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Observations of mortality in farmed bison in the Canadian prairies: 2103 – 2016

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ABSTRACT

The present study is a continuation of a previous mortality study on Saskatchewan bison farms with special emphasis on Malignant Catarrhal Fever. The updated objective of the study was to estimate the most common causes of mortality in farmed bison herds in Western Canada. Results were compared to the previous Saskatchewan study to assess the similarities and differences in the etiology associated with farmed bison deaths across the Prairie Provinces of Canada. The most common cause of death was respiratory disease associated with *Mycoplasma bovis*, although this was restricted to Alberta and Saskatchewan farm locations. This was in contrast to the previous Saskatchewan based study which did not identify any deaths involving this pathogen. An updated overall assessment of the risks of Malignant Catarrhal Fever in farmed bison at various proximities to sheep operations further confirmed the low risk of occurrence on farms within a 1 km boundary fence distance.

1. Introduction

Commercial bison (*Bison bison*) farming is a relatively new agricultural activity in North America and private herds created to serve a growing demand for bison meat are increasing in number and size (CBA, 2017; NBA, 2017). Both breeding and feeding operations similar to beef cattle production systems can be found in the industry. There is very little available peer reviewed published information characterizing the losses from disease and other causes of on-farm bison mortality in these operations. In 1999, a cross sectional survey of producers and veterinary diagnostic laboratories was carried out by researchers at the Western College of Veterinary Medicine (Berezowski and Woodbury, 2000). The resulting report included information on reproductive losses and mortalities by age class. Trauma from handling events was the overall leading cause of mortality but respiratory disease and mineral (copper) imbalance led the list of infectious and non-infectious causes, respectively (Berezowski and Woodbury, 2000). In 2012, a prospective observational study of bison farm mortality was carried out on selected Alberta bison operations by Burrage et al. (2012). Findings featured mostly respiratory disease (*Mycoplasma bovis*, *Mycoplasma bovis* plus another significant pathogen, *Mannheimia* sp.), as well as clinical parasitism, reproductive disorders, and malignant catarrhal fever (MCF) (Burrage et al., 2012). This research was pivotal in alerting the bison industry to an emerging international epidemic of *Mycoplasma bovis* related disease, the effects of which are still being experienced by bison producers. In 2014, the USDA published the results of a survey of producer-reported health and management practices on U.S. farmed bison operations, in which producers responded to questions about disease and death losses. Approximately 40% of farms had bison die or be euthanized due to health issues, injury or trauma, or predation. The number of farms that had bison die increased as herd size increased, ranging from 12.6% in very small operations to 82.1% in large operations. Overall, 2.3% of the survey bison inventory died of natural causes or were euthanized. The survey identified parasitism and undiagnosed diarrhea as the most frequently encountered problems but it did not ask specific questions about mortality or laboratory confirmed disease occurrence (USDA, 2016). In 2016, Epp et al. published the results of a prospective mortality study aimed at discovering the significance of malignant catarrhal fever (MCF) and sheep ranches to Saskatchewan bison herds (Epp et al., 2016).

The present study is a continuation of that study with broader objectives. The main objective of this surveillance study was to estimate

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the proportional mortality and relative frequency of the most common causes of mortality in farmed bison herds in Western Canada. This was compared to the previously completed bison mortality study to assess the similarities and differences in the etiology associated with farmed bison deaths in the Prairie Provinces of Canada. An overall assessment of the risks of MCF in farmed bison at various proximities to sheep operations was included.

2. Materials and methods

2.1. Herd recruitment

Commercial bison herds were recruited from across western Canada [Manitoba (MB), Saskatchewan (SK), Alberta (AB) and British Columbia (BC)] and enrolled into the study with the help of the Canadian and Provincial Bison Associations and local veterinary clinics in selecting suitable herds. Preference for enrolment was given to farms with more than 50 adult bison in their herd but to increase sample size, smaller farms were not excluded. The desired study sample size was at least 50 farms. Each producer completed a baseline questionnaire to gather information on herd size and management and identified a veterinarian willing to conduct necropsies according to a standard protocol provided by the researchers.

Methods for the collection of herd information were approved by the University of Saskatchewan Behavioral Ethics Board (BEH 12-266). Animal use protocol approval is not required for studies involving animals dying from natural causes or being euthanized on farms at the discretion of the owner.

2.2. Diagnostic procedures

The study was conducted over a period of 18 months, between July 1 st, 2015 and December 15th, 2016. Any bison that died, including stillbirths and abortions, in a study herd during that period and was in a suitable condition (not too scavenged or decomposed) received a full necropsy by a licensed veterinarian. A protocol and laboratory submission form was provided to the veterinarian and collection of a standardized set of samples for diagnostic purposes was carried out. The protocol included gathering information on animal identification, gender, age and a detailed clinical case history together with herd management information, clinical signs observed and treatments, if any. The protocol requested an external body examination to estimate the body condition score and gross abnormalities. A detailed internal examination and tissue sample collection for major organ systems was required. Samples from the musculoskeletal system including tongue and diaphragm, together with skin and lymph nodes were obtained. Thoracic (trachea, heart, lung), and abdominal organs including the digestive tract, liver, spleen, and urogenital tract (bladder, kidneys) were examined and sampled. Additional samples listed as optional such as feces, eye, spinal cord or brain and affected joint or joint fluid were taken depending on any abnormalities or pathology found. Tissues samples were fixed in formalin and/or included as fresh or frozen samples as indicated on the submission form. Any additional diagnostic samples (those not requested on the submission form) were based on the initial post-mortem findings by the attending veterinarian. All samples were appropriately shipped to a veterinary diagnostic laboratory [Prairie Diagnostic Services (PDS)] together with the history and summary of the gross post-mortem findings.

When suitable tissues were received each underwent standardized post-mortem testing including fecal flotation for internal parasites, standard liver mineral panel testing, and polymerase chain reaction (PCR) testing for Ovine herpesvirus 2 (OHV-2) and *Mycoplasma bovis*. The mineral and vitamin panel analysis was carried out on fresh liver or kidney and included magnesium, manganese, iron, cobalt, copper, zinc, selenium, and molybdenum as well as vitamin A and E determination following a previously published protocol (Waldner and Blakley, 2014). Where a fecal sample was available, a fecal flotation (Wisconsin technique) was performed to identify gastrointestinal parasites. Other testing suggested by specific pathology was done at the discretion of the attending pathologist to help determine a cause of death. All formalin-fixed tissues were examined histologically for abnormalities.

To continue surveillance for MCF initiated in a previous mortality study (Epp et al., 2016), regardless of history or necropsy findings real-time polymerase chain reaction (PCR) was used to identify MCF (OVH-2) DNA in fresh or frozen pooled liver, lymph node, kidney or spleen (sensitivity, 97%; specificity, 100%) from all cases (Traul et al., 2007; Li et al., 2011). The case definition of an MCF death was any bison submitted for necropsy with a positive PCR test and histopathological evidence supporting a diagnosis of MCF with or without specific clinical symptoms in the history (i.e. “found dead” could have been the only clinical sign listed). To identify *Mycoplasma bovis* DNA, PCR was conducted on available fresh or frozen lung tissue, where available. Where lung was not available, other tissues deemed suitable by the pathologist were tested. The case definition for a *M. bovis* death was any bison submitted for necropsy with a positive PCR test with or without specific clinical symptoms in the history.

Individual animal necropsy results were reported to the herd veterinarian, who then communicated the findings to the producer. Any subsequent management or treatment decisions were the responsibility of the herd owner in consultation with their veterinarian.

3. Statistical analysis

3.1. Descriptive analysis

Data were compiled in an Excel database; descriptive analysis included calculation of herd mortality risks (proportions) with exact 95% confidence intervals and the overall proportional mortality of the most frequent causes of death. The mortality risk was calculated using the total number of bison deaths during the study period divided by the total bison herd size at the start of the study period. The total number of animals that died per farm, including necropsied and non-necropsied reported deaths, was reported. The proportional mortality was calculated for the most common defined causes of death or by affected body system as a fraction of the total deaths submitted for necropsy. Age of bison was classed categorically; abortion, neonate (less than 3 days old), calves (differentiated as under 5 months and 5 months to a year of age), and adults (anything over 1 year of age which includes yearlings, sub-adults and adults).

The average number of bison in each herd was calculated as the average of total number of bison in the herd from the pre- and post-study surveys. The average size of each bison herd was then categorized as small (1–150 animals), medium (151–500 animals) or large (more than 500 animals); however, for simplicity of the majority of analysis, a cut-point of either 150 bison or 500 bison was used. Bison herds were classified into 2 groups based on proximity to sheep; no sheep operation boundary within 5.0 km (negligible exposure group) and a sheep operation boundary within 5.0 km (at risk group). Herds at risk were further classified into 2 groups; those less than 1.0 km from a sheep operation boundary (high exposure group) and those between 1.0 to 5.0 km from a sheep operation boundary (low exposure group). Distance was based on closest point of contact between bison and sheep as determined by fence line boundaries of the pastures.

Other specific management factors collected in the surveys included the use of vaccines and deworming medications, use of a chute to capture animals within the study time period and whether pregnancy testing occurred in the study time period, and use and types of supplements.

3.2. Comparison to previous mortality study

A total of 26 herds were enrolled in a previous bison mortality study

Table 1
Major morphological diagnoses (specific etiology by system noted) for necropsied bison deaths within the study period (July 1, 2015–December 31, 2016) for calves (bison under a year of age) and neonates.

Categories of deaths	Morphological diagnoses	Neonates < 3 days	Calf < 5 months	Calf > 5 months	Total (PM[a])	Herds (N)
Undetermined causes	No morphological diagnoses possible	2	1	9	**12 (17.9%)**	**9**
Non-infectious causes	Calving, nutritional or trauma	1	1	0	**2 (3.0)**	**2**
	Mineral deficiencies or toxicosis	0	2	3	**5 (7.5)**	**2**
Infectious causes	Whole body - septicemia	0	2	2	**4 (6.0)**	**3**
	GI (*Cl. Perfringens*, Coccidiosis)	1	2	3	**6 (9.0)**	**4**
	Heart (bacterial)	0	0	2	**2 (3.0)**	**2**
	Liver (lipidosis)	0	0	1	**1 (1.5)**	**1**
	Lung (bacterial, *Mann. haemolytica*, *M. bovis*, *P. multocida*)	2	0	27	**29 (43.3)**	**4**
	Multi-organ	0	0	1	**1 (1.5)**	**1**
	Polio	0	1	0	**1 (1.5)**	**1**
	Musculoskeletal (blackleg, *actinobacillus* abscess)	0	3	1	**4 (6.0)**	**2**
Total					**67**	**17**

[a] PM = proportional mortality calculated as the number of deaths by specific cause divided by all deaths; expressed as a percentage.

from December 2012 to May 2014, focusing on herds within Saskatchewan only (Epp et al., 2016). A total of 76 deaths were necropsied and deaths were classified by body system affected in order to compare to the current study time period. For both time periods, the proportional mortality was calculated by body system or conditions: undetermined causes, non-infectious (calving, trauma and malnutrition), mineral or vitamin deficiencies, major body systems (liver, heart, gastrointestinal, brain, renal, multiple organs, blood, or lung), fetal or abortions, and MCF.

The information on herd MCF status in this previous study time period was combined with the current study time period to gain an overall assessment of MCF. A herd was classified as MCF-positive if, during either study time periods, at least one bison from that herd was reported to have died of MCF, based on PCR test result. The association between proximity of bison herds to sheep (< 1.0 km compared to > = 1.0 km) and whether MCF was identified by necropsy within that herd (dichotomous outcome; positive or negative) during the combined study time periods (current and previous mortality study time periods) was investigated using exact logistic regression (STATA-MP 13.1, Statacorp LP; College Station, Texas, USA). The estimate for all analyses was reported as an odds ratio (or median unbiased estimate, OR-MUE), with P-value calculated by the probability test.

4. Results

4.1. Description of study herds

Of the 54 herd owners who expressed interest in enrollment, 48 herds completed the enrollment process (consent form and pre-survey). There were 6 herds that did not return the post-survey forms, of which 2 also did not submit any animals for necropsy throughout the course of the study. Therefore, only 42 herd owners fully completed all of the study requirements. Thus the remainder of the results will be reported for either 42 fully completed farms or all deaths from 46 enrolled herds who submitted samples for necropsy. Of the 42 herds completing the study, 17 were located in AB, 18 in SK, 4 in MB and 3 in BC. The average number of bison per herd ranged from 28 to 5500 (median: 291). Of the 16 herds with more than 500 bison, 8 were within 5.0 km of sheep operation boundaries. All herds were considered primarily farmed bison operations where calves were produced and the majority were grazed or fed on pasture when available.

Of the 42 herds to complete the study, 8 were within 1.0 km of sheep operation boundaries (high exposure group), 17 were within 1.0 to 5.0 km of sheep operation boundaries (low exposure group), and 17 were at distances greater than 5.0 km from sheep operation boundaries (negligible exposure group). Two of the herds with low exposure changed status midway through the study, moving from or to the negligible exposure group; they were classed as low exposure since that was the highest level of risk at any point during the study time period. The remaining 4 herds which did not complete the end survey but which submitted animals for necropsy were classed only upon entry into the study; 1 high exposure, 2 low exposure and 1 negligible exposure. The size of the sheep operations ranged from 1 to more than 500 sheep (4 bison herd owners could not define sheep operation size), but most contained between 1–150 sheep. All but two sheep operations, as classed by bison producers were typical commercial or hobby sheep flocks.

4.2. Descriptive analysis - mortalities

During the study time period, the mortality risk using 379 necropsied and non-necropsied deaths from 42 farms with complete data was 2.2%; 3.2% for small sized farms (1–150 animals), 2.5% for medium sized farms (151–500 animals) and 1.1% for large sized farms (500+ animals). The overall proportion of deaths on farm (from 42 farms with complete data) that were necropsied was 45.8%; 17.3% for small sized farms (1–150 animals), 37.7% for medium sized farms (151–500 animals) and 74.3% for large sized farms (500+ animals). There were 4 farms in the study that did not record any deaths (9.5% of 42 farms), neither necropsied nor non-necropsied; 83.8% (10/12) of small sized farms, 85.7% (12/14) of medium sized farms and 100% (16/16) of large sized farms experienced at least one death. There were an additional 9 farms that did not submit any dead bison for necropsy but reported anywhere from 1 to 6 non-necropsied deaths on farm; these were either small or medium sized farms.

There were 217 dead animals with samples submitted for necropsy; for 212 deaths, the results were broken down by age groupings. There were 3 abortions from 2 farms; for which one fetus was from a dam that died of MCF but the fetus contained no evidence of the virus within the tissues (liver sample) submitted. The major morphological diagnoses for deaths in neonates and calves are reported in Table 1; the etiologic diagnosis was undetermined for only 14% (8/55) of calf losses with a morphologic diagnosis. The major morphological diagnoses for adults are reported in Table 2. There was no morphological diagnosis identified in 10% (14/142) of adult deaths; there was no etiologic diagnosis for 13% (17/128) of adult losses with a morphological diagnosis.

For 138 deaths sent for necropsy the veterinarian provided a preliminary etiologic or morphological diagnosis; the remainder were left blank or listed as undetermined on the submission form. The necropsy diagnosis confirmed the veterinarian's specific etiologic suspicions for 0 of the 5 anthrax, 2 of the 4 blackleg, 1 of the 2 Johne's disease, 2 of the 3 MCF and 45 of the 48 mycoplasma diagnoses. The majority of deaths (142/217) were submitted between July and November; 76% (20/26) of undetermined cases were submitted during the same months. MCF

Table 2
Major morphological diagnoses (specific etiology by system noted) for necropsied bison deaths within the study period (July 1, 2015–December 31, 2016) for adult bison (over 1 year of age).

Categories of Deaths	Major Morphological diagnoses	Death total	Proportional Mortality	Herds (N)
Undetermined Causes	No morphological diagnoses possible	14	**9.8%**	**10**
Non-infectious causes	Calving, trauma or malnutrition	10	**7.0**	**9**
	Mineral deficiencies or toxicosis	14	**9.8**	**7**
Infectious causes	Whole Body - Anthrax	1	**0.7**	**1**
	GI (hardware, coccidiosis, ostertagiasis, bacterial)	18	**12.7**	**15**
	Heart (failure, bacterial)	5	**3.5**	**4**
	Liver (lipidosis, abscess, tumor)	7	**4.9**	**6**
	Lung (*M. bovis*, *Mann. haemolytica*, bacterial)	58	**40.8**	**8**
	Lymphatics	1	**0.7**	**1**
	MCF	12	**8.5**	**5**
	Multi-organ (bacterial)	1	**0.7**	**1**
	Muscoloskeletal (bacterial)	1	**0.7**	**1**
TOTAL		**142**		**26**

deaths were identified in February (1), April (1), June (2), July (1), August (2), October (1), November (3) and December (1). The majority of mycoplasma deaths were also submitted during July to November (55/73, 75%).

Eleven of the 12 positive MCF bison were from farms within the high risk group; the remaining MCF case was brought onto the farm (which was more than 5 km away from sheep) within the preceding 6 months. One farm had 50% (6/12) of the MCF cases found in this study and another had 25% (3/12) of MCF cases. Overall, only 25% of necropsied animals on these 2 farms were due to MCF; other top causes of death within these 2 herds were non-infectious (i.e. mineral toxicities, calving and/or trauma). Overall, the MCF risk in high risk herds was 1.8 per 1000 bison over the 18 month study period (95% CI: 1.0 per 1000, 3.2 per 1000) and was 0 per 1000 bison for those in the low and negligible herds (95% CI: 0 per 1000, 1.5 per 10,000). Of the 14 deaths for which no morphological diagnosis could be made, 4 were from herds that had MCF cases; only 1 of these animals could not be tested for MCF while the remainder had a negative result.

Mycoplasma bovis was detected in 73 deaths from 6 herds; 4 were large herds (500 + animals) and 2 were medium sized herds (151–500 animals). The most common clinical signs reported in these cases were coughing, weight loss, lethargy and poor doing. In 36% (26/73) of deaths, the animal had reportedly received an autogenous Mycoplasma vaccine in its history, either recently or within the last year.

Of the 136 deaths that had a fecal floatation completed, 22 deaths had no parasites identified while the remainder had at least one species; Trichostrongyle-type eggs were the predominant parasite species identified. This was an incidental finding for most animals; enteropathy (colitis) associated with *Eimeria* spp. or more generally Coccidiosis was listed for 5 deaths. Of those deaths with vitamin and mineral testing completed, 50.3% (77/153) were marginal or deficient in Vitamin A, 18.7% (29/155) were marginal or deficient in Vitamin E and 74.4% (119/160) were marginal or deficient in at least one mineral. The top three deficient/marginal minerals were cobalt, copper and manganese; however, copper was the primary deficient mineral (33 deaths) and was identified in the etiology for 9 deaths (4 of which also involved parasitism). Thirteen deaths were reported as having toxic levels of one mineral; 8 copper, 2 iron, 2 selenium and 1 molybdenum. Only one herd reported not using a mineral supplement in the initial enrollment survey.

There were 177 deaths from 42 farms that were not submitted for necropsy but reported on the post-study survey by herd owners during the study period. Of deaths not necropsied, 37% (65/177) were attributed by owners to non-infectious causes such calving, injury, weather, old age, nutritional issues and mineral deficiencies. An additional 39% (69/177) were missing, only bones left, or too badly decayed/scavenged to determine a cause of death. For two of the herds that had confirmed MCF positive deaths on necropsy, owners reported MCF as the primary cause of deaths not necropsied. Anthrax, mycoplasma and woody tongue were causes identified by owners based on other mortality experiences within the herd or at the discretion of the local veterinarian.

4.3. Comparison to previous mortality study

A previous mortality study was conducted in SK herds during the period of December 1, 2012 to May 31, 2014 (Epp et al., 2016). The largest difference between the study time periods involved lung lesions (7% in first study and 41% in the second study), which was driven by the diagnosis of *M. bovis* within the current study time period but not the previous study time period (Fig. 1).The other notable difference was 'calving, trauma and malnutrition'; in both study time periods, many of the deaths that were not necropsied were attributed by owners to calving, trauma, nutritional or old age causes. Additionally, despite the majority of bison producers citing the use of mineral supplements in the second study, there was not much change in the proportion of deaths due to mineral or vitamin abnormalities.

A total of 60 herds were used in the analysis of MCF and proximity to sheep; 26 farms from the previous mortality study time period and 42 farms from the current study time period, with 8 farms continuous between the 2 time periods. Of the 14 farms within 1 km of a sheep operation boundary fence, only 5 farms had MCF confirmed deaths during at least one of the 2 study time periods (Table 3). The unadjusted primary relationship between MCF herd status and distance to sheep produced an OR-MUE of 30.1 ($P = 0.0007$). When adjusting for bison herd size, the OR-MUE for MCF herd status and sheep distance dropped to 27.8 ($P = 0.009$) for herd size cut-off of 150 and 23.9 ($P = 0.002$) for herd size cut-off of 500.

5. Discussion

This study was a continuation of an earlier investigation into the causes of farmed bison deaths in western Canada (Epp et al., 2016). The main objectives were to estimate the proportional mortality and relative frequency of the most common causes of mortality in farmed bison herds in Western Canada and to compare the results to a previously completed bison mortality study in Saskatchewan. The risks of MCF at various proximities to sheep operations was also assessed.

Compared to the first study which followed 26 herds from Saskatchewan (Epp et al., 2016) the herd sample size of this study was almost twice as large with more than 3x the number of necropsied bison and included farms in all four western Canadian provinces. The overall mortality risk in the studied herds was low (≤3%) regardless of herd size. When considering the mortality risk for all deaths (necropsied and

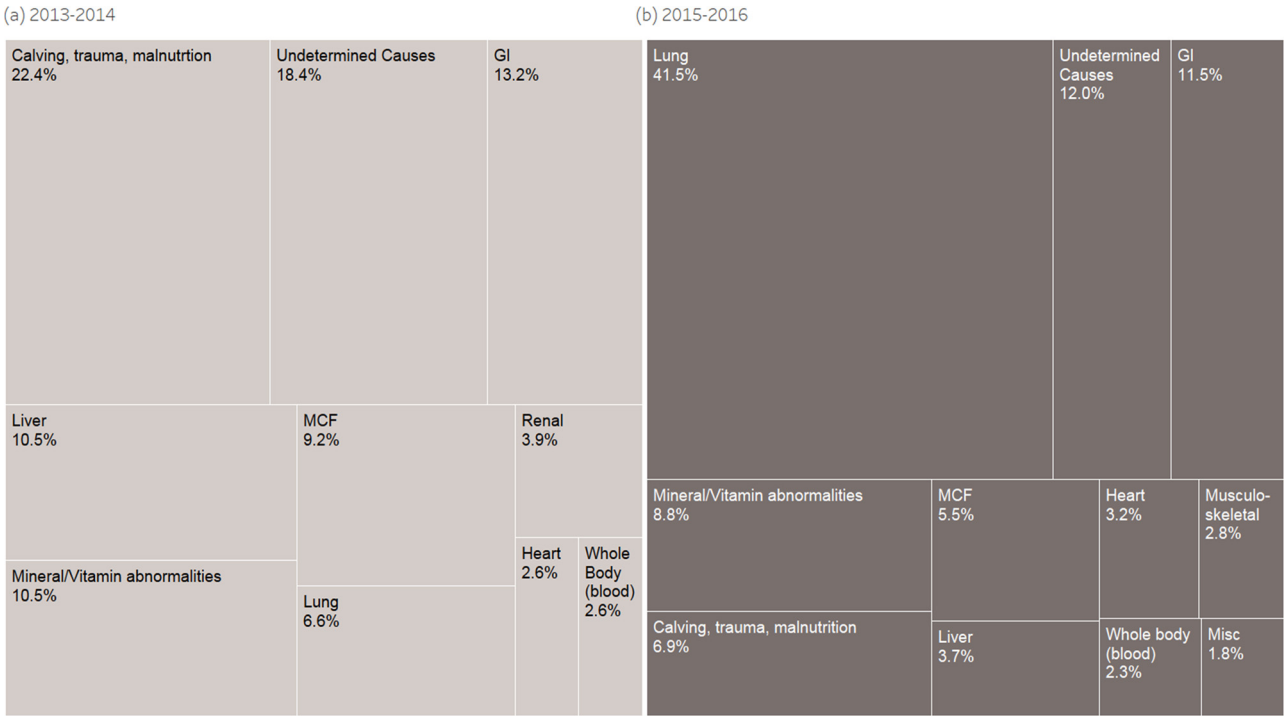

Fig. 1. Proportional mortality by body system or condition for: 76 bison deaths on 26 study bison farms for the period of December 1st, 2012–May 31st, 2014 and 217 bison deaths on 46 study bison farms for the period of July 1st, 2015–Dec 15th, 2016.

Table 3
Malignant Catarrhal Fever (MCF) herd status by bison herd size, sheep operation size and distance to sheep. Combination of data from mortality studies in 2 time periods, one specific to Saskatchewan herds (26 herds) and the other opened up to western Canada herds (42 herds). For herds that were enrolled in both study time periods (n = 8), data was compared and combined to create only one entry per herd in the dataset.

Sheep Distance	Sheep Herd Size	MCF positive farm		MCF negative farm		Total
		< 500 Bison	500 + Bison	< 500 Bison	500 + Bison	
Sheep within 1 km of bison	**< 50**	0	1	4	2	14
	51–500	1	1	2	1	
	500 +	0	1	0	0	
	unknown	1	0	0	0	
Sheep more than 1 km away from bison[a]	**< 50**	0	0	5	2	46
	51–500	0	0	10	1	
	500 +	0	0	0	0	
	unknown	0	0	20	8	
Total		5		55		60

[a] One herd more than 5 km away from sheep had a MCF death (animal was brought onto the farm within 6 months of its death); it was not considered an exposure on farm where death occurred. For farms more than 5 km away from sheep, no information was available on sheep operation size and was recorded as unknown.

non-necropsied) and regardless of herd size, the risk was somewhat bigger (2.2%) with a tendency for decreased risk in larger herds (e.g. 3.2% in farms with < 150 bison compared to 1.1% in farms with > 500 bison). Within the study, only 4 small or medium sized farms experienced no deaths at all. This is similar to the findings from the 2014 USDA survey where the frequency of farms reporting bison deaths (during a 12-months period) ranged from 12.6 to 82.1% and increased with increasing herd size (USDA, 2016). Although the categorization of herd sizes differed markedly between this and the USDA study (example: operations considered large were ≥100 bison in the USDA study but > 500 bison in this study), the death risk was still markedly lower in herds of all sizes in our study with almost all herds experiencing at least one death in a 18 month time period. The risks were, however, similar to the first mortality study conducted by Epp et al. (2016) where the overall risks ranged from 1.1 to 1.9% for necropsied bison and all bison that died during the study period (necropsied or non-necropsied), respectively. Differences in management, herd composition, nutrition (including pasture quality), climate and environment may account for the different mortality risks between western Canada and the US.

The main cause of death in the necropsied bison, regardless of age, was attributed to lung lesions primarily caused by *Mycoplasma bovis*. Unfortunately, *Mycoplasma bovis* was not specifically investigated in the first mortality study and, therefore, no direct comparisons can be made. However, in the previous mortality study, if lesions were indicative of potential lung involvement, testing for *M. bovis* was done (using the same test as the second mortality study), and all were negative. In a prospective study on farmed bison deaths conducted in Alberta, Canada, in 2012, however, respiratory disease associated with *M. bovis* was also a prominent cause of death (Burrage et al., 2012); in that study, not all *M. bovis* deaths had prominent lung lesions. Multiple other studies in farmed bison have highlighted the emergence of this important and often fatal pathogen in the North American industry (Dyer et al., 2008; Janardhan et al., 2010; Dyer et al., 2013; Register et al., 2013). Although it was not an objective of this study to identify risk factors for *M. bovis* and, therefore, specific information was not collected, some available literature investigated potential risk factors in farmed bison. For example, in Alberta, Canada, herd-level *Mycoplasma* spp. seroprevalence in bison > 1 year old from 19 herds was 79% and was also present in herds that did not report any history of clinical disease associated with *M. bovis* (Bras et al., 2017). This may suggest that *M. bovis* is present subclinically in herds or may go undiagnosed which is also supported by an outbreak in a farmed bison herd in Kansas, where no new animals were introduced into the herd in the 4 years prior to the outbreak (Janardhan et al., 2010). The risk also increased with increasing herd size, in cows > 3 years and yearlings, if a feedlot unit was part of the bison operation, if there was regular trailer traffic to the farm and if the herd was vaccinated annually (Bras et al.,

2016). Interestingly, most of the *M. bovis*-related deaths here occurred in adult bison, from only 6 participating farms, all of which were either medium- (151–500 head) or large-sized (> 500 head) herds.

Gastrointestinal disease and mineral abnormalities were the next most common causes of death in necropsied bison. Gastrointestinal parasites were very common (114 of 136 fecal flotations were positive for at least one species) but only coccidiosis (or *Eimeria* spp.) was considered as the final diagnosis in 5 animals. Information on internal parasites in farmed bison is scarce. Although a similar number of animals was diagnosed with parasitic abomasitis in the previous mortality study (Epp et al., 2016), the total number of necropsied bison was only about 1/4 of the total number of bison necropsied here. In an earlier survey of bison herds in Manitoba and Saskatchewan, Canada, 100 and 95% of tested herds were positive for trichostrongyle and *Eimeria* species, respectively (Woodbury et al., 2014). Similarly, 100% of 22 bison herds from Alberta, Canada, had at least one animal test positive for trichostrongyle species (Dies and Coupland, 2001). The overall egg count was low in both surveys. Although the study populations or data collection methods are not comparable between our study and these herd-level surveys, it appears that although internal parasites are common, clinical disease is likely rare given the low egg counts identified in the two herd-level studies and the few animals in which parasitism was considered the cause of death in this study. This may differ somewhat from the 2014 USDA survey of bison producers, where 19% reported the presence of (internal) parasites in their bison and 5.3% directly linked bison death to (internal or external) parasitism (USDA, 2016). It is perhaps noteworthy that, in comparison to two previous reports from farmed bison in Manitoba (Woodbury et al., 2012; Woodbury et al., 2014), no *Toxocara* spp., and specifically, no *Toxocara vitulorum* was identified in any of the bison calves necropsied here. This is notable because *T. vitulorum* has only been reported for the first time in North America in 2012, when it was also suspected to have caused clinical signs and fatalities in affected bison calves (Woodbury et al., 2012). However, it is perhaps not surprising as to date only 4/59 farms in Manitoba (but none in Saskatchewan) tested positive for this parasite (Woodbury et al., 2012; Woodbury et al., 2014); the current study included only 5 production sites in Manitoba.

Most of the animals with a completed mineral panel were marginal or deficient in at least one mineral and half of the animals tested for vitamin concentrations were marginal or deficient in vitamin A and/or E. Similarly, copper deficiency in farmed bison was also frequently identified in the previous mortality study (Epp et al., 2016). In nine animals here, it was considered the main cause of death but the deficient concentrations in the other bison could have contributed to their death. Copper deficiency is also a common problem in cattle in western Canada (Van De Weyer et al., 2011). Except for one bison producer, all reported that they provided a mineral supplement for their bison; however, supplementation is likely not the only factor determining the copper status of farmed bison. Sulfate concentration in water, soil type, pasture quality and composition, season and general animal health/infection status influence the copper concentration of an individual (Smart et al., 1992; Van De Weyer et al., 2011; Suttle, 2012). Interestingly, toxic levels of copper, iron, selenium and molybdenum were also found. While copper toxicoses were identified in eight animals from seven farms, the other toxicoses were all in different animals from one farm which also experienced copper toxicoses. This further suggests the presence of specific herd-level factors that contribute to the variability in mineral status between herds.

No comparative literature on vitamin concentrations in bison could be identified. Comparisons to the cow-calf industry, however, are probably justified based on similar management practices and highlight that vitamin A and E deficiencies are common in young stock (Waldner and Blakley, 2014; Waldner and Uehlinger, 2017) but somewhat less in adults (Van de Weyer et al., 2010; Van De Weyer et al., 2011). Fresh, green forage are the primary sources of vitamin E and the precursor, β-carotene, for vitamin A in ruminants (Frye et al., 1991; Puls et al., 1994). Higher vitamin A concentrations at the end of the grazing season were demonstrated in beef cows by Van de Weyer et al. (2010). In the current study, the majority of dead bison were submitted for necropsy between July and November each year, a period during which most bison have preferred access to pasture grasses. It is therefore possible that the identified deficiencies are an underestimation of the prevalence of vitamin deficiencies and their contribution to mortality in bison. Vitamin A and E deficiencies in adult beef cows were not associated with adverse pregnancy outcomes in one study (Van De Weyer et al., 2011), but inadequate concentrations of these vitamins in the dam affect their concentrations in the offspring which is dependent on colostrum and milk intake as a source of vitamin A and E (Frye et al., 1991; Puvogel et al., 2008). A lack of vitamin A and E in calves has been associated with increased morbidity and mortality (Frye et al., 1991; Puvogel et al., 2005; Waldner and Rosengren, 2009; Moosavian et al., 2010; Waldner and Uehlinger, 2017), and this is supported in the current study by the relative frequency in which they contributed to death in the necropsied bison.

It is necessary to consider that only approximately half of bison that died during the study period were submitted for necropsy. While the reported causes of death in some of these bison may indeed have been obvious to the producer or attending veterinarian as reported (e.g. calving-related and other injuries or when infectious disease testing was conducted animal-side (such as anthrax)), some are likely based on speculation only (e.g. nutritional issues, mineral deficiencies). Reasons for not submitting these animals to the study may have included advanced autolysis of the carcass, severe scavenging of the carcass with insufficient useful tissues available for submission, frozen carcasses with inability to collect useful tissues, or other logistical issues such as e.g. inability to have a veterinarian attend within a reasonable time period. In some situations, veterinarians and/or owners were also probably certain of their diagnosis and did not consider it beneficial to submit samples to the study.

Similar to the previous mortality study (Epp et al., 2016), the category of 'undetermined causes' was the second most common necropsy finding, tied with gastrointestinal disease and following respiratory (*M. bovis*) disease. However, the usefulness of performing a necropsy is still supported in the current study by the overall few open morphologic or etiologic diagnoses. Some reasons for the inability to identify a possible cause of death at necropsy were overt autolysis of submitted tissues and insufficient tissues or lack of specific samples to conduct certain testing. Overall, bison producers should continue to be encouraged to ask for this service while veterinarians should make it a routine procedure for any dead bison because in the majority of necropsied bison, useful information was gained with regards to an underlying disease process.

Except for one death from MCF, all were on farms within 1 km of a sheep operation boundary fence. The risk of MCF on high risk farms was still very low (1.2 per 1000 bison per year) overall with the majority (75%) of cases occurring on 2 separate larger bison operations located within Saskatchewan and British Columbia. Compared to previously published outbreaks of MCF in bison herds, this study highlights that there may be a three contributing components to whether a herd experiences a case of MCF or not, bison herd size, sheep operation size and exposure distance to sheep (Schultheiss et al., 2000, O'Toole et al., 2002, Li et al., 2006). Not all herds within close proximity to sheep operations experience MCF, but for those that do, the resulting outcome, as described by producers, is both devastating and costly. Further insights into high risk herds that experience extra-ordinary cases of MCF are worth investigating in more depth to determine what other factors may predispose bison to clinical manifestations of the disease.

Declaration of interests

None.

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