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The demographic pattern of infection with chronic wasting disease in reindeer at an early epidemic stage

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Abstract. Infection patterns linked to age and sex are crucial to predict the population dynamic effects of diseases in long-lived species. How such demographic patterns of infection arise is often multifactorial, although the cause is commonly seen as a combination of immune status as well as variation in pathogen exposure. Prion diseases are particularly interesting, as they do not trigger an adaptive immune response; hence, differences in pathogen exposure linked to behavior could be the prime determinant of the pattern of infection. In cervids, the fatal prion disease, chronic wasting disease (CWD), is spreading geographically, with economic and cultural consequences in affected areas in North America, and all infected individuals eventually die from disease-associated sequelae if they live long enough. Understanding the causes of the demographic pattern of infection with CWD is therefore urgent but is limited by the fact that reported data primarily come from related deer species in North America. The recent (detected 2016) emergence of CWD among wild alpine reindeer (*Rangifer tarandus*) in Norway with a different social organization, that is, no home range behavior and no matrilineal female groups, offers an opportunity to advance our understanding of how behavior influences the infection patterns. Testing of 1081 males and 1278 females detected 19 animals positive for abnormal prion protein in brain and/or lymphatic tissues. No calves and only one male yearling were infected, with the remaining positives being adults (representing 1.5% of adult males and 0.5% of adult females). We found a strong sex-biased infection pattern in reindeer (with infection 2.7 times more likely in adult males), which is similar to the results reported in mule deer and white-tailed deer. The hazard of being detected as positive increased with age in males. There was no close genetic relatedness among positive animals. The results were consistent with the within-group contact of males being a possible major route of transmission. We discuss the demographic pattern of infection with CWD in view of the lack of stable home range behavior and other key behavioral traits of reindeer relevant to understanding pathogen exposure in general.

Key words: direct contact; disease ecology; environmental reservoirs; pathogen exposure; population dynamics; prions; social organization; transmission.

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Introduction

Parasites and pathogens are usually unevenly distributed among individuals in a population. Physiological and behavioral differences are expected to be major drivers of the skewed appearance of many diseases (Guerra-Silveira and Abad-Franch 2013). Differential investment in and development of parts of the immune system can create age- and sex-specific patterns of infection. A common pattern among mammals is higher infection levels in males compared to females (Schalk and Forbes 1997, Córdoba-Aguilar and Munguía-Steyer 2013, Metcalf and Graham 2018) and in the young and senescent compared to prime-aged individuals (Hayward et al. 2011, Abolins et al. 2018, Benton et al. 2018). However, there are many exceptions to these main demographic infection patterns (Vicente et al. 2007, Smyth and Drea 2016, Sparks et al. 2018). Differences in behavior and contact rates affect the likelihood of pathogen exposure, and such variability differs predictably between sexes and age classes (Smyth and Drea 2016, Silk et al. 2018). It is therefore often difficult to unravel the relative role of variation in immune defenses and pathogen exposure in the demographic patterns of disease infection.

Prion diseases are a particularly interesting group of diseases in this context, as prions do not trigger an adaptive immune response (Prusiner 1998). Chronic wasting disease (CWD) is a fatal neurodegenerative prion disease affecting cervids (Williams and Young 1992). Hence, the demographic pattern of CWD can shed light on the role of pathogen exposure as a basis upon which to understand demographic infection patterns of wildlife diseases in general. Chronic wasting disease was first observed in captive mule deer (*Odocoileus hemionus*) in the late 1960s in Colorado, USA. Chronic wasting disease has since spread to 26 states in the United States and reached three provinces of Canada. All individuals infected with CWD eventually die from the disease if they live long enough. The CWD epidemic has come to the point of causing population declines in white-tailed deer (*Odocoileus virginianus*; Edmunds et al. 2016) and mule deer (DeVivo et al. 2017) in some well-studied endemic areas. Understanding its demographic pattern of infection is crucial to understanding the population dynamic impact of CWD (Potapov et al. 2012, Samuel and Storm 2016). Further, the demographic pattern of infection can shed light on the mode of transmission (Potapov et al. 2013) and hence provide keys to mitigation.

There is a strong age-specific pattern of CWD infection (Samuel and Storm 2016). Calves are rarely found infected, and yearlings have less than half the chance of infection relative to that of adults (Miller and Conner 2005, Heisey et al. 2010, Samuel and Storm 2016). Chronic wasting disease has an incubation period of 1.5–2.5 yr in mule deer (Fox et al. 2006) and 2–5 yr in elk (*Cervus canadensis*), depending on the prion protein gene (PRNP) polymorphism (Moore et al. 2018). The lower infection prevalence in young animals probably results from the shorter time at risk of exposure combined with the lag between the time of prion infection and detection by standard diagnostic tests (Viljugrein et al. 2019). In both mule deer (Miller and Conner 2005) and white-tailed deer (Heisey et al. 2010), the prevalence of CWD was 2–3 times higher in males compared to females. Most likely, the sex effect is mainly driven by differences in pathogen exposure (Potapov et al. 2015) and, therefore, strongly depends on the social organization or behavior of a given species. However, our understanding of how the sex-specific infection pattern arises is limited by the fact that the data come from two closely related species, mule deer and white-tailed deer, while the most detailed demographic CWD infection studies of elk do not include males (Sargeant et al. 2011, Monello et al. 2014).

In 2016, the first natural cases of CWD in reindeer (*Rangifer tarandus*) and in Europe were reported (Benestad et al. 2016). The different social organizations of reindeer compared to other cervids offer a unique opportunity to learn more about the factors causing the demographic infection pattern of CWD and the general role of pathogen exposure. The lack of both matrilineal grouping and stable home range behavior in reindeer can shed light on the possible transmission routes and infection pattern. The population was surveyed for CWD during annual hunts in 2016 and 2017 and during population eradication that finished in April 2018 (Mysterud and Rolandsen

2018). We herein report the sex and age distribution of the reindeer positive for the abnormal prion protein (PrP^{Sc}) relative to the demographic composition of the population, and we estimate the genetic relatedness of positive individuals relative to the rest of the population. We test whether there is a sex bias in infection probability, as seen in mule deer and white-tailed deer, and whether infection probability increase with age among adults. If mother–offspring is the main route of transmission, we predict many infected individuals of 1.5–2.5 yr old, a time similar to the anticipated incubation period, and closer genetic similarity among the positives than expected from random in the population. Mule deer and white-tailed deer form matrilineal groups, resulting in higher infection levels among closely related females. In contrast, reindeer do not form similar matrilineal groups and relatedness is unlikely to increase horizontal contact rates required for prion transmission. We hence predict no stronger genetic relatedness among positive reindeer females than for a random sample of the population.

Materials and Methods

The study area

The data derive from the Nordfjella wild reindeer management area in the counties Sogn & Fjordane and Buskerud, Norway (between 60°37′–61°02′N and 07°14′–8°59′E). The Nordfjella area comprises a northern territory (zone 1) of approximately 2000 km^2 and a southern territory (zone 2) of approximately 1000 km^2, parted mainly due to a road (FV50 Hol-Aurland). Chronic wasting disease has only been detected in zone 1. The Nordfjella Mountains have a steep and rugged terrain. Most of the area is in the mid- and high-alpine zones above 1500 m a.s.l., with peaks extending to 1900 m a.s.l. This mountain range has a harsh and volatile climate due to the high elevation and it being situated on a climatic divide with a strong coastal influence in the west (wetter and warmer) and more of an inland climate in the east (drier and colder). The tree line is at approximately 800–1000 m a.s.l. The reindeer are alpine but occasionally use the surrounding birch (*Betula* spp.) forest, in particular during spring and early summer. During summer, over 60,000 domestic sheep (*Ovis aries*) graze in the area (VKM et al. 2018). Red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), and moose (*Alces alces*) use the surrounding forests and, occasionally, the alpine habitat.

Reindeer data: sampling

In total, 2424 reindeer were tested for CWD in the Nordfjella reindeer management area, zone 1, in the period of March 2016–May 2018 (Appendix S1: Table S1). We excluded animals with unknown sex (n = 94) and/or unknown age class (n = 68) leaving 2365 reindeer (1085 males and 1280 females) for analysis (Table 1). The data originate from (1) hunting in 2016 (20 August–30 September), (2) extended hunting in 2017 (10 August–30 October), (3) culling (7 November 2017–1 May 2018) performed by marksmen, and (4) fallen stock from the index case in March 2016 to the last animal removal in May 2018 (Appendix S1: Table S1). Tissue samples for CWD testing were brain, as the *medulla oblongata*, and lymph nodes, which were mainly retropharyngeal (RLN), but in a few instances, mandibular lymph nodes or tonsil tissue was used. Hunters provided reindeer heads to be sampled by trained veterinarians, or in cases of culling, sampling was performed by the marksmen. Personnel sampling the tissues also provided jaws with teeth for age determination.

Testing for CWD

All brain and lymph node samples were sent to the Norwegian Veterinary Institute in Oslo for CWD testing. The primary test was an ELISA (TeSeE ELISA SAP; Bio-Rad, Hercules, California, USA) for the detection of PrP^{Sc}, hereafter designated prions. A positive or inconclusive result was confirmed by Western blot testing (TeSeE Western Blot, Bio-Rad). The analytical test sensitivity of the ELISA was evaluated by Hibler et al. (2003) to be 92.5% (81.8–97.9) for the obex (part of the brainstem) and 98.8% (93.5–99.97) for the RLN compared to immunohistochemistry of the same tissues. The analytical tests have close to perfect specificity (European Food Safety Authority [EFSA] 2005). Due to economic and logistical constraints, samples of RLN and brain tissue from the same individual were pooled in primary testing, slightly lowering the test sensitivity for RLN. More profound variation in the diagnostic sensitivity is due to individual variation in the stage of infection (Viljugrein et al. 2019).

Table 1. An overview of reindeer with known sex and age tested for CWD, by the presence of PrP^{Sc}, during the epidemic outbreak in the Nordfjella reindeer management area, zone 1, in Norway, 2016–18.

		Age																
Source	Sex	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Unknown	Sum
Hunt 2016	Males	40	13	16	19	13	15	3	5	3				1			1	21	150
	Females	36	20	35	20	15	6	9	8	7	6	4	1	2	1			3	173
Hunt 2017	Males	67	36	73	41	25	25	18	19	9	1	5		2				3	324
	Females	45	19	39	35	32	20	11	21	10	5	9	1	4	2	1	1	2	257
Marksmen 2017–18	Males	133	100	68	74	46	20	17	9	9	3	2						71	552
	Females	157	122	94	88	67	45	36	28	18	23	6	6	2	2	1	1	143	839
Fallen stock 2016–18	Males	1	1		1	3	1											48	55
	Females		3		1													5	9
Sum	Males	241	150	157	135	87	61	38	33	21	4	7		3			1	143	1081
	Females	238	164	168	144	114	71	56	57	35	34	19	8	8	5	2	2	153	1278
PrP^{Sc} positives	Males		1	2	3	2	2	1	1	1									13
	Females				3	3													6

Notes: In 2016, animals were not marked with zone and there may be included up to 35 hunted animals in 2016 from Nordfjella reindeer management area, zone 2. Note that exact age will differ depending on time of harvest. There were excluded 100 animals due to missing information on sex and/or age class, see Appendix S1: Table S1. For 87 of 479 calves (age 0) and 33 of 270 yearlings (age 1), the age class had been determined by the hunter and not been confirmed by official age determination.

Determination of the age

The standard procedure for aging reindeer in population surveillance programs at the Norwegian Institute for Nature Research is to separate calves and yearlings from older reindeer by tooth eruption patterns, while counting of cementum annuli in stained tooth sections is used to age older reindeer (Hamlin et al. 2000). In cases of uncertain counts of cementum annuli, a qualitative judgment of the mandible including the dentition pattern and wear is also used as a guide to ascertain the most likely correct age (Solberg et al. 2017). For the hunter harvest, half of the mandible was available. For the marksmen culling, only the incisive part was extracted and not the whole mandible. This may have made the separation of yearlings from older reindeer less accurate, but we do not expect any systematic over- or underestimation of age. We also note that the index case was aged based on tooth eruption and wear (as we did not receive the incisors from this reindeer) to be above 2.5 yr, probably 3–4 yr old (Benestad et al. 2016), and was thus concluded as 3 yr old in Table 1 and in the analysis. Age data were lacking from individuals found dead or injured (Appendix S1: Table S1).

Microsatellite marker analysis

Genomic DNA was isolated from brain samples from 19 cases and 41 controls using the DNeasy Blood and Tissue Kit (Qiagen, Oslo, Norway) as indicated by the manufacturer's protocol. Samples were analyzed for 18 microsatellite loci: NVHRT01, NVHRT03, NVHRT16, NVHRT31, NVHRT48, NVHRT66, NVHRT73, BM4513, BM6506, Oheq, DeerC89*, RT 1, RT 7, RT 9, RT 27, RT 30, OarFCB193, and MAF46 (Appendix S1: Table S2). The microsatellites were amplified in six-multiplex PCR using fluorescent-labeled forward primers (Appendix S1: Table S2). Each PCR contained 1.0 μL of genomic DNA as a template, 1 μL of dNTPs mix (4 × 2.5 mmol/L, VWR), 3 pmol of forward and reverse primers, 1.0 μL of Key Buffer (15 mmol/L $MgCl_2$, VWR), 0.05 μL of Taq DNA polymerase (5 U/μL, VWR), and purified water to a 10 μL volume. PCR conditions were set as an initial denaturation at 95°C for 2 min; then 26 amplification cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s; and finally, extension at 72°C for 10 min. The multiplex PCR products were pooled into three panels and run individually in a 3500xL Genetic Analyzer (Applied Biosystems, Schwerte, Germany). The fragment peaks were scored with GeneMapper version 5.0 (Applied Biosystems).

GenAlEx 6.5 (Peakall and Smouse 2012) and Arlequin v3.5 (Excoffier and Lischer 2010) were used to estimate genetic variation within and between cases and controls. The related package in R (Pew et al. 2015) was used to calculate the pairwise genetic relatedness between individuals

by Lynch and Ritland's (LR) method (Lynch and Ritland 1999). We tested whether individuals within cases and controls were more related than was randomly expected by permuting individuals between groups in 1000 iterations.

Statistical analysis

We used logistic regression to test for age- and sex-specific patterns of CWD infection in R vs. 3.5.1 (R Development Core Team 2018), using only data with known sex and age information (Table 1). Due to the low number of cases and slow epidemic development of CWD, we pooled data across years and included only adults (≥2 yr). We used the Akaike information criterion (AIC) to compare models.

We also ran a Cox regression (proportional-hazards regression) to estimate age- and sex-specific patterns of CWD infection. Cox regression is the most widely used method for modeling the relationship of covariates to a survival outcome (Therneau and Grambsch 2000). An advantage of the Cox model over ordinary regression models is that the inference procedures can easily handle right-censored responses, that is, cases in which individuals are removed from the study population before the event is observed. The coefficients in a Cox regression relate to hazard—a positive coefficient indicates a worse prognosis (shorter time to the event), and a negative coefficient indicates a protective effect of the variable with which it is associated, which, in our case, is the hazard of becoming infected. The hazard ratio associated with a predictor variable (multiplicative change in risk) is given by the exponent of its coefficient. We set the starting point of the observation period to the first month after the index case was reported (April 2016), as all animals in the population that were found dead or hunted were tested for PrP^{Sc} after the index case. The index case was therefore not included in the Cox regression. The study ended when the whole population was terminated and the last fallen stock from avalanches was tested (14 May 2018). Through the recruitment of calves, new animals were included in the study population during the time of study. In the Cox regression, we only included tested animals with known ages older than calves (Table 1). The potential covariates included were sex, age class (calves vs. yearlings or adults at the start of the study), and/or age (in years). Age class or age was included as the value the individual had at the time of inclusion in the study. In this analysis, we were assuming a calving date of 15 May for changing from one age/age class to another (Reimers 2002). To check for consistency, we repeated the analysis on the extended data set including 341 tested animals with known age classes but missing information on exact age. When age information was lacking for adults tested in 2017 or from the marksmen culling in 2017–2018, age classes at the time of inclusion in the study were imputed based on the category corresponding to the mean age of adults tested in 2017 or in the marksmen culling in 2017–2018.

Apparent (observed) prevalence is the proportion of animals from a representative sample of the population that are positive with the diagnostic method used (see Testing for CWD). Infected cases were modeled according to the hypergeometric distribution to obtain credibility intervals for the apparent prevalence. Population sizes were set to the total numbers hunted or found dead from the start of the hunting season in 2017 to the end of the marksmen culling. For this period, all adults, except 20–30 males and females, were registered tested at the Norwegian Veterinary Institute. Animals with unknown age class were distributed according to the population proportion of animals with known age classes. With perfect test specificity, true prevalence equals apparent prevalence divided by test sensitivity. We used a Bayesian framework to estimate the true (informed) prevalence from the apparent prevalence, taking into account the modeled diagnostic test sensitivity being dependent on stage of infection (Viljugrein et al. 2019). By simulating infected individuals of each age class with a random stage of infection, the modeled test sensitivity becomes a stochastic distribution and is dependent on assumed development of infection, the length of the incubation period (set as 2 yr), and tissue sampling regime (for details see Viljugrein et al. 2019). The stochastic distribution of the test sensitivity was accounted for by running our model in jags with r-package R2jags.

Results

Demographic infection pattern

A total of 19 animals with PrP^{Sc} out of 2359 tested reindeer were detected from 2016 to 2018

(Table 1; Appendix S1: Table S1). No calves and only one male yearling were found to be infected. The Bayesian apparent prevalence was 1.6% (95% credibility interval [CI] 1.4%, 1.8%) in adult males and 0.5% (95% CI 0.5%, 0.7%) in adult females in the last period from 10 August 2017 to 1 May 2018. The true prevalence that accounts for imperfect detectability with the given test regime was estimated as 1.8% (95% CI 1.5, 2.6) in adult males and 0.6% (95% CI 0.5%, 0.9%) in adult females. There was a strong male bias among infected reindeer, with 68.4% (13) being males and 31.6% (6) being females despite testing more females overall. Infection was detected among adult males of up to 8 yr of age (3.0% of males ≥5 yr old infected), whereas there was no positive among females 5 yr or older (Table 1). Among adults of known age (n = 1270), the logistic regression model confirmed that males were 2.7 (95% CI 1.0, 7.2) times more likely to test positive for PrP^{Sc} than were females (Z = 1.96, P = 0.05). The best-fit model only included sex and had a weight of evidence superior to the sex+age and age*sex models (Table 2).

The Cox proportional-hazards model confirmed effects of the sex and age categories on the hazard of being tested positive (Fig. 1A, Table 3, n = 1583). The hazard rate was approximately five times higher for males compared to females. The hazard rate of testing PrP^{Sc}-positive was higher for individuals who were already adults in the spring of 2016 compared to that of individuals maturing into the adult age class later in the study period. This may reflect that some adults were infected already at the onset of the observation period. The main results were robust upon extending the analysis to known age class (n = 1879). The model selection supported the inclusion of an age category term (Table 2). Models with ages in years or with an interaction term for sex and age in years resulted in less parsimonious models

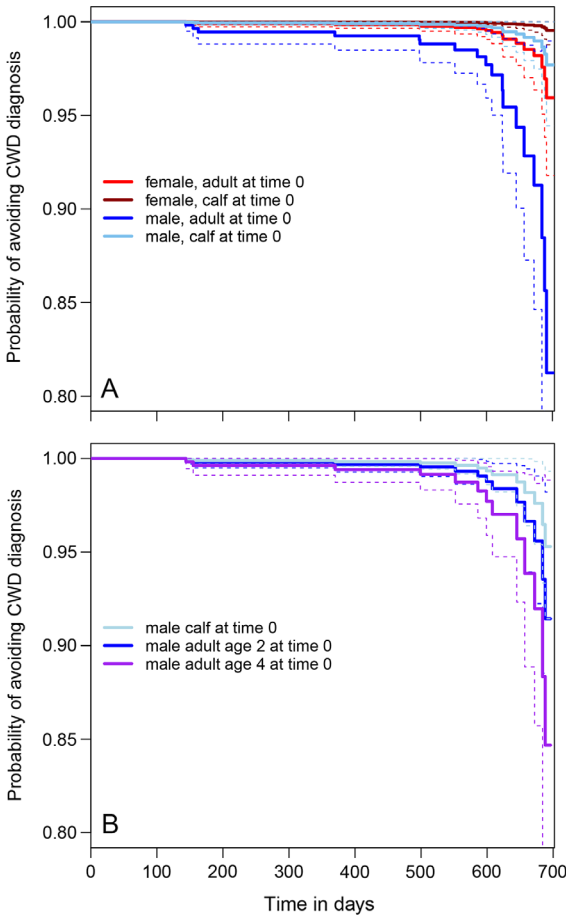

Fig. 1. The hazard of being tested PrP^{Sc}-positive of (A) male and female reindeer and (B) males of increasing ages from the Nordfjella population, zone 1, Norway, based on Cox regression models. The hazard was (A) higher in males than in females and (B) increased with age for males.

Table 2. Model selection with Akaike's information criterion (AIC) using logistic regression and Cox regression models to determine the age- and sex-specific pattern of CWD infection in reindeer from the Nordfjella reindeer area, zone 1, Norway, 2016–18.

Model parameters	AIC	ΔAIC
Logistic regression model		
Sex	188.85	0
Sex + age cat	190.78	1.93
Sex + age cat +sex:age cat	190.91	2.06
Cox proportional-hazards model		
Sex	225.30	7.01
Sex + age cat	218.29	0
Sex + age in years	221.89	3.60
Sex + age in years + sex:age	222.41	4.12
Age in years + (age in years)^2	217.69	−0.60
Males only		
Age in years	138.81	0
Age cat	141.10	2.29

Note: Age cat is age category (calf, yearling, and adult).

Table 3. Parameter estimates from the best Cox regression model of the hazard of being tested PrP^{Sc}-positive among reindeer from the Nordfjella reindeer area, zone 1, Norway, 2016–18.

Parameter	Coef.	SE (coef)	exp(coef)	Lower 0.95	Upper 0.95	*Z*	*P*
All							
Sex (male vs. female)	1.611	0.535	5.01	1.75	14.3	3.01	0.002
Yearlings vs. calves	1.571	0.871	4.81	0.87	26.5	1.80	0.071
Adults vs. calves	2.185	0.791	8.89	1.89	41.9	2.76	0.006
Males only							
Age in years	0.310	0.104	1.36	1.11	1.67	2.98	0.003

Note: The age parameter refers to the age class or age at the start of the study.

(Table 2). A model including age^2 was competitive ($\Delta AIC = -0.60$) but was only driven by the infected young females and did not fit the data for males. In a model on the male subset of the population, the hazard of testing PrP^{Sc}-positive over time increased with age (Fig. 1B, Table 3).

Genetic relatedness

The mean number of alleles was 6.4 (SE = 0.40) among the PrP^{Sc}-positive ($n = 19$) and 7.0 (SE = 0.51) among the PrP^{Sc}-negative ($n = 41$) reindeer. The mean observed heterozygosity was 0.722 (SE = 0.037) and 0.741 (SE = 0.025) for the two groups, respectively, while the mean expected heterozygosity was 0.741 (SE = 0.023) and 0.756 (SE = 0.018). There was no difference in genetic variation between the samples of positives and negatives ($F_{ST} = 0.000$, $P = 0.65$). The variation in relatedness estimators was similar for the cases ($n = 171$, mean = −0.022, SE = 0.007) and controls ($n = 820$, mean = −0.018, SE = 0.003). The mean relatedness within the groups was not higher than expected for the cases ($P < 0.959$) or for the controls ($P < 0.667$). The two positives with the closest genetic relatedness (0.388) differed in two microsatellite loci. These loci did not share alleles and were heterozygous in both animals, which suggests that these individuals could not have a parent–offspring relationship.

Discussion

The emergence of CWD in reindeer, which have a social organization contrasting from that of mule deer, white-tailed deer, and elk, offers an opportunity to learn more about how behavioral differences in pathogen exposure affect infection patterns. We found a 2.7-time higher infection rate in adult males compared to adult female reindeer, which is similar to the results reported in most mule deer and white-tailed deer populations (Miller and Conner 2005, Heisey et al. 2010, Rees et al. 2012, Samuel and Storm 2016). The current observations were consistent with frequent transmission in male–male groups at this expected early epidemic stage.

Demographic patterns of infection

Prion diseases, by the absence of an adaptive immune response, represent a rare case of how the demographic pattern of infection can arise from differences in pathogen exposure. For CWD, absent or low infection prevalence in calves and markedly lower infection prevalence in yearlings compared to adults have been documented for mule deer (Miller and Conner 2005), white-tailed deer (Heisey et al. 2010, Samuel and Storm 2016), and elk (Robinson et al. 2012, Monello et al. 2014, 2017). Our results in reindeer support this main pattern of prevalence across age classes. Prions are not detectable in early infection stages. The pattern of infection across age classes likely arises due to differences in the prion exposure period since birth and the long incubation period of the infection before it can be detected. Prevalence levels often continue to increase moderately with age in the adult stage, in particular, for males (Samuel and Storm 2016). A decline in infection among the oldest males was reported in both white-tailed deer and mule deer in Saskatchewan, Canada (Rees et al. 2012). We found an increasing hazard of becoming PrP^{Sc} infected with age in adult reindeer males (Fig. 1B).

All six PrP^{Sc}-positive females were 3–4 yr of age; however, females 5 yr and older comprised 41.0% of the adult (≥2 yr) females in the population. This clustering of infection in young adult females may be a random event, as the age and

sex interaction was not significant. Nevertheless, since the infected reindeer females were all 3–4 yr old, they were not old enough to be mothers of most PrP^{Sc} positives. Any such mother–offspring relations were denied by the confirmed lack of close genetic relatedness among the cases. We cannot exclude the possibility that older infected females died before sampling, but the low relatedness, together with the age distribution of all positives, suggests that mother–offspring contacts were not the main mode of transmission. Mother–offspring (vertical) transmission of CWD has been experimentally proven in muntjac (*Muntiacus reevesi*; Nalls et al. 2013), but horizontal transmission is regarded as the main mode of transmission among North American deer under natural conditions (Miller and Williams 2003). The PrP^{Sc}-positive prevalence of 1.5% in males and 0.5% in females indicates an early epidemic stage, and the demographic pattern of infection is consistent with mainly horizontal transmission of CWD.

Infection pattern and mode of transmission

Understanding transmission routes is critical for disease management, but establishing this information for CWD has proven difficult due to both direct transmission from animal to animal by contact with saliva, urine, or feces (Mathiason et al. 2006) and indirect transmission through environmental contamination (Miller et al. 2004). Direct contact is assumed as the main transmission route in the early epidemic stages of CWD and likely plays a near-constant role following behavior, throughout an epidemic, while environmental contamination becomes more important and increases transmission rates in later epidemic stages (Almberg et al. 2011). Female reindeer live in much larger groups than do males during the seasons in which they are sexually segregated. In the affected Nordfjella reindeer population in Norway, female groups were often in the range of 100–200, while male groups rarely exceeded 20–40 individuals. Hence, the broad levels of sociality and group sizes were poor predictors of the demographic infection pattern, suggesting that environmental contamination was not the primary mode of transmission, as expected in an early epidemic stage.

A largely unresolved issue in the CWD literature is the cause of the approximately 2–3 times higher infection prevalence in adult males than in females. This pattern was reported for white-tailed deer in Wisconsin (Heisey et al. 2010, Jennelle et al. 2014, Samuel and Storm 2016) and Illinois (Samuel and Storm 2016), for mule deer in Colorado (Miller and Conner 2005, Miller et al. 2008, Wolfe et al. 2018) and Wyoming (DeVivo et al. 2017), and for mule deer and white-tailed deer pooled in Saskatchewan, Canada (Rees et al. 2012). Female white-tailed and mule deer form matrilineal groups with stable home ranges, with minimum overlap with other matrilineal groups. There was a higher prevalence of CWD among genetically related females in the matrilineal social groups of both white-tailed deer (Grear et al. 2010) and mule deer (Cullingham et al. 2011) compared to unrelated females. The higher prevalence in adult males could be explained by males visiting many groups of females, increasing the overall likelihood of visiting an infected group (Grear et al. 2010). Reindeer are an interesting contrast, as they do not form matrilineal groups, nor do they use stable home ranges. Rather, their space use is characterized as being nomadic in large groups of related and nonrelated individuals. Hence, the strong sex bias in infection that also occurred in reindeer with this different spatial organization suggests that direct contact rates may be sufficiently frequent to yield a sex bias in CWD infection. The strongest associations in mule deer were among males pre-rut and between males and females during rut (Mejía-Salazar et al. 2017). Direct contact in the form required for pathogen transfer is most likely during male–male combat and female–male courtship (Potapov et al. 2013).

Interestingly, though the data published to date are limited, there appeared to be no sex bias in CWD infection in elk (Sargeant et al. 2011, Monello et al. 2014). The reasons for this are uncertain; however, one possible explanation is that environmental transmission may play a more important role in locations where sexual segregation is rather low and densities are high, such as winter ranges where elk may rut and spend the majority of the year (R. Monello, *personal communication*). Another main exception was white-tailed deer in Wyoming, with 28% of males and 42% of females being positive for CWD (Edmunds et al. 2016), suggestive that more environmental transmission in late

epidemic stages may erode the sex-specific infection in some areas.

Implications of the demographic infection pattern

Male-biased infections have several important implications. In polygynous species, males are not limiting for population growth unless sex ratios become extremely skewed (Mysterud et al. 2002). Any causes of mortality affecting adult females, however, are likely to have a strong impact on population growth (Gaillard et al. 1998). Therefore, the demographic patterns of parasites and disease may influence their impact on population dynamics (Miller et al. 2007). For CWD, the dynamic impact will be most strongly linked to the lowered survival of adult females. Infected females reproduce at close to normal rates until the late disease stages (Dulberger et al. 2010, Blanchong et al. 2012); hence, the effect of CWD on reproduction is expected to have a weaker impact on population dynamics. On the downside, males have a wider space use, and the male-biased infection may increase the chances of geographic spread. The harvesting of males can lead to more stable population dynamics under the threat of CWD (Jennelle et al. 2014), increase disease detection, and limit the risk of geographic spread (Lang and Blanchong 2012). This insight can guide the harvest management of adjacent populations with uncertain disease status in Norway and elsewhere.

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