73% to agents for brucellosis, neosporosis, BVD, and IBR, respectively. Segura-Correa *et al.* (2016) also recently surveyed beef operations in Tamaulipas and found seroprevalence of 48% and 68% for agents of BVD and IBR, respectively. Certain risk factors for exposure were common to both studies, and included herd size and introduction of new cattle to the herd. High seroprevalence in elk to several of these disease causative agents suggests that introduction or co-occurrence of elk may be an additional risk factor for unvaccinated herds in Mexico.

For example, IBR was the only disease for which the causative agent (BHV-1) showed exposure in all elk populations (Table 2). While potentially affecting a variety of systems in cattle, IBR is primarily of concern because of the potential to cause abortions regardless of the severity of disease or whether the disease is present in respiratory or ocular form (Fraser & Mays, 1986). Because wild ruminants frequently do not display clinical signs of IBR infection, the disease is primarily considered a concern only for sympatric cattle (Castro, 2001). Our data supports this conclusion; despite a wide range of exposure (4–43%), hierarchical logistical analysis indicated that probability of pregnancy and calf survival to weaning were both unrelated to exposure to BHV-1. However, because exposure to BHV-1 was seen in all tested elk populations, it has the highest likelihood of the agents we surveyed of being present in elk and potentially transferred to cattle. While seroprevalence to BHV-1 is high and widespread in cattle in Mexico (Milián-Suazo *et al.*, 2016, Segura-Correa *et al.*, 2016), it appears less common in areas near the central and western USA border (Milián-Suazo *et al.*, 2016) where introductions of elk are most likely.

Similarly, reproductive diseases can be transmitted to elk from cattle. Of greatest concern in Mexico would be brucellosis, as it is widely distributed in Mexico (Peel *et al.*, 2010; Milián-Suazo *et al.*, 2016), has demonstrated negative impacts on elk productivity, and once infected elk can serve as a reservoir for the disease, increasing the difficulty of eradication programs (Cheville *et al.*, 1998; Thorne, 2001). Presence of brucellosis in cattle thus can compromise the success of elk introductions or translocations. Similarly, elk that are translocated from areas where brucellosis is endemic in Mexico to areas that are free of brucellosis should be tested for the presence of *B. abortus*,

and translocations should not proceed if brucellosis is present in elk. Such movements may complicate ongoing efforts to eradicate brucellosis in Mexico (Milián-Suazo *et al.*, 2016).

Last, as previously noted, positive serology indicates antibody presence and thus exposure to a disease causative agent, not necessarily active infection (Calisher & Taylor, 1993). Consequently, the lack of effect of screened bovine reproductive diseases on elk productivity that we observed may have been due to past exposure or past infection, and not current infection. However, longitudinal persistence in positive serology is indicative of disease presence (Calisher & Taylor, 1993; Bender *et al.*, 2003), and several of our study populations (i.e., CC, LNF) have shown long-term persistence of positive serology. Moreover, high seroprevalence where vaccination is not present suggests that the actual prevalence of the disease is high (Milián-Suazo *et al.*, 2016). For example, Morales *et al.* (2001) found that cattle herds in Mexico with higher seroprevalence for neosporosis had a greater number of abortions; seroprevalence was 72% for herds with >13% abortions, but 36% for herds with <12% abortions. Thus, while a lack of observed effect of exposure to bovine reproductive diseases may have been due to lack of active infection, high longitudinal seroprevalence in many populations suggests that it is likely that the causative agents in the bovine abortion screen did not impact pregnancy or calf survival in free-ranging elk. Again, the exception to this would be brucellosis, which was not present in our study populations as it is endemic only to the greater Yellowstone area in North America (Cheville *et al.*, 1998).

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