

Investigation of *Neospora caninum* seroprevalence and potential impact on reproductive success in semi-free-ranging Père David's deer (*Elaphurus davidianus*)

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ABSTRACT

This study aimed to determine the prevalence and reproductive impact of *Neospora caninum* in a herd of semi-free-ranging Père David's deer (*Elaphurus davidianus*) at a conservation facility, following a suspected abortion storm in 2008. Serum samples (n=103) collected from 60 individuals between 2002 and 2011 were evaluated using competitive ELISA for the detection of *N. caninum*-specific antibodies. Of the 103 samples, 73 (70.9 per cent) were positive for *N. caninum* antibodies, with a mean±sd competitive inhibition (CI) of 77.6±19.8 per cent. Thirty (29.1 per cent) samples were seronegative, with mean CI of 15.2±9.70 per cent. A significant negative linear relationship was noted between the proportion of seropositive animals and age ($R^2=0.228$, $P<0.001$). Calving rates of adult females from 2005 to 2011 ranged from 15.4 per cent to 52 per cent (mean 35.5±11.3 per cent), and did not correlate with mean CI values (Spearman's rank correlation coefficient=0.072, $P=0.878$). While both horizontal and transplacental transmission are likely contributing to persistent herd seropositivity, the role of *N. caninum* in reproductive success in this and other populations of endangered ungulates remains to be explored.

INTRODUCTION

Père David's deer (*Elaphurus davidianus*) are endemic to China, and are currently classed as extinct in the wild by the International Union for the Conservation of Nature (Zhigang and Harris 2008). Since 1939, successful captive propagation of the species and subsequent reintroduction to protected natural habitat reserves in China (Jiang and others 2000, Hu and Jiang 2002) have lent promise to the future of this species, but the maintenance of breeding herds in human care remains critical to its survival.

Neosporosis, caused by the coccidian parasite *Neospora caninum*, is a major cause of

abortion worldwide affecting reproductive output in beef and dairy cattle (Dubey and Lindsay 1996, Dubey 2003). The life cycle of *N. caninum* is characterised by three known infectious stages: (a) oocysts, shed by canid definitive hosts and infectious to ungulates (McAllister and others 1998); (b) tachyzoites, the active and abortion-causing stage and (c) bradyzoites, the inactive stage found in ungulate tissues and ingested by canids (Gondim and others 2003, Dubey and others 2007). Ungulate intermediate hosts become infected through (a) the ingestion of food or water contaminated by sporulated oocysts (Dubey and others 2007), (b) transplacental transmission of tachyzoites or (c) ingestion of tachyzoites in milk or colostrum (Moskwa and others 2007).

The pathogenesis of neosporosis is related to maternal and fetal immune responses (Innes and others 2002). During mid-gestation, a natural shift from Th1 (cell-mediated) to Th2 (humoral) maternal cytokines allows the fetus to evade maternal rejection, but predisposes the pregnant animal to reactivation of *N. caninum* bradyzoites to tachyzoites (Innes and others 2003, Quinn and others 2002, Kano and others 2005). Reactivation in persistently infected dams is associated with high antibody titres, and results in transplacental transmission, causing either abortion or the birth of persistently infected calves. Antibody titres can, therefore, be used as a marker of tachyzoite reactivation and abortion risk in infected herds (Quintanilla-Gozal and others 2000, Guy and others 2001, Innes and others 2002, Frössling and others 2005, Nogareda and others 2007).

The Wilds, a 10,000 acre conservation facility in Southeastern Ohio, manages a herd of

57 semi-free-ranging Père David's deer. An abortion storm was suspected in this herd in 2008 after detecting a significantly lower calving rate than expected and the finding of an aborted fetus in pasture. Dams of calving age were evaluated, and a high degree of seropositivity to *N. caninum* was diagnosed after evaluating for various abortifacive agents. The incidence and prevalence of diseases that may impact herd health and annual recruitment are important in the maintenance of this endangered population. Despite high antibody titres being reported in captive Père David's deer in European zoos, the relationship between seropositivity and reproductive output has not been investigated in this species (Sedlák and Bártošová 2006). The current study was conducted to evaluate the prevalence and potential reproductive impact of *N. caninum* in a herd of Père David's deer and to improve the understanding of the impact and ecology of *N. caninum* at the interface between wild and managed species.

MATERIAL AND METHODS

Study animals and sampling

The Wilds Père David's deer herd was established in 1995 following the acquisition of 15 founder animals (seven females and eight males, age range from 2 months to 18 years) from a North American zoological facility. In 2011, the herd consisted of 57 animals (25 females, 32 males) maintained in an approximately 500-acre pasture with multiple lakes. The pasture is shared with three other managed species (Sichuan takin (*Budorcas taxicolor tibetana*), Grevy's zebra (*Equus grevyi*) and banteng (*Bos javanicus*)).

At the Wilds, blood sampling occurs during neonatal, annual, diagnostic and postmortem examinations. Neonates are handled between one and three days of age for a physical examination, blood collection and identification (microchip and ear tag). Herd examinations are performed annually in the spring using a mechanical hydraulic restraint device (Fauna Research); during which time, physical examinations and hoof trims are performed, vaccines are administered (rabies, multivalent Clostridium and tetanus toxoid) and blood is collected for total protein, packed cell volume, fibrinogen and serum banking. Serum is collected at post-mortem examination from the heart for diagnostics and/or serum banking.

Serologic testing

Serum samples (n=103) were collected from 60 individuals between 2002 and 2011 (12 neonate and 91 adult samples from 21 males, 37 females and 2 unsexed individuals). Serum was submitted to the Animal Disease Diagnostic Laboratory, Ohio Department of Agriculture, for competitive ELISA for the detection of antibodies specific to *N. caninum*. A positive result was defined as a competitive inhibition percentage (CI) of ≥ 30 per cent,

based on the cut-off used to determine seropositivity in domestic cattle using this assay.

Data analysis

The proportion of positive samples is reported as a percentage with 95 per cent confidence limits (CL). Linear regression analysis was used to determine the association of CI and age, and Spearman's rank correlation coefficient was used to evaluate the significance of suspected relationships. All statistical tests were carried out in SPSS Statistics V.22.0.

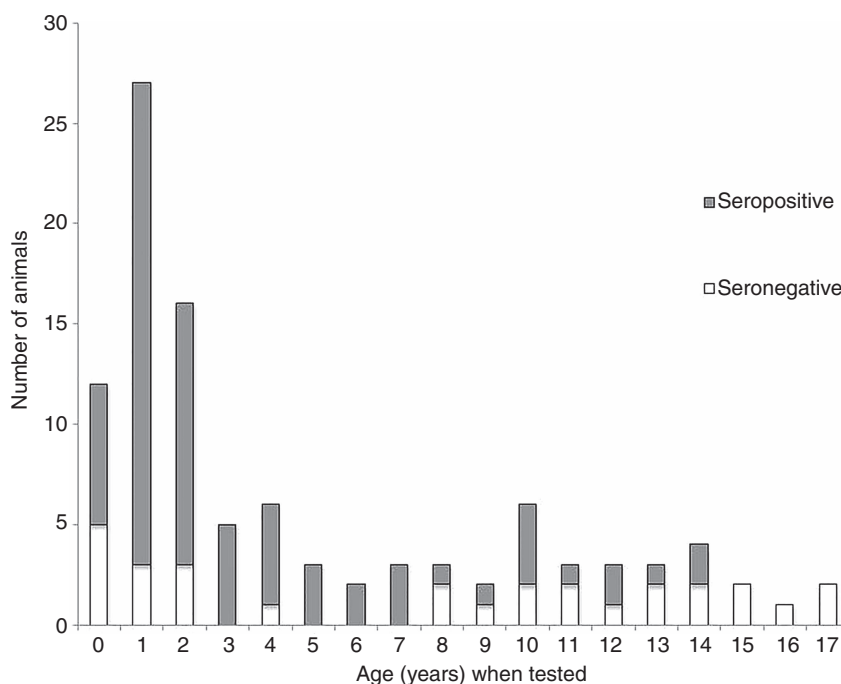
RESULTS

Of the 103 samples tested, 73 (70.9 per cent, 95 per cent CL 61.5, 78.8 per cent) tested positive for *N. caninum* with CI ≥ 30 per cent. Mean \pm sd CI for seropositive samples was 77.6 ± 19.8 per cent. Thirty (29.1 per cent, 95 per cent CL 21.2, 38.5 per cent) samples tested seronegative with mean CI of 15.2 ± 9.70 per cent. The proportion of seropositive animals appeared to decline with age at testing (Fig 1), and CI was noted to decline as the population aged (Fig 2, $R^2=0.228$, $P<0.001$). Of the 12 neonates sampled, seven (58.3 per cent) were seropositive (mean CI 84.0 ± 13.2 per cent) and five (41.7 per cent) were seronegative (mean CI 7.04 ± 3.83 per cent). Fig 3 demonstrates the proportion of seropositive and seronegative animals over the years sampled. Calving rate from seven consecutive years varied from a low of 15 per cent to a high of 52 per cent, but was not correlated with mean CI values (Spearman's rank correlation coefficient=0.072, $P=0.878$) (Table 1). Individual deer sampled repeatedly between 2005 and 2011 did not show seroconversion (Table 2).

DISCUSSION

The level of *N. caninum* seropositivity in this semi-free-ranging population of Père David's deer (70.9 per cent) was considerably higher than that reported in a survey of domestic cattle in the USA (16 per cent, Rodriguez and others 2002), and is suggestive of endemic neosporosis in this herd. White-tailed deer, considered to be one of the most important wildlife reservoirs of *N. caninum* in the USA, are present in high density in the study area, and provide a rich source of infection for dogs and coyotes, the known definitive hosts (Gondim and others 2003, Dubey and others 2009). Coyotes, also abundant in the region, occasionally enter managed areas, and 68.2 per cent of coyote scats collected on Wilds property were found to be positive for *Neospora*-like oocysts using faecal flotation (S. Gupta, unpublished data). Coyote presence in hay fields used to grow forage for managed species may provide an additional route of horizontal transmission. Both vertical (transplacental) and horizontal transmission are likely contributing to the high level of seropositivity in this herd as only 58.3 per cent of neonates tested demonstrated high levels of *N. caninum* antibodies. However, seronegativity at birth does not rule out

FIG 1: Graph demonstrates numbers of animals and serological status against age (in years) when individuals were tested (103 serum samples)



congenital infection as one study of domestic cattle showed that 4 per cent of transplacentally infected calves were seronegative for 9–18 months postpartum, but seroconverted by 25 months of age (Hietala and Thurmond 1999). The immunological mechanism behind the decrease in *Neospora* seropositivity with age within this population is not understood. However, this phenomenon has been demonstrated in domestic cattle, in which *N. caninum* infections can persist throughout life while antibody titres may decline to low levels over time (Fioretti and others 2003, Frössling and others 2005).

This is the first report of persistent herd-level seropositivity, likely representing endemic infection with

N. caninum, in semi-free-ranging Père David's deer. Although transplacental transmission is primarily responsible for maintaining endemic infection and seropositivity within a herd, vertical transmission alone is unlikely to sustain endemic infection. Therefore, reducing canid faecal contamination of pastures and food material (through improved security of pastures, hay-fields and food storage areas) should reduce the prevalence of infection (French and others 1999, Hall and others 2005). Control measures in domestic cattle also include culling and intensive reproductive management strategies, which are not feasible in a herd of endangered fractious cervids, such as Père David's deer.

FIG 2: Competitive inhibition of all 103 samples plotted against the age of the individual tested. Linear regression is demonstrated by the line, which indicates a significant negative relationship between competitive inhibition and age ($R^2=0.228$, $P<0.001$)

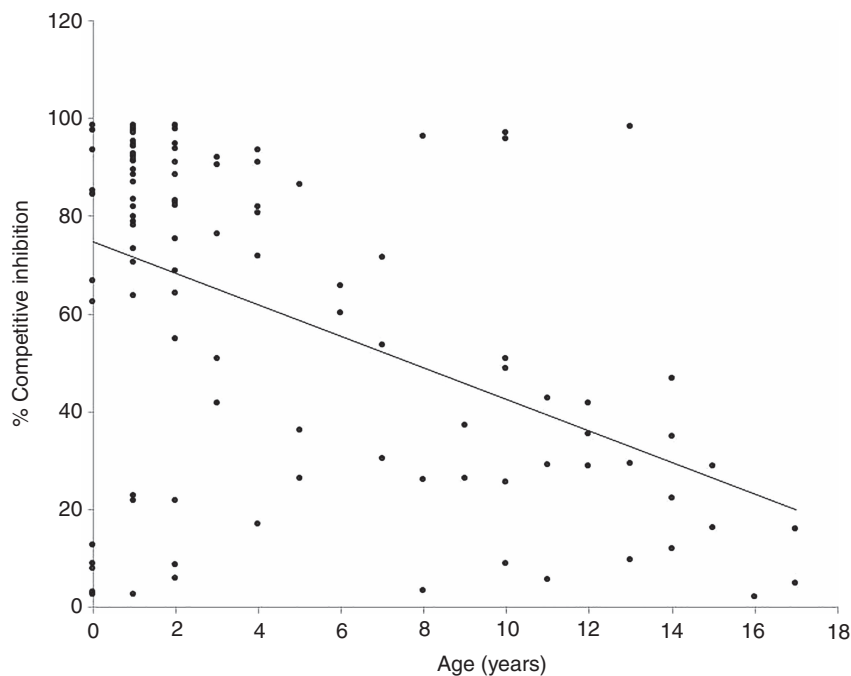


FIG 3: Serological status of Père David's deer (*Elaphurus davidianus*) sampled between 2002 and 2011. Values in parentheses on x-axis indicate the proportion of seropositivity in that tested year

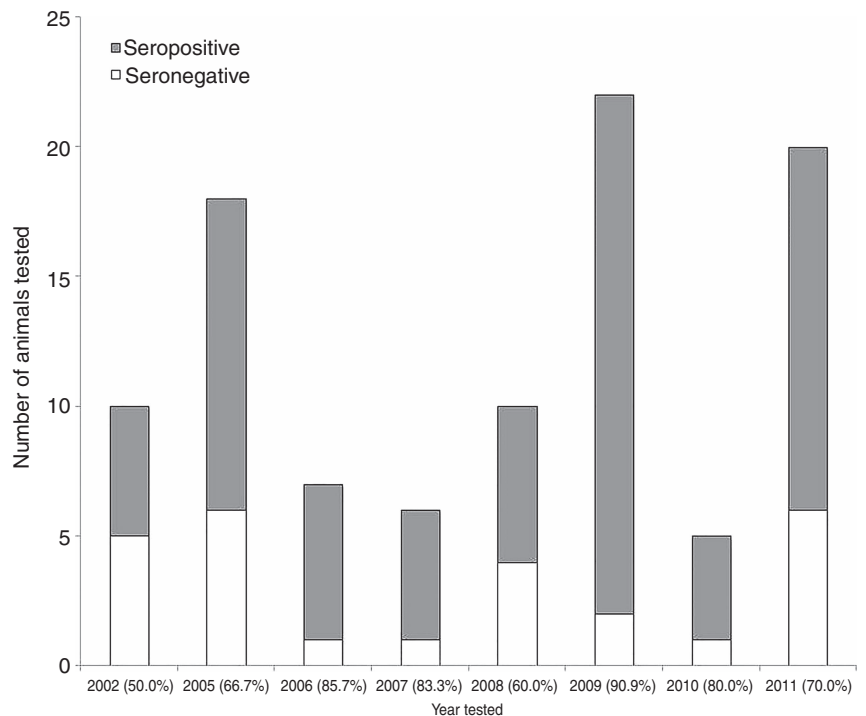


TABLE 1: Number of fertile females, calving rates and mean and sd of CI from 2005 to 2011

Year	Number of fertile females	Calves born	Calving rate (%)	Mean±sd of CI (%)
2005	12	4	33.3	44.4±27.0
2006	13	2	15.4	76.6±21.1
2007	17	6	35.3	66.3±27.8
2008	18	8	44.4	55.0±31.7
2009	23	8	34.8	63.8±28.2
2010	25	13	52.0	78.4±37.9
2011	24	8	33.3	67.1±39.8
Total	132	49	37.1	54.3±31.6

CI, competitive inhibition

The calving rate of 37.1 per cent in this herd for the 7-year study period is low compared with the report of [Wemmer and others \(1989\)](#) who found that, between 1975 and 1987, a herd of 27 adult Père David's does under

similar housing conditions in Virginia demonstrated a calving rate of 89.2 per cent. Interestingly, the coyote population has been estimated to have increased in Ohio fivefold between 1990 and 2011 (<http://wildlife.ohiodnr>.

TABLE 2: Individual results from samples repeated in successive years (2005–2011)

Individual ID (age at first sample in years)	CI 2005	CI 2006	CI 2007	CI 2008	CI 2009	CI 2010	CI 2011	Mean±sd of CI (%)
104003 (1)	79.7	88.4	90.5	81.7	86.3			85.3±4.05
106005 (1)			22.9	21.8	16.9			20.5±2.61
106026 (1)			70.5	64.2	50.7			61.8±8.26
950605 (9)	26.4	25.2		35.5	29.4	35.0	28.8	31.8±5.44
950607 (9)	53.6	50.8			46.7			50.4±2.83
950611 (10)	8.9			9.7			2.0	6.87±3.44
950612 (8)	3.3			5.7			16.3	8.43±5.65
980402 (7)	80.6			71.5			42.7	65.0±16.2
990303 (3)	76.2			65.5			48.8	63.5±11.3
MM0404 (2)	68.8		36.2				37.3	42.2±15.9

No individual seroconverted
CI, competitive inhibition

gov/species-and-habitats/species-guide-index/mammals/coyote), and the population of white-tailed deer has grown from an estimated 17,000 in 1970 to 700,000 in 2006 (<http://wildlife.ohiodnr.gov/portals/wildlife-/pdfs/publications/wildlife%20management/pub087.pdf>). However, despite the apparent increased wildlife exposure, demonstrated high level of *N. caninum* seropositivity and the low calving rate in this herd, neosporosis cannot be definitively identified as the cause of the low reproductive output as no aborted tissues were recovered. This is presumably due to the large enclosure size and rapid scavenging by vultures. Demonstration of abortion due to *N. caninum* will be necessary in future studies to distinguish it from other causes of low recruitment such as decreasing genetic diversity. In fact, the authors have yet to understand the long-term impact of *Neospora* and other reproductive pathogens on genetically depauperate populations of endangered species in the wild or in human care. Further research is needed in order to confirm transmission pathways and identify the impact of neosporosis and potential control measures in this and other critical cervid populations.

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