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Herd-level risk factors associated with chronic wasting disease-positive herd status in Minnesota, Pennsylvania, and Wisconsin cervid herds

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ABSTRACT

The objective of this study was to evaluate risks related to introduction of Chronic Wasting Disease (CWD) to farmed cervid herds in Minnesota (MN), Pennsylvania (PA), and Wisconsin (WI). This was the first study to evaluate risk factors related to multiple pathways of CWD transmission to farmed cervid herds. Participating herds in this case-control study included 22 case and 49 control herds identified through participation in the respective mandatory State CWD herd program. Data was voluntarily collected from white-tailed deer (WTD) study herds using a questionnaire and state animal health agency databases. Univariable analyses identified associations between CWD-positive herds and variables representing different transmission pathways, including direct contact with infected farmed cervids (imported from a herd that later tested positive for CWD) odds ratio (OR):7.16, 95 % confidence intervals (CI):1.64–31.21 and indirect contact with infected wild cervids (access of domestic cats to pens or feed storage area) OR:4.07, 95 % CI:1.35–12.26, observed evidence of mammalian scavengers inside or outside of fenceline in the previous 12 months OR:6.55, 95 % CI:1.37–31.32, $\leq$ 5 km distance to nearest detected CWD-positive wild cervid OR:3.08, 95 % CI:1.01–9.39, forested area crosses the perimeter fenceline OR:3.54, 95 % CI:1.13–11.11, $\leq$ 0.3 m distance of water source to fenceline OR:4.71, 95 % CI:1.60–13.83, and water source shared with wild cervids (running or standing water) OR:4.17, 95 % CI:1.34–12.92. Three variables from univariable analyses that represented different biological transmission pathways were placed in a Firth's penalized maximum likelihood multivariable logistic regression to evaluate associations between transmission pathway and CWD herd infection status. For the issue of low sample size and overfitting, 95 % CIs for estimated coefficients for the three variables were computed via bootstrapping of 10,000 independent bootstrap samples. The three biological variables were significantly associated with herd CWD infection status: imported cervids from a herd that later tested positive for CWD (OR:5.63; 95 % CI:1.1–28.2), $\leq$ 0.3 m distance of cervid water source to perimeter fenceline (OR:4.83; 95 % CI:1.5–16.1), and $\leq$ 5 km distance to nearest detected CWD-positive wild cervid (OR:4.10; 95 % CI:1.1–15.2). The risk factors associated with CWD herd status identified in this study indicated the importance of transmission through direct contact pathways with infected cervid herds (introduction of cervids from herds later identified as CWD-infected) and indirect contact pathways with infected wild cervids that could be related to other animals through the perimeter fence. Further studies are needed to confirm and clarify understanding of indirect pathways to allow development of improved biosecurity practices to prevent CWD introduction to cervid herds.

1. Introduction

Chronic wasting disease, one of several diseases known as Transmissible Spongiform Encephalopathies, is an infectious, fatal disease affecting both wild and farmed cervids. Resulting from infection with an abnormally folded prion protein, CWD is an important emerging disease affecting the cervid industry in North America. In the USA, cervids enclosed by fences are born and raised in captivity and not captured from the wild. They are considered farmed cervids in many states, and are required to be completely enclosed by a fence with most states requiring a minimum height of 8 feet, with varying fencing material used. There are different purposes of farmed cervid herds, including (1) breeding for sale of offspring (cervids kept and bred to sell offspring, both males and females), (2) meat/slaughter (cervids raised and harvested for meat), (3) hunting preserve (cervids kept in an enclosure for hunting, and may breed own stock or purchase from an outside source), (4) trophy buck or bull sales (male cervids bred and sold specifically for certain genetic qualities such as body or antler size), (5) pets/pleasure (cervids kept as pets or for owner enjoyment), or (6) exhibition (cervids kept for educational purposes, showing to public, or zoos). Cervid herds provide venison, velvet and hard antlers, trophy buck and bull sales, recreation, and pleasure to farmers and consumers, with significant economic benefits to their communities (Keckhaver, 2012). CWD poses a major threat to cervid herds due to continued disease spread (Derreumaux, 2005; Benestad and Telling, 2018). To date, CWD has been detected in farmed cervids in 18 USA states and 3 Canadian provinces and in wild cervids in 31 USA states and 3 Canadian provinces (Richards, 2023).

Transmission is possible through multiple direct and indirect exposure pathways to susceptible herds. Direct contact occurs when susceptible cervids have direct bodily contact with an infected farmed or wild cervid, while indirect contact occurs when infectious prion material from an infected farmed or wild cervid contaminates objects or environment that is shared with other cervids.

Current CWD regulatory policies are focused on postmortem testing of cervids and preventing exposure of farmed cervids to infective prion protein through practices such as introduction of cervids from CWD-positive herds, fencing breaches that allow direct contacts between infected wild cervids and susceptible farmed cervids, and movement of infected cervid carcasses through hunting or taxidermy practices which Kincheloe et al. (2021) classifies as known high-risk practices. For each state, the type of CWD program the herd participates determines the percentage of cervids in which postmortem testing is performed. In MN, 100 % of cervids > 12 months old that die or are slaughtered are tested for CWD in herds in both Certified and Not certified programs (MNBAH, 2023). In PA, herds participating in the Herd Monitored Program are required to test 50 % of cervids > 12 months old that die for any reason (hunting/slaughter/natural) whereas herds in the Herd Certified Program are required to test 100 % of cervids > 12 months old that die or are slaughtered (PDA, 2023). In WI, herds in the Enrolled program < 5 years are required to test 100 % of cervids > 12 months that die (WIDATCP, 2023). Herds in the Enrolled program > 5 years are required to test 100 % of all cervids > 12 months that die or are killed and 25 % of those > 12 months that are shipped directly to slaughter (WIDATCP, 2023). Herds in the Nonenrolled program are required to test 100 % of cervids > 16 months that die by accident, natural causes, or escapes, 50 % of cervids > 16 months that are hunted or killed intentionally, and 25 % of cervids > 16 months that are shipped directly to slaughter (WIDATCP, 2023). In addition, many cervid producers also implement voluntary biosecurity practices to reduce their risk of CWD introduction. However, CWD continues to be detected in cervid herds across the US. In MN, PA, and WI alone**,** 84 (MN 8, PA 47, WI 29) newly detected CWD infected herds have been identified since 2016, primarily in WTD herds. Once identified as infected, CWD-positive cervid herds are quarantined and many are depopulated, as there are no proven methods to eradicate prion infection from test-positive herds without removal of animals from

contaminated properties. Miller et al. (2004) demonstrated that CWD prions can remain in the environment for > 2 years, suggesting that, even with removal of cervids from a location, the risk of CWD is still present for a considerable amount of time. Environmental persistence of prions within pens and the presence of potentially infectious farmed cervids also pose a threat to susceptible wild cervids in the surrounding area.

While one focus of CWD research to date has been the characterization of CWD transmission in wild cervid populations, relatively little CWD research has been performed to assess transmission risks to farmed cervids, other than through between-herd movements of cervids (Argue et al., 2007; Rorres et al., 2018). While between-herd movements are one potential CWD exposure pathway, other exposures have been identified. Based on information generated from a systematic literature review, Kincheloe et al. (2021) developed a risk framework to identify transmission exposures to cervid herds through different direct and indirect contact pathways, including (1) direct contact with an infected wild cervids (2) direct contact with an infected farmed cervid, (3) indirect contact with an infected cervid carcass, and (4) indirect contact with infected farmed or wild cervid. Through an evaluation of CWD-infected herds, the study indicated the potential importance of indirect exposures between infected wild and farmed cervids (through sharing equipment, contaminated feed and water, or scavengers as fomites), especially for recently detected CWD-positive herds located near CWD-detected wild deer (Kincheloe et al., 2021).

Identification of important risk factors associated with CWD-positive cervid farm status would facilitate better understanding of disease transmission pathways and are critical to improving disease prevention efforts. When available, this information provides the opportunity for cervid producers to reduce their own risks of CWD introduction to their herds using a targeted approach. The purpose of this study was to identify herd-level factors associated with CWD-positive herd status in MN, PA, and WI cervid herds detected since 2016 to inform improved farmed cervid biosecurity practice implementation.

2. Materials and methods

2.1. Study design

The observational epidemiologic study design used was a case-control study, with a goal of three control herds for each case farm. This study was conducted in the upper midwestern and eastern states of MN, PA, and WI, where CWD exists in wild WTD populations, recognizing potential differences between the behavior and ecology of wild WTD and other wild cervids from other regions with respect to CWD transmission. In addition, since most recent CWD-positive cervid herds in these states have been composed of predominantly WTD, predominantly WTD herds served as the study target population.

2.2. Case and control selection

CWD herd test status information from farmed herds in MN, PA, and WI from 2016 to 2022 was obtained from state animal health agencies (Board of Animal Health or Dept of Agriculture, depending on the state). For this study, study herds were defined as following:

2.2.1. Case herds

WTD (all cervids in the herd are WTD) or WTD-mixed (mixture of majority WTD and any other cervid species in the herd) CWD test-positive farmed cervid herds detected in a participating state since 2016.

2.2.2. Control herds

WTD or WTD-mixed farmed cervid herds with zero test-positive results for CWD prior to and during data analysis based on mandatory State CWD Control Program surveillance. Control herds were selected by matching to cases within their own state to account for the differing

surveillance programs within each state. Controls were also matched to case herds by the following criteria: surveillance program status at time of matched case detection (Herd Certified Program vs Herd Monitored Program for PA, Enrolled vs Nonenrolled for WI, and Certified vs Not Certified for MN), adult cervid population size ($\leq$ 15 or > 15 cervids older than 12 months of age), in operation when the matched case herd was identified as positive and remaining in operation, and within 80 km (expanded up to 112 km if no control herds within 80 km were available or agreed to participate). If more than 3 control herds met the matching requirements, the first three that agreed to participate were included in the study.

2.3. Data collection

Sources of information for this study included participating cervid herds and state animal health agencies. Using a CWD transmission framework modified from Kincheloe et al. (2021), we identified the following variables by transmission pathway for evaluation (Fig. 2):

1. *Direct contact with infected wild cervid:* Fencing breaches/escapes and nose-to-nose contact through single fencing.
2. *Indirect contact with infected wild cervid:* Contaminated feed/plants/ water, and through other animals/scavengers/birds.
3. *Direct contact with infected farmed cervid:* Farmed cervid introductions to herds through cervid movements.
4. *Indirect contact with infected farmed cervid:* Equipment/vehicles, clothing/boots, feed, semen/embryos.
5. *Indirect contact with infected cervid carcasses*: Hunting or taxidermy practices.

Specifically, information on farmed cervid movements, regulatory infractions, CWD test results, and distance to nearest wild and farmed cervid CWD-positive case was obtained for study herds from each state's animal health agency database. Distance from the closest perimeter fenceline to the nearest detected CWD-positive wild cervid data was obtained from state agencies for the time period from January 2016 to December 2021. For case herds, the distance to the nearest detected CWD-positive wild cervid was for the time frame from five years before to 12 months after the case herd tested positive, while for control herds, the closest detected CWD-positive wild cervid was from January 2016 to December 2021. To capture use of herd management practices related to potential CWD transmission pathways, a 74-question study questionnaire was developed, and pretesting was performed with a small sample of cervid herds in each participating state. An interview (in-person, video conference, or via telephone) was conducted to administer the questionnaire to each participating cervid producer to collect

information about cervid management and biosecurity practices between March to September 2020. Steps were taken to prevent information bias when collecting cervid producer data. Two study investigators conducted 80 % of the questionnaires (33 and 24 questionnaires respectively) with the remaining 14 questionnaires collected by 6 people. A descriptive informational sheet explaining how to phrase questions with further details was developed for personnel conducting the questionnaire to ensure data quality. Participation by cervid producers was voluntary and individual producer information was kept confidential. To account for changes in landscape, biosecurity practices, and cervid numbers (due to depopulation), managers of case herds were asked to provide information for the time frame prior to identification of their index CWD-positive cervid. Control cervid producers were asked to provide information for the present time frame.

2.4. Statistical analysis

For identification of CWD herd-level risk factors, data was analyzed using SAS® statistical software (SAS Institute Inc., Cary, NC, USA). In the first stage of data analysis, associations between CWD-herd status (case/control) and each categorical and continuous variable were identified using univariable analyses to determine crude ORs (double-sided t-test or Cochran-Mantel-Haenszel (CMH) test). See **Supplemental Sheet 1** with description of important variables. Significant continuous variables were also categorized into binary variables and associations were evaluated as categorical variables. For several variables in the same biologically related transmission pathway (e.g., multiple variables pertaining to scavengers), the variable with the strongest biological plausibility was identified using univariable analyses.

To prevent overfitting due to small sample size, a maximum of three variables from univariable analyses were included in multivariable logistic regression, due to the rule of thumb of 1–2 predictors per 10 events (Laupacis et al., 1997), with one variable with a biologic relationship to a different transmission pathway found to be associated with CWD herd status from univariable logistic regression. These variables were therefore selected based on their statistical significance in conjunction with biological importance. Of the independent variables identified as associated with CWD-positive herd status ($p < 0.1$), one variable was manually selected for multivariable logistic regression to represent each biological transmission pathway of CWD, with a total of three pathway variables selected. We accounted for multicollinearity among variables in several ways, by including only one variable associated with each biological pathway from univariable analyses and checking the variance inflation factor for each independent variable. Spearman's correlation coefficients were also calculated to evaluate correlation between the 13 significant independent variables.

The crude odd ratios were adjusted and compared to determine if there was effect modification by $\leq$ 5 km distance to nearest detected CWD-positive wild cervid, using the Mantel-Haenszel procedure to produce an adjusted OR (Mantel and Haenszel, 1959). For the adjusted OR, farmed cervid herds were grouped into strata ($\leq$ 5 km or > 5 km) and the resulting OR and 95% CI for the two selected biological variables were calculated. Breslow-Day test of homogeneity was used to determine if the stratified OR were homogenous and if effect modification resulted in ORs to be reported by stratum and not pooled (Breslow and Day, 1980). If the stratum ORs were homogenous, then an adjusted OR was calculated and compared to the crude OR to determine if distance to nearest detected CWD-positive wild cervid was a confounder.

Firth penalized maximum likelihood method was used in the multivariable logistic regression model to address small sample size and reduce bias. Case/control herd status was the dependent variable in the model. Bootstrapping, a resampling observational method with replacement, was performed using the method described by Wicklin (2018) to estimate mean OR and 95% CIs. First, 10,000 bootstrap samples with a sample size of 71 with replacement were created from our study population. Firth logistic regression analysis was performed
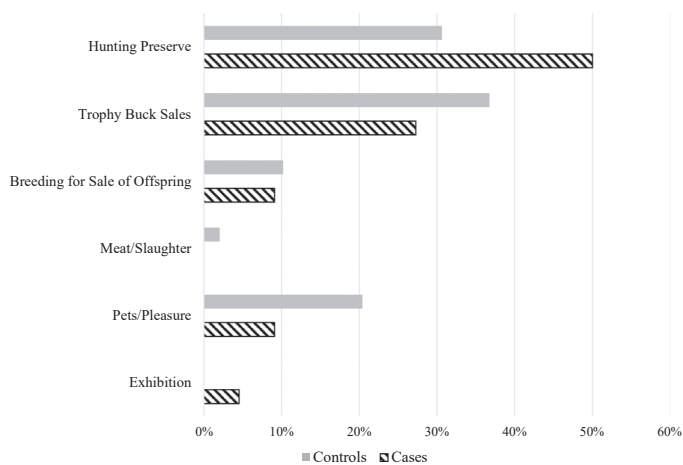

Fig. 1. Proportion of participating WTD or WTD-mixed study herds in MN, PA, and WI by primary herd type (percent) for case and control herds (Primary herd types defined in Introduction).
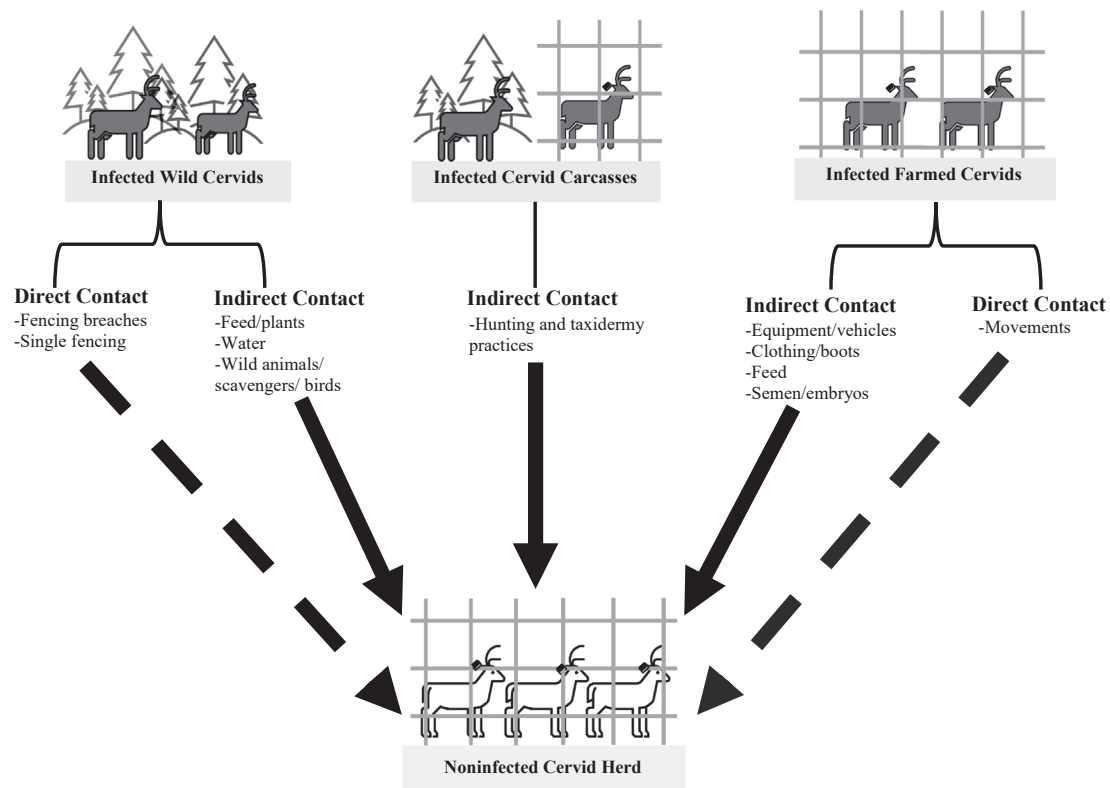
Fig. 2. Indirect and direct contact transmission pathways.

on each of the 10,000 bootstrap samples, resulting in 10,000 estimated coefficient estimates for each variable. The mean OR and 95% CIs for the estimated ORs were constructed from the distribution of the 10,000 bootstrapped coefficient estimates after exponentiation. For verification, this bootstrapping process was replicated numerous times to ensure the resulting 95% CIs for each variable were similar.

Finally, we evaluated the biologically relevant variables from the logistic regression model using a General Linear Mixed Model (GLMM), considering the three variables as fixed effects, while State and Primary Herd Type were considered as random effects. These two variables were considered random effects because States have different regulatory surveillance programs and management systems varied between herd types.

3. Results

3.1. Descriptive data

Study herds included a total of 71 herds (22 case herds and 49 control herds) in the three states. We initially identified 18 case herds, but four cervid herds selected initially as control herds tested CWD-positive during the study period and were therefore converted to case herds. Control herds in the study fit the matching criteria to serve as control herds for these new case herds. PA had the highest number of study herds (this state had the highest number of eligible cervid herds positive for CWD; Table 1). Among the 71 herds participating in this

Table 1
Number of participating case and control WTD herds by state.

State	Case herds	Control herds	Total herds
Minnesota	3	9	12
Pennsylvania	12	23	35
Wisconsin	7	17	24
Total	22	49	71

study, case herds had a median of 79 total cervids (range 6–3000) compared to control herds with a median of 60 cervids (range 2–358). Case herds had a median of 61.5 (range 2–1299) cervids tested for CWD from five years prior to detection of index CWD case up to the index case with control herds testing an average of 19 (range 1–249) from 2016 to 2021. The primary herd type percentage was similar for cases and controls (Fig. 1). Hunting preserves and trophy buck sales accounted for 50 % and 27 % (total of 77 %) for case herds and 31 % and 37 % (total of 68 %) for control herds, respectively. Breeding for sale of offspring accounted for 9 % of case and 10 % of control herds. The median distance to the nearest detected CWD-positive wild cervid was 11.8 km for cases (range 0.5–140) and 15.5 km for controls (range 0.3–161). Sixty-eighty percent (15/22) had a CWD-positive wild deer detected within this distance prior to the index farmed cervid detected with median distance of 10 km in this group of herds.

3.2. Univariable Analyses for the Case-Control Study

Variables not associated with CWD herd status in univariable analyses ($p < 0.1$) included primary herd type, total number of movements/loads of cervids moved onto the premises in past 5 years, cervid escapes in the past 5 years, number of fencing breaches in the past 5 years, height of outer perimeter fence, outside hunted cervid parts or whole carcasses brought back to property in the past 5 years, processing of hunted cervid carcasses in any way on site, taxidermy or other trophy display preparation involving cervids performed onsite in past 5 years, use of artificial insemination or embryo transfer in the past 5 years, number of compliance infractions in the past 5 years, maximum density of cervids in one pen at a given time of year, and use of CWD resistance genotype testing performed on cervids.

Overall, 32 variables were associated with CWD-positive herd status ($p < 0.1$), including several biologically similar variables. As one example, there were six variables associated with CWD herd status relating to the presence of avian and mammalian scavengers in and around the herd premises. After selection of a single variable among

biologically similar variables, 13 variables associated with positive herd status were identified (Table 2). Using the framework of CWD transmission pathways previously characterized through systematic literature review (Kincheloe et al., 2021), we identified potential risk factor variables within these pathways, with some variables having the possibility to be in multiple pathways:

Direct contact with farmed cervids pathway: imported from a herd that later tested positive for CWD.

Direct or indirect contact with wild cervids pathway: farm location within 5 km to the nearest detected CWD-positive wild cervid, does not use double fencing, does not inspect complete outer perimeter fence daily, and observed wild cervids along the perimeter fenceline in the previous 12 months.

Indirect contact with wild cervids pathway: access of domestic cats to pens or feed storage area, observed evidence of mammal scavengers inside or outside fenceline in the previous 12 months, on-site disposal of carcasses of resident cervids that died naturally in compost or waste pile, store feed/bedding on the farm, forested area crosses the perimeter fenceline, ≤ 0.3 m distance of water source to perimeter fenceline, and water source shared with wild cervids (running or standing water).

Other: > 40 adult males greater than 12 months of age.

Table 2

Results from comparison of case and control herds using univariable CMH analysis of association between herd-level variables and CWD herd status ($p < 0.10$).

Variable	% Cases	% Controls	CMH Crude OR	CMH 95% CI	p-value
Imported from a herd that later tested positive for CWD	31.8	6.1	7.16	1.64–31.21	0.004
Does not use double fencing	86.4	67.4	3.07	0.79–11.92	0.097
Does not inspect complete outer perimeter fence daily	75.0	51.0	2.88	0.91–9.16	0.069
Observed wild cervids along the fenceline in previous 12 months	86.4	63.3	3.68	0.95–14.17	0.050
Access of domestic cats to pens or feed storage area	72.7	39.6	4.07	1.35–12.26	0.011
Observed evidence of mammal scavengers inside or outside of fenceline in the previous 12 months	90.5	59.2	6.55	1.37–31.32	0.010
On-site disposal of carcasses of resident cervids that died naturally in compost or waste pile	27.3	10.2	3.30	0.88–12.32	0.068
Store feed/bedding on the farm	90.9	73.5	3.61	0.74–17.64	0.098
≤ 5 km distance to nearest detected CWD-positive wild cervid	40.91	19.37	3.08	1.01–9.39	0.045
Forested area crosses the perimeter fenceline	77.3	49.0	3.54	1.13–11.11	0.027
≤ 0.3 m distance of water source to fenceline	63.6	27.1	4.71	1.60–13.83	0.004
Water source shared with wild cervids (running or standing water)	45.5	16.7	4.17	1.34–12.92	0.011
> 40 adult males greater than 12 months of age	47.6	24.5	2.80	0.96–8.22	0.058

3.3. Stratification analysis

Breslow–Day statistic was not statistically significant ($p > 0.05$) which indicated that the stratified measures of association were not significantly different and that there was no significant effect modification of distance to nearest detected CWD-positive wild on the two biological variables. The homogeneity of the stratified results indicates that an adjusted CMH OR should be calculated and reported (Table 3). There were only small differences between crude and adjusted odd ratios ($< 20\%$ as the cutoff) indicative of lack of evidence for confounding (Pourhoseingholi et al., 2012).

3.4. Multivariable analyses for the case-control study

Of the 13 different independent variables identified as associated with CWD-positive herd status, mild to moderate correlation was identified among some variables, with most Spearman's Correlation Coefficients below 0.43. No significant correlation was identified between the three variables used in the regression models. This logistic regression model (Table 4) included the following variables: imported from a herd that later tested positive for CWD, ≤ 0.3 m distance of water source to perimeter fenceline, and ≤ 5 km distance to nearest detected CWD-positive wild cervid. In this model, all three variables in the model were associated with herd infection status (Table 4).

3.5. Bootstrap analysis

The results from bootstrapping analysis were similar to those from Firth logistic regression, and mean OR and 95 % CI were generated from the first bootstrap analysis (Table 4). In this model, all variables were associated with herd infection status (Table 4).

3.6. GLMM analysis

The three selected variables were placed in a GLMM with State and Primary Herd Type as random effects. These two variables were selected to account for the variability between the three States and the different primary herd types. The results showed agreement with the Firth logistic regression and bootstrapped model results (Table 4).

4. Discussion

This study represented the first herd-level inferential epidemiologic assessment of CWD transmission risks to cervid herds and may be indicative of transmission pathways of CWD to cervid farms. Specifically, it explored CWD introduction risks to WTD herds in MN, WI, and PA using a case-control observational epidemiologic study design for a comparison of factors that differ between test-positive (case) and non-test-positive (control) herds. This study design can be useful to identify associations that may be causal in nature, and is useful for generating hypotheses for future study.

Univariable study results identified associations with variables from at least two different primary pathways associated with CWD-positive herd status: (1) direct contact with other farmed cervids through cervid movements with herds later found to be CWD-positive and (2) indirect contact with infected wild cervids through several factors, including distance to nearest CWD-positive wild cervid, presence of other animals or scavengers in the vicinity, and factors that may attract these animals to the cervid farm, including water source location, forest area surrounding fenceline providing shelter for animals, or carcass disposal location attracting scavengers. Use of single perimeter fencing and proximity of CWD-infected wild cervid to the herd, variables identified from univariable analysis, could also be placed in the pathway of direct contact with infected wild cervids.

Table 3
Results from stratified univariable CMH analysis comparing case and control herds by distance to nearest detected CWD-positive wild cervid (≤ 5 km and > 5 km).

Variable	≤ 5 km OR	95% CI	p-value	> 5 km OR	95% CI	p-value	Adjusted OR	95% CI	Adjusted p-value
≤ 0.3 m distance of water source to fenceline	10.00	0.9–117.0	0.052	5.06	1.3–19.7	0.015	6.02	1.8–19.6	0.002
Imported from a herd that later tested positive for CWD	2.29	0.2–31.0	0.539	11.88	1.9–72.4	0.002	6.48	1.5–28.6	0.005

Table 4
Results from comparison of case and control herds using multivariable Firth logistic regression, GLMM, and Firth bootstrapping case-resampling models.

Variable	Model OR	Model 95% CI	Model p-value	GLMM[a] p-value	Bootstrap Mean OR	Bootstrap 95% CI
≤ 0.3 m distance of water source to fenceline	4.83	1.5–16.1	0.010	0.008	5.69	1.7–22.7
Imported from a herd that later tested positive for CWD	5.63	1.1–28.2	0.035	0.045	6.83	1.1–71.0
≤ 5 km distance to nearest detected CWD-positive wild cervid	4.10	1.1–15.2	0.035	0.042	4.77	1.2–22.1

[a] GLMM with State and Primary Herd Type as random effects

4.1. Direct contact with farmed cervids

This study identified that importing cervids from a herd that later tested positive for CWD poses a risk of CWD introduction through direct contact between an infected farmed cervid with susceptible farmed cervids. These movements of infected cervids are a risk for CWD introduction to a herd, which could later pose potential risks to wild cervids in the area surrounding the herd. CWD risks from farmed cervid movements have been well-documented in case investigations and a Canadian observational study (Argue et al., 2007), and state regulations are in place to prevent CWD transmission after detection in cervid herds. In the US, current mandatory routine surveillance for CWD involves testing adult farmed cervids after natural death, hunter-kill, or slaughtered (percentage of the latter two variable by state); these post-mortem test results through time accumulate to represent the overall CWD herd status of the herd. Even with this ongoing surveillance, however, the possibility exists that another herd may become infected with CWD by introducing farmed cervids from an infected herd not yet detected. In this study, only 32 % of the participating case herds had introduced cervids from herds later found to be infected. Introductions of farmed cervids onto or off other farmed cervid herds were not identified as associated with CWD-positive herd status, and the remaining case herds (68 %) that did not import cervids from a herd later detected as CWD-positive were likely infected through other transmission pathways.

4.2. Indirect contact with farmed cervids

No variables associated with indirect contact with farmed cervid transmission pathway were identified in this study. This included use of shared equipment, trailers, and artificial insemination or embryo transfer in the past 5 years. Semen has long been recognized as a potential source of transmission, and recently CWD prions have been detected in semen (Kramm et al., 2019). That study reported the presence of CWD prions in semen and reproductive tissues from naturally CWD-infected WTD bucks. Although prions were detected, further research is needed to determine if CWD can be transmitted by artificial insemination (Kramm et al., 2019).

4.3. Direct contact with wild cervids

Results from our study indicate marginally increased odds of CWD-positive herd status for producers who observed wild cervids along the fenceline in previous 12 months, did not inspect their fenceline daily, and used single perimeter fencing. It is not clear from this study, however, if these risks are through direct or indirect contact transmission. Results from one previous study showed the likelihood of direct nose-to-nose contacts between wild and farmed WTD occurs very infrequently. VerCauteren et al. (2007) examined direct nose-to-nose contact of wild

WTD and farmed WTD in a Michigan study of six WTD herds and found only 2 direct naso-oral contacts that occurred during $> 77,000$ h of camera monitoring of perimeter fences. That study suggests that, while theoretically an important risk, direct contact with wild cervids through perimeter fencing occurs minimally and other indirect methods should be examined. Double fencing, although expensive, provides a buffer zone from feed, water, and other attractants being placed along the outer perimeter fence, acting as a deterrent to other wild animals (e.g., scavengers) from easily gaining access to cervid pens. Factors not associated with CWD-positive herd status in our study included fencing breaches in the previous five years and wild cervids entering pens or farmed cervids escaping pens.

4.4. Indirect contact with wild cervids

Results from this study identified the potential importance of indirect contact pathways of CWD to cervid herds from infected wild deer. Proximity to CWD-infected cervids is a necessary component of indirect contacts, and in this study, 41 % of case and 19 % of control herds were located within 5 km of a detected CWD-positive wild cervid. The effect of this factor was even greater in multivariable regression models. This is an indication that both proximity to infected wild deer and other variables (e.g., presence of scavengers) may be necessary to convey risk to cervid herds.

Indirect contact transmission from infected wild deer to farm cervids, however, is little understood, and results from this study warrant additional investigation. CWD-positive cervids shed prions into the environment through feces, urine, and decaying tissues after death (unless the carcass is removed from the area by hunting or another method; (Miller et al., 2004). Plants have been shown to uptake prions from contaminated soil and transport them to the leaf and stem parts of the plant (Pritzkow et al., 2015). In one study, wheat grass sprayed with prion-contaminated excretions was found to be contaminated with prion material on leaves for at least 49 days after exposure (Pritzkow et al., 2015). Indirect spread of infectious prions from these areas may spread to susceptible cervids on herds through movement of scavengers feeding on infected carcasses, other wild animals, or harvesting feeds from this area.

Further, univariable results indicated that the presence of wild animals, cats, and other scavengers inside or near the perimeter fence was associated with CWD-positive herd status, as well as practices that may attract these animals to cervid pens. Carcasses disposed of via waste or compost piles above ground on the property, water or feed location, and land features may act as attractants to scavengers and wild animals. Coyotes and crows that ingest infected material may pass infectious prions in their feces, and it is plausible that other scavengers could also spread prions this way (VerCauteren et al., 2012; Nichols et al., 2015). Forested areas crossing and surrounding the fenceline may provide

shelter to wild animals and scavengers and access for these species to enter pens. Studies of landscape features in WI and Illinois identified that compact forests, lower elevations closer to rivers, and small forest patches increased the risk of CWD in wild cervid populations, and perhaps may increase risks to farmed cervids through also attracting other animals (O'Hara Ruiz et al., 2013; Rivera et al., 2019). Importantly, the congregation of wild cervids in forested areas may lead to environmental contamination in the areas surrounding the fenceline, providing an opportunity for wild animals acting as vectors to bring prions into the herd through fenceline.

Our study results indicate the potential importance of farmed cervid water location and source. Placing the water source next to the outer perimeter fenceline may act as an attractant to wild cervids or scavengers. In addition to location of water, 45 % of cases shared their running (e.g., creek, stream, river) or standing (e.g., pond, lake, drainage) water source with wild cervids, compared to 12 % of controls. In areas with CWD in wild populations, water may also serve as a route of transmission. Nichols et al. (2009) detected CWD prions from water samples in a CWD endemic area, which, although detected below the infectious dose in that study, showed potential risk of transmission of CWD through water sources. In this study, one water source with CWD prions was runoff from an area with CWD in the wild population, indicating that being downstream from a CWD endemic area may pose a higher risk then being upstream. Further research is needed to determine the risk water poses in CWD transmission, especially the relationship between the water flow and location of wild and farmed cervids.

4.5. Indirect contact with cervid carcasses

There were no variables in the indirect contact with cervid carcasses (both wild and belonging to other cervid farms) through hunting or taxidermy practices that were associated with herd status. These variables include bringing hunted cervid parts or carcasses onto the property, location of where these hunted cervid parts or carcasses came from, and performing taxidermy or other trophy display preparations on-site in the previous 5 years. The lack of significant variables in this pathway could be based on the limited number of herds who partake in hunting or taxidermy in our study population.

Identification of the most important factors related to transmission pathways in this study was challenging. Despite current challenges to herd recruitment related to sensitivities in the current CWD regulatory climate, we recruited voluntary participation from 71 cervid herds in the three study states, including 22 case (CWD-positive) herds and 49 control herds. Initially, 18 case and 53 control herds were identified within the three states, but during data collection, four control herds were confirmed as positive for CWD. These four newly detected herds were included as cases because they fit case definition; controls already in the study fit matching study criteria to serve as controls for these new cases. With a sample size of 71 herds, bias due to overfitting during multivariable logistic regression analysis and multicollinearity can occur. To address these issues, we used multiple analytic methods, including Firth penalization during logistic regression, and bootstrapping to assess the validity of the final biological model. Despite these methods, our ability to use multivariable methods to identify the most important risk factors was limited. We selected three variables for multivariable analyses based on statistical and biological transmission pathway relevance, which limited the statistical power to evaluate interactions with other variables. This model generated wide 95% CIs, which could be due to multicollinearity not identified between our variables and to relatively small sample size. Therefore, our primary focus for the study is on the univariable results which was used to help prioritize the most important transmission pathways.

While our study evaluated direct and indirect pathways of transmission, one study limitation was examination of specific risk factors at a set point in time and specific to the population of participating herds. We recognize that CWD may be transmitted through multiple potential pathways, and risk factors associated with our study herds may not be important in cervid herds in other locations and for other farmed species. Another limitation was slight differences in time frame for herd practices between case and control herds. During the data collection stage, case farm producers were asked about the time frame before they tested positive for CWD since after detected as positive, cervid herds were quarantined, and their herd management practices changed. This time frame adjustment could have resulted in recall bias from study participants, especially if cases were detected early in the study timeframe, as it could be challenging for producers to remember exactly what was occurring on their farm at that time. In addition, we recognize the possibility for changes in farm practices and the environment through time that could have contributed to measurement bias between case and control herds. However, in MN, the average time from date of CWD detection to date of interview was 2.7 years (median 2), PA average of 1.7 years (median 1.5), and in WI an average of 3.6 years (median 4). Overall, 60% of case herds in the study were detected after 2018, leading to a relatively short time difference between case and control herds in most cases.

Finally, we were not able to evaluate the role of genetic susceptibility to CWD of farmed deer as a confounding effect in this study, as genetic testing was performed in only 27.3 % of case herds and 30.6 % of control herds, and only three case and control herds each had tested > 75 % of their herd. Future studies may need to examine the potentially confounding role of genetic susceptibility in the association between CWD herd status and other herd factors.

In case-control studies, it is critical to select control herds carefully to ensure they are as similar to case herds as possible, other than for the study outcome (CWD-herd status) and exposures of interest (CWD transmission-related factors). We matched control to case herds by state, herd size, location within 112 km, and surveillance intensity. These types of studies evaluate associations between different risks and the outcome of interest, and magnitude of association is a key criterion for determining causation (Hill, 1965). While case-control studies have limitations (subject to bias and confounding, due to lack of study investigator control of exposures, lack of ability to evaluate temporality of associations, and other factors), these studies can be very useful to provide direction for future research. Our study results demonstrate the need for research into indirect transmission pathways from infected free-ranging deer to cervid herds, and specifically, the roles played by wild animals, water, and land cover involved.

5. Conclusion

In this study, we evaluated multiple factors involved with all currently known pathways of CWD transmission in a population of study herds in MN, PA, and WI. Based on results of this study, we conclude that CWD-positive herd status is associated with direct contact with infected farmed cervids (importing cervids from a herd that later tested positive for CWD) and multiple variables associated with indirect contact with infected wild cervids. This study provides justification to direct future research to better understand indirect pathways of CWD transmission from wild cervids to cervid herds. These results also support the ongoing efforts to control CWD in wild cervid populations, which will be beneficial in the control of CWD in farmed cervids. Improved understanding of the risks of CWD introduction to farmed cervids provides the opportunity for development of successful CWD biosecurity protocols to reduce the risk of CWD introduction to farmed cervid herds.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prevetmed.2023.106000.

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