



Fluke abundance versus host age for an invasive trematode (*Dicrocoelium dendriticum*) of sympatric elk and beef cattle in southeastern Alberta, Canada



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ABSTRACT

Epidemiological parameters such as transmission rate, rate of parasite-induced host mortality, and rate of development of host defenses can be assessed indirectly by characterizing the manner in which parasite burdens change with host age. For parasites that are host generalists, estimates of these important parameters may be host-species dependent. In a cross-sectional study, we determined age-abundance profiles of infection in samples of sympatric free-ranging elk and domestic cattle infected with the lancet liver fluke, *Dicrocoelium dendriticum*. This parasite was introduced into Cypress Hills Provincial Park in southeastern Alberta, Canada in the mid 1990s, and now occurs in 60–90% of co-grazing elk and beef cattle examined at necropsy. The livers of 173 elk were made available by hunters during the 1997–2011 hunting seasons and livers from 35 cattle were purchased from ranchers. In elk, median worm abundance peaked in 6–24 month-olds (median = 72, range = 0–1006) then significantly declined to <10 worms/host in 10–16 year olds. The decline in fluke burden with age is not consistent with an age-related decline in exposure to metacercariae in intermediate hosts and high rates of fluke-induced host mortality are unlikely. Rather, the pattern of peak fluke burdens in elk calves and juveniles, followed by a decline in older animals is consistent with the development of a protective immune response in older hosts. There was no pattern of worm accumulation or decline in sympatric cattle, although statistical power to detect a significant effect was low. These results highlight the complexity and context-dependent nature of epidemiological processes in multi-host systems.

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1. Introduction

Heterogeneity in parasite burdens within and among host populations is one of the hallmarks of ecological epidemiology. Even under laboratory conditions where features such as parasite exposure rate, host immune status, host condition, and environmental characteristics are tightly controlled, heterogeneity in parasite burdens between hosts is often very high (Hudson and Dobson, 1995; Tompkins et al., 2011; Wilson et al., 2002). Not surprisingly, when among-host heterogeneity is evaluated within naturally-occurring host–parasite interactions, differences between hosts can vary by several orders of magnitude. In these complex, natural systems, epidemiological parameters such as transmission rate, rate of parasite-induced host mortality, and rate of development of host defenses

can be assessed indirectly by characterizing the manner in which parasite burdens change with host age. Thus, the determination of the relationship between host age and parasite abundance has important epidemiological and management implications (Boag et al., 2001; Hudson and Dobson, 1995). These implications can be expected to be especially important for generalist parasites that are shared among sympatric host species, especially for those species of parasite that have been introduced outside their native range. Yet our understanding of general epidemiological processes, including the relationships between host age and parasite burden, are poorly understood for generalist parasites in multi-host systems (reviews by Tompkins et al., 2011; Wilson et al., 2002).

The lancet liver fluke, *Dicrocoelium dendriticum* (Trematoda: Dicrocoeliidae), is a parasite of the bile ducts of grazing mammals. Evidence from experimental exposures of domestic stock to metacercariae (review by Manga-González and González-Lanza, 2005), together with molecular sequencing of worms collected from several species of domestic and wildlife hosts (van Paridon et al., unpublished observations), have confirmed that *D. dendriticum* is a host

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generalist. This species has been widely introduced outside its native range in mainland Europe, including into various locations in western and eastern North America (Goater and Colwell, 2007; Lewis, 1974; Mapes, 1951). In Cypress Hills Interprovincial Park (CHP) in south-eastern Alberta, where *D. dendriticum* was introduced in approximately the mid 1990s, prevalence now ranges from 60% to 90% in sympatric deer, elk, and beef cattle each year, with intensities frequently exceeding 1000 worms per host (Goater and Colwell, 2007). In samples of hosts from this location, the opportunity exists to evaluate the association between host age and worm abundance in different host species within a region of known sympatry. The purpose of this study is to evaluate relationship between host age and *D. dendriticum* abundance in sympatric free-ranging elk and cattle in CHP.

2. Materials and methods

2.1. Cypress Hills Interprovincial Park

The Park straddles the southern Alberta-Saskatchewan border in south western Canada (49°37.5'N, 110°W). It is a complex habitat mosaic that encompasses two distinct ecological sub-regions embedded within the extensive 'Grassland Natural Region' (Downing and Pettapiece, 2006). Vegetation characteristic of the mixed grass natural sub-region is dominant in sites below approximately 1100 m in CHP, including extensive stands of plains rough fescue, western porcupine grass, sedges, and various forbs. At elevations >1100 m, vegetation characteristic of the montane natural subregion dominates, with characteristic grasses and forbs, interspersed with stands of lodgepole pine (*Pinus contorta*), white spruce (*Picea glauca*), trembling aspen (*Populus tremuloides*), and balsam poplar (*P. balsamifera*). Together, the combination of these two sub-regions that co-occur within the relatively narrow confines of the Park (531,000 hectares) creates an island-like habitat that is distinctive from the adjacent and highly cultivated lowland grasslands (the extensive dry mixed grass natural subregion that is characteristically known as 'prairie'). Regions of the park above approximately 1250 m were unglaciated by the Wisconsinan Laurentide Ice Sheet (Stalker, 1965).

The community of sympatric large herbivores on the Alberta side of the CHP includes approximately 700 elk, 300 mule deer (*Odocoileus hemionus*), 300 white-tailed deer (*Odocoileus virginianus*), and 4000 beef cattle (Alberta Tourism, Parks and Recreation, 2013). Elk (*Cervus canadensis*) were re-introduced into the Park in 1938. Annual fall hunts have been regulated and monitored by Park's personnel since 1978 to establish a density of 350–700 resident elk. Beef cattle have grazed within almost all regions of the Park since 1918, typically grazing cow-calf pairs or yearlings that are introduced in early June and removed in late October (Hegel et al., 2009). Upon removal from the Park cattle receive a dose of broad-spectrum antiparasitic drugs (i.e. ivermectin, moxidectin) which are ineffective against *Dicrocoelium* infection (Ballweber and Baeten, 2012).

2.2. Worm counts

Opportunistic collections of whole elk livers from hunters began in autumn 1997 and continued haphazardly until 2005. Collections occurred annually, each fall, from 2009 to 2011. During each hunting season, data on kill site, gender, and approximate age (calves ≤ 6 months, juveniles = 6–24 months, adults ≥ 24 months) are collected by Park's personnel for all elk shot within the Park. Calf, juvenile, and adult cattle were purchased opportunistically from two of the three CHP stock associations between 2003 and 2013. All sampled cattle grazed within the park from early June to late October each year since birth. Data on host age and sex were made available from stockowners.

Livers from elk were harvested within 4 h of host death and frozen whole; cattle livers were examined immediately after slaughter. The dissection of individual livers for enumeration of worms

followed methods described by Goater and Colwell (2007). Each liver was thawed, weighed, and cut into approximately 5-mm-wide strips. The sections were placed into saline-filled pans and gently palpated to dislodge flukes from the hepatic and bile ducts. Once all sections were processed, the pans were examined for the presence of flukes. Flukes were enumerated using a dissection microscope and then preserved in ethanol.

2.3. Analyses

We follow the terminology of Bush et al. (1997) to describe parasitological parameters. Prevalence is the proportion of infected hosts within a sample of hosts and abundance is the total number of parasites in an infected host, including those that were uninfected. We used mean or median abundance to describe the central tendency of worm counts within a sample of hosts. Due to limited sample sizes associated with the opportunistic sampling program, analyses involved data pooled between years. The distribution of worms within a sample of hosts was estimated by exponent k of the negative binomial distribution (NBD) using Quantitative Parasitology 3.0 (Reiczigel and Rozsa, 2005). The overall distributions of worms within the samples of elk and cattle were highly aggregated, and worm counts could not be transformed to normality. Thus, for comparison of worm counts between pairs of samples, we used non-parametric Mann-Whitney U tests. Kruskal-Wallis tests were used to evaluate differences in medians between multiple samples.

The initial analysis of the association between host age, liver weight, and fluke abundance focused on 61 elk livers collected during the 2009–2011 hunting seasons. These hosts were aged by evaluation of the cementum of a single I-1-lower incisor tooth per animal (Matson, 1981). Previous studies have confirmed that this method is the most accurate for ageing elk and other grazing ungulates (Hamlin et al., 2000). Ageing was completed in a commercial laboratory (Matson Laboratories Inc., Missoula, Montana) by two independent technicians that were blind relative to the source and identity of each animal. There was no significant difference in median abundance in relation to host gender (number of males = 115, number of females = 51; $p = 0.705$) so the genders were pooled for all subsequent analyses unless stated otherwise.

For analyses, data on the relationship between host age and worm counts were fitted to a generalized linear model with an underlying negative binomial error distribution using maximum likelihood in R (R Core Team, 2014). This analysis is appropriate for count data that is highly aggregated and for data sets that involve unequal and small sample sizes (Pacala and Dobson, 1988). Associations between elk liver weight and worm abundance for each gender were also evaluated using maximum likelihood methods.

We analyzed a second sample of 112 elk livers collected opportunistically during the 1997–2005 hunting seasons to complement our assessment of age-related effects. Elk collected during this period were not aged via analysis of the cementum and therefore cannot be fitted to the age-infection profile used in the previous analysis. Rather, count data from individual hosts collected during this period were pooled with data from cementum-aged hosts (total $n = 173$), and then assigned into the broad age categories described in Goater and Colwell (2007): calf (~6 months old); juvenile (~6–24 months old); and adult (24+ months old). Confidence intervals for prevalence values were calculated using the Wald method (Vollset, 1993; $p \pm z\sqrt{pq/n}$, where $z = 1 - \alpha/2$ of the standard normal distribution and $q = 1 - p$).

Variation in median worm abundance with cattle age was assessed from a sample of 35 cattle that had grazed from early June to end of October in CHP for consecutive years. Differences in median intensities were assessed using non-parametric Mann-Whitney U tests. Data on the relationship between host age and worm abundance were fitted to a generalized linear model with an underlying

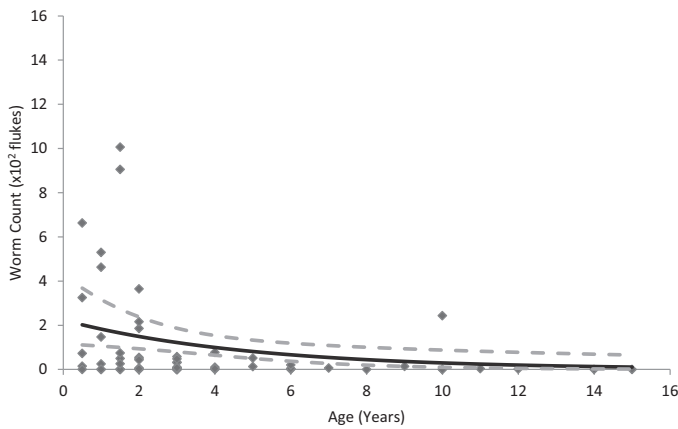


Fig. 1. Age–abundance profiles for the trematode, *D. dendriticum* in a population of elk sampled from 2009 to 2011 from Cypress Hills Park, Alberta. The solid line represents the negative binomial distribution model fit using maximum likelihood; the dashed lines represent the 95% confidence intervals.

NBD using maximum likelihood following the approach described earlier.

3. Results

The overall distribution of *D. dendriticum* within the sample of 61 elk aged via cementum analyses did not differ significantly from the theoretical model of NBD ($\chi^2 = 12.7, p > 0.05; k = 0.192$). For the sample of 61 elk of known age, worm prevalence did not significantly differ ($\chi^2 = 2.70, p = 0.136$) between juveniles (76%, 95% CI: 60–91%) and adults (65%, 95% CI: 49–82%). However, worm abundance varied significantly with host age ($\chi^2 = 5.3, p < 0.01$), with data fitting the NBD ($\ln(Y_i) = 5.18 - 0.24X_i; D_i = -539.2; p < 0.001$; Fig. 1). The results also showed that some individuals were exposed to metacercariae within their first grazing period at approximately 3–6 months of age (Fig. 1). Worm counts were highly variable within 0.5- to 2-year olds (Fig. 1), with some individuals accumulating up to 1000 worms. Beyond 2 years of age, there was a sharp decrease in worm numbers, such that by approximately age 5, few hosts contained greater than 100 worms.

For data pooled from 1997 to 2011, median worm abundance was significantly higher in calves and juveniles ($n = 73$) compared to adults ($n = 100, p < 0.001$; Table 1). Approximately 70% (95% CI: 62–76%) of the total sample of hosts contained less than 100 worms; 5% (95% CI: 3–9%) contained greater than 1000 adult worms (Fig. 2). Of the nine animals harboring over 1000 worms, five were calves and three were juveniles, with four of the five calves containing greater than 2000 worms. In contrast, 90 of the 120 animals containing less than 1000 worms were adults.

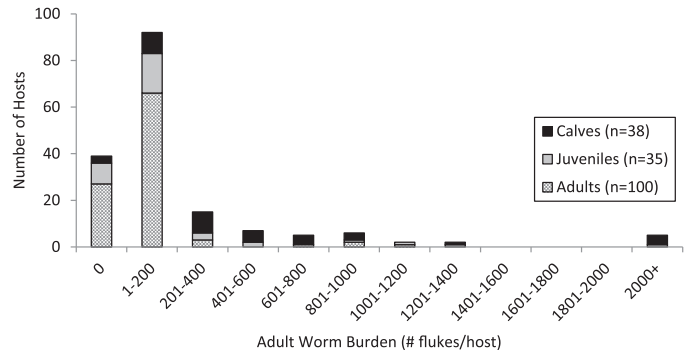


Fig. 2. Stacked frequency distribution of adult *D. dendriticum* in calf, juvenile, and adult elk collected between 1997 and 2011 from Cypress Hills Park, Alberta.

The distribution of worm counts within cattle did not differ significantly from the theoretical model of NBD ($\chi^2 = 11.9, p > 0.05; k = 0.393$). Median worm abundance did not significantly differ between males and females (number of males = 6, number of females = 29, $p = 0.312$). Approximately 84% (95% CI: 71–98%) of cattle under the age of 2 are infected with *Dicrocoelium* while all adult cattle included in our sample were infected. Worm abundance did not significantly vary with cattle age ($\ln(Y_i) = 5.3 + 0.1X_i; D_i = -436; p > 0.05$; Fig. 3). The power (Cohen, 1992) to detect the effect of age in cattle was very low (power: 0.24, $n = 35, p < 0.01$) as a result of low sample sizes and highly variable worm counts, particularly for >2 year old hosts. Fluke intensities were highly variable in cattle regardless of age (Fig. 3); ranging from 9 to 983 worms in calves and juveniles and from 1 to 1490 in adults. The highest counts (1490 worms) occurred in a 4-year old cow.

Adult elk had significantly higher median liver weights than calves/juveniles, for both males ($n = 76, p < 0.001$) and females ($n = 28, p < 0.01$). Liver weight in cows increased non-linearly with age ($Y_i = 3.7 - 2.54 \times \exp(-0.42 \times X_i); R^2 = 0.82, df = 10, SSE_R = 0.36$), with livers tending toward an asymptote in adults older than 6 years (Fig. 4). Livers were not available for bulls older than 6 years of age. For <6 year old bulls, liver weight increased linearly with age ($Y_i = 2.75 + 0.33X_i; R^2 = 0.54, F_{1,42} = 34.22, p < 0.001$). For bulls, worm abundance significantly declined as liver weight increased ($\ln(Y_i) = 9.27 - 1.30X_i; D_i = 36.47; p < 0.05$). This association was not statistically significant for cows ($D_i = 14.62; p > 0.05$).

4. Discussion

Results from the analyses of the two elk data sets indicate that the general pattern of worm counts with host age involves a peak at approximately 2 years of age, followed by a decline. These results are consistent with the earlier observations by Goater and Colwell

Table 1
Prevalence and abundance of *Dicrocoelium dendriticum* in hunter-shot elk from Cypress Hills Park between 1997 and 2011.

| Age | Years | N | Prevalence ($\pm 95\%$ CI) | Mean abundance ($\pm SD$) | Median | Range |
|---|-----------|-----|-----------------------------|-----------------------------|--------|--------|
| All | 1997–2000 | 17 | 94 \pm 11 | 91 \pm 169 | 17 | 0–653 |
| | 2003–2005 | 95 | 79 \pm 19 | 428 \pm 769 | 37 | 0–4343 |
| | 2009–2011 | 61 | 71 \pm 22 | 136 \pm 239 | 10 | 0–1006 |
| Calves and juveniles (≤ 2 years of age) | 1997–2000 | 5 | 80 \pm 19 | 277 \pm 270 | 194 | 0–653 |
| | 2003–2005 | 39 | 90 \pm 14 | 774 \pm 988 | 278 | 0–4343 |
| | 2009–2011 | 29 | 76 \pm 20 | 237 \pm 300 | 38 | 0–1006 |
| | Pooled | 73 | 84 \pm 8 | 548 \pm 811 | 192 | 0–4343 |
| Adults (>2 years of age) | 1997–2000 | 12 | 100 | 29 \pm 47 | 14 | 1–167 |
| | 2003–2005 | 56 | 71 \pm 22 | 126 \pm 264 | 11 | 0–1102 |
| | 2009–2011 | 32 | 66 \pm 23 | 31 \pm 53 | 4 | 0–243 |
| | Pooled | 100 | 73 \pm 0.9 | 82 \pm 203 | 35 | 0–1102 |

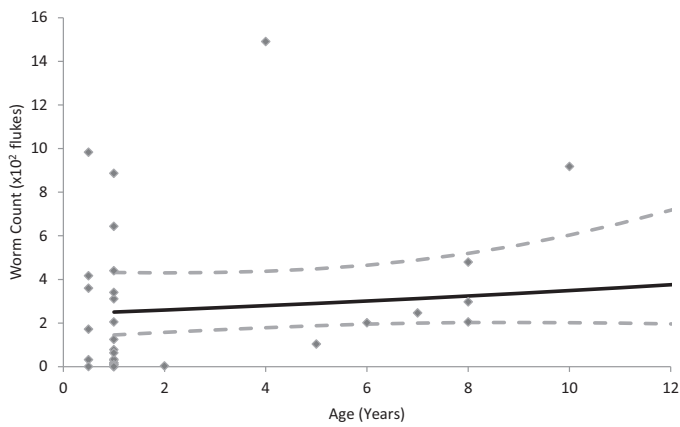


Fig. 3. Age–abundance profile of infection for the invasive trematode, *D. dendriticum* in beef cattle sampled from 2003 to 2013 from Cypress Hills Park, Alberta. The solid line represents the negative binomial distribution model fit using maximum likelihood; the dashed lines represent the 95% confidence interval.

(2007) on a restricted sample of elk collected from the same region. The results are also consistent with those from longitudinal field studies involving *D. dendriticum* in domestic cattle (González-Lanza et al., 1993) and sheep (Manga-González et al., 1991) in which fecal egg counts were higher in calves and juveniles compared to adults. These results indicate that, for elk, there is a rapid increase in worm numbers during the first 1–2 years, followed by a significant decline thereafter. In contrast, the clear age-related pattern in elk was absent in cattle that co-grazed on the same pasture. These contrasting results in elk versus cattle highlight the context-dependent nature of key epidemiological processes for generalist parasites in multi-host systems.

Our analysis of the cementum-aged elk provides the strongest evidence for a significant age versus abundance relationship. These results indicate a rapid rise in worm abundance in 1–2 year old elk, with the accumulation of worms following single or multiple exposures to metacercariae between June and September. Unpublished data from our laboratory indicate that the density of metacercariae infected ants in CHP peaks in June and early July, coinciding approximately with the initiation of grazing that occurs 2–3 months after birth (Robbins et al., 1981). The density of infected ants on vegetation then declines on pasture toward the end of summer (Goater, unpublished data). On average, individual ants collected clinging to plants in the same areas of CHP as the hosts necropsied in this study harbor from 1 to 255 metacercariae ($\bar{x} = 27 \pm 22$, $n = 143$; Goater,

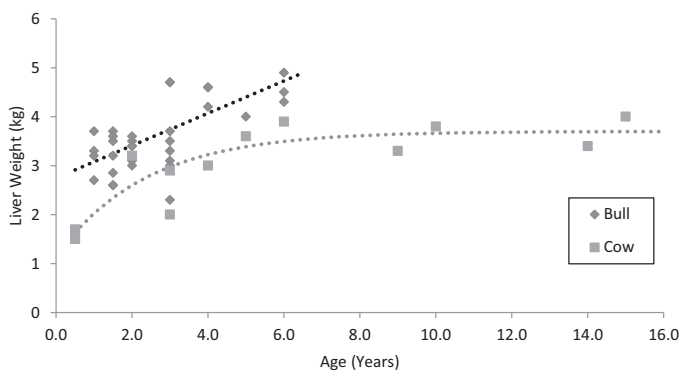


Fig. 4. Relationship between liver weight and host age for elk sampled from 2009 to 2011 from Cypress Hills Park, Alberta. Regression lines are maximum likelihood estimates.

unpublished data). Thus, the high variation in fluke abundance that was observed in naive calves and also yearling elk is best explained by chance ingestion of infected ants that contain variable numbers of metacercariae. This pattern of stochastic encounter with infected ants likely repeats during their second spring/summer, when prevalence reaches 90% in 2-year-olds.

Several mechanisms have been proposed to explain peaked patterns of abundance with host age (Anderson and Gordon, 1982; Duerr et al., 2003). The simplest explanation for the observed pattern in elk lies in the stochastic encounter of 2–3 month old calves with metacercariae in a small number of infected ants, followed by worm senescence at 1–2 years. Experimental studies involving sheep exposed to known numbers of metacercariae show that adult worms live for at least 270 days (Hohorst and Lammler, 1962). Similar studies in our laboratory involving sheep and cattle show that 150-day-old worms continue to produce eggs (Colwell, unpublished data). Thus, it is conceivable that a single period of exposure of calves to metacercaria during their first grazing season, followed by worm senescence at 1–2 years could explain the peaked pattern of fluke abundance in elk.

There are two reasons why this simple exposure/death scenario is unlikely in this system. First, there is no supportive evidence for an age-related change in habitat or diet that could lead to reduced rates of exposure to metacercariae in ants. Results from field studies on individual elk, including those in Cypress Hills Park (Lee, 1979), have shown that grasses and forbs dominate the diets of both juvenile and adult elk (Baker and Hobbs, 1982; Cook et al., 1996). Further, Wickstrom et al. (1984) showed that elk increase their intake of grasses and forbs as they age. Second, experimental exposures of sheep and cattle to known numbers of metacercariae have consistently shown rates of recovery between 10% and 20% (Campo et al., 2000; Colwell, unpublished data). Such low recovery of adults implies that many metacercariae within individual ants are not infective, or that the rate of metacercariae mortality during ingestion/migration/establishment is high. These results mean that single, or at least restricted, encounters with infected ants are unlikely to lead to the intensities of infection that we observed in elk. Rather, these hosts are probably frequently exposed to metacercariae throughout each grazing season, and rates of exposure increase due to increased vegetation intake as hosts age.

Fluke-induced host mortality, concentrated in older hosts, can also explain peaked age–abundance patterns (Anderson and Gordon, 1982; Duerr et al., 2003). There exists no evidence that *D. dendriticum* contributes to host mortality, although the key experiments have not been done in wildlife. Experimental studies in sheep indicate that mortality is rare in infected hosts. Further, mortality has not been documented for naturally infected sheep located on the island of Coll, Scotland where fluke counts are significantly higher than observed in CHP (Sargison et al., 2012). Subtle and chronic effects range from minor irritation of the mucosal lining of the bile ducts, to mass fibrosis and cholangitis of liver tissue (review by Manga-González and González-Lanza, 2005). In extreme cases, hepatobiliary changes in infected sheep cause reductions in weight gain and milk production, and can cause necrosis of the skin and other pathologies associated with general inflammatory defenses (Sargison et al., 2012). Although cases of mortality associated with *D. dendriticum* appear to be rare, interactions with other forms of mortality such as predation, hunting, malnutrition, other parasites and pathogens, and extreme weather could result in the removal of heavily infected hosts. These interactions would have to occur consistently within almost all elk >2 years of age to produce the decline in abundance observed in our data. Nonetheless, a key implication of the age–abundance pattern observed in elk is that studies aimed to understand the effect of *Dicrocoelium* on wildlife should initially focus on calves, where peak-intensities coincide with small liver sizes.

Exposure-dependent acquired immunity could also explain the decline in worm abundance at 2 years in elk. Exposure-dependent immunity of this type has been invoked to explain age-related declines for macroparasites of humans, domestic stock, and laboratory animals (Woolhouse, 1998). The results of experimental studies indicate that established macroparasites often promote the production of anti-parasite antibodies, leading to protection against subsequent exposures (Srisawangwong et al., 2011). In natural systems, a delayed antibody-based response to nematodes has been documented in wood mice, *Apodemus sylvaticus* (Quinnell, 1992), and Soay sheep, *Ovis aries* (Gulland and Fox, 1992). In both of these cases, a delayed immune response best explained convexity in the age-abundance pattern in field-collected hosts. Although anti-*Dicrocoelium* antibodies have been detected in experimentally and naturally infected sheep (Manga-González and González-Lanza, 2005; Revilla-Nuín et al., 2005) and cattle (Colwell and Goater, 2010; Wedrychowicz et al., 1995), the protective nature of these antibody responses and their role in determining liver fluke burdens requires further research, particularly within wildlife hosts.

The lack of an association between age and fluke burdens in beef cattle must be interpreted with caution due to low statistical power. But despite the low numbers of >2 year old cattle in our sample, it is striking that two to three of these hosts were among the most heavily infected animals in the overall sample of hosts. The most heavily-infected cow was a 4-year-old that had grazed within the Park since birth. Likewise, the heavily-infected 10-year-old cow had also grazed in the Park since birth. These anecdotal observations on a few hosts contrast with the pattern observed in elk in which only 1 of 32 hosts that was >2 years of age contained more than 100 flukes. One explanation for these results is that these older, heavily-infected cattle were not exposed to metacercariae during earlier grazing seasons. Given the high prevalence in our sample of 1- and 2-year olds, this is unlikely. An alternative explanation is that in contrast to elk, some cattle accumulate worms as they age, despite the presence of anti-*Dicrocoelium* antibodies (Colwell and Goater, 2010). This interpretation is consistent with the results of studies involving domestic stock exposed to *Fasciola hepatica*, in which the presence of anti-fluke antibodies was not associated with protection from further infection (Bossaert et al., 2000; Cleary et al., 1996).

As expected, the distribution of liver flukes was highly aggregated within the samples of cattle and elk. This characteristic pattern means that a restricted proportion of both populations of hosts will be responsible for the contamination of pasture in CHP with liver fluke eggs. Our results indicate that host age, at least for elk, contributes to this pattern of aggregation. Crude calculations that take into account our data on worm prevalence, worm abundance, and estimates of host population size can be used to estimate the relative roles of the cattle population versus the elk population in the dissemination of eggs onto pasture. Winter aerial surveys in CHP indicate a fairly stable population of approximately 800 elk (Hegel et al., 2009). Based on studies completed on other well-studied elk populations, calves comprise approximately 20% of a total herd (Bender and Piasecke, 2010). Thus, in CHP, the subpopulation of calf elk (about 160 hosts) will harbor about 60% of the ~60,000 worms in the total worm population. In contrast, for cattle, approximately 2000 calves and their 2000 mothers introduced into CHP each spring will harbor approximately 1.1×10^6 worms a few months later, with both age classes contributing roughly similar numbers of fluke eggs onto pasture. These crude calculations imply that the cattle population, on account of its larger size and the absence of a reduction in worm counts with host age, contributes roughly 95% of the total population of fluke eggs that are produced from these two species of hosts. Further empirical studies involving more species of potential definitive host, together with the application of epidemiological models, could increase our precision regarding the

relative roles of different species of host in disseminating infective stages of generalist parasites.

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