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Original paper

Infectivity of gastrointestinal nematode parasites of sika deer (*Cervus nippon*) for calves and lambs

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ABSTRACT. Sika deer, mainly of Japanese origin, have been introduced into the British Isles and central Europe and established free-ranging populations, expanding in several countries. Introduction of the sika deer was associated with the transfer of *Spiculopteragia houdemeri* which has been reported for the first time in 2003 from Germany and thereafter from sika deer and other cervid species in some countries in Europe. Surveys of livestock parasites have shown that cervid-specific gastrointestinal nematodes of native deer occur in natural infections in cattle and sheep, usually at low level, and infections were experimentally transferred. However, to date there is no such information for sika deer-specific nematodes to livestock. To investigate the establishment of sika deer-derived gastrointestinal nematodes in domestic ruminants, three calves and two lambs were challenged with mixed burdens of infective larvae (~90% ostertagids, ~10% *Oesophagostomum*) cultured from the faeces of free-ranging sika deer; calves received 20,000 or 30,000 larvae, lambs 12,000 or 13,000. Establishment rate of ostertagids varied from 0.4% to 3.1% in the calves and was 1.3% and 8.4% in the lambs. *Spiculopteragia houdemeri*, index ostertagid of Japanese sika deer, was the dominant species, recovered from all animals. In addition, *Ostertagia leptospicularis* and *Spiculopteragia boehmi*, index ostertagids of native roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*), respectively, were isolated from calf and lamb and *Cooperia pectinata* from one lamb. *Oesophagostomum venulosum* established in the lambs at ~6% but no *Oesophagostomum* was found in any calf. This investigation indicates that both calves and lambs are permissive to the sika deer-specific *S. houdemeri* and other deer-derived nematodes which reached maturity, but susceptibility to infections was apparently low.

Keywords: sika deer, cattle, sheep, gastrointestinal nematodes, *Spiculopteragia houdemeri*, experimental transmission

Introduction

For several reasons, there has been an increase in the study of diseases and disease transmission, including parasite-sharing patterns, at the interface between wildlife and domestic animals over the past decades. One area of interest are infections with gastrointestinal nematodes, which can be, in principle, exchanged between ruminant livestock and wild cervids as both frequently share habitats and resources, i.e., well-managed pastures as well as extensive rangeland and may have ecological, epidemiological, management and economic implications [1–6].

Research on the transfer of pasture-transmitted nematode infections between free-ranging and domestic ruminants based on experimental inoculation and natural exposure (cross-grazing) studies has a long history for cervids native to Europe [7–14]. There is, however, apparently only very limited knowledge on the potential role of the introduced sika deer (*Cervus nippon*) in that regard. Sika deer form currently well-established free-ranging populations in Europe which, like those of native cervid species, tend to increase in distribution and abundance in several countries [15–20]. With sika deer translocated from the mainland of eastern Asia, *Ashworthius sidemi*, a haemonchine nematode considered typical for cervids originating from that region, was transferred and found for the first time in the 1970s in former Czechoslovakia and Ukraine [21]. With sika deer originating from Japan, *Spiculopteragia houdemeri*, the index ostertagid nematode of Japanese sika deer [22] was introduced

into Europe and reported there for the first time in 2003 from sika deer in Germany [23]. While the establishment of *A. sidemi* in sheep was proved by experimental inoculation [24] and, using molecular methods, bulk nematode larval samples cultured from feces of pastured cattle in Poland tested positive for *A. sidemi* DNA [25], there is apparently no reported research on the infectivity of *S. houdemeri* for livestock.

A survey of the endoparasite fauna of wild sika deer from populations in Germany and Austria [22,26] gave an opportunity to study the susceptibility of calves and lambs to sika deer-derived gastrointestinal nematodes, including *S. houdemeri*, by experimental inoculation.

Material and Methods

Experimental animals

Three male Brown Swiss calves, approximately four months of age, and two male ruminating Merino Landrace lambs, approximately five months of age, raised indoors and confirmed to be helminth-negative by repeated faecal examination prior to inoculation with sika deer-origin gastrointestinal nematode larvae were used for the study. During the study, the animals were housed in two pens by species. They were offered hot air-dried grass for ad libitum consumption and had free access to water.

Preparation of infective larvae and larval challenge

Rectum faeces were collected from sika deer harvested in two sika deer populations in Austria (Tullner Donauauen, Ostrong) and one sika deer population in Germany (Arnsberger Wald) according to the hunting regulations in the years 2003 and 2004 while surveying the endoparasite status of sika deer from free-ranging populations in Germany and Austria [22,26]. The faecal material, pooled by sika deer origin, was mixed with vermiculite and water and incubated at approximately 25°C, stirred every other day with water added as needed to maintain appropriate moisture levels. After seven days the larvae were harvested with a standard Baermann apparatus. Several coproculture runs were performed, according to the availability of faecal material, and the harvested larvae were combined to one lot per source and stored in the dark at approximately 8°C for five to eight months until use for inoculation. Before inoculation, 100 larvae per bulk culture were morphologically identified to 'ostertagids' (*Ostertagia* type) or *Oesophagostomum* based on a standard identification key [27]; no larvae of other nematodes were identified.

All animals were dosed orally with the sika deer-derived nematode larvae on the same day. The number of larvae and composition of the inoculum given is given in Table 1.

Parasitological techniques

Individual faecal samples were collected from the experimental animals as described in Table 1 and eggs per gram (EPG) counts were determined by a modified McMaster technique with one egg counted representing 10 eggs per gram of faeces [27]. Two slides per sample were examined to achieve a multiplication factor of 5.

For nematode recovery and count, experimental animals were slaughtered 21, 40 or 62 days after inoculation and the contents of the abomasum, small and large intestines were collected individually. One 10% aliquot of the abomasum content and one 20% aliquot of the contents of both small intestine and large intestine were examined for nematodes. To facilitate isolation and counting of nematodes, organ contents were screened over sieves of appropriate mesh size.

Nematodes were identified to species and developmental stage based on their morphology after clearing in lactophenol, using published descriptions [28–30]. However, adult female ostertagid nematodes were not identified to the species level instead total counts per species were estimated by proportionally assigning adult females to each species based on the adult male counts. In addition, the presence of eggs in the uteri of adult females was noted, if observed.

Ethical approval

The study procedures complied with the appropriate local animal welfare regulations and were approved by the institutional animal welfare and ethics committee.

Results and Discussion

The results of the study regarding faecal egg shedding, recovery of nematodes, establishment rates and percentage of ostertagid females with eggs in the uteri are summarized in Table 1. No clinical signs or other health problems were observed in any animal in response to the infection. However,

Table 1. Inoculation, faecal egg counts and nematode recovery from calves and lambs dosed third-stage gastrointestinal nematode larvae cultured from rectum faeces of free-ranging sika deer

		FEC[1] … dpi[2]			Nematode recovery							
					Species recovered and nematode count							
Host inoculated	Source[A] and number of larvae given orally; percentage composition of inoculum	21	40	62	Slaughter … dpi	*Spiculopteragia houdemeri*	*Spiculoptergia boehmi*	*Ostertagia leptospicularis*	*Cooperia pectinata*	*Oesophagostomum venulosum*	Percentage take	Percentage ostertagid females with eggs
Lamb 1	TD, ~13,000 larvae; ~89% ostertagids, ~11% *Oesophagostomum*	5	–	–	21	150	0	0	0	90[3]	Ostertagids, ~1.3%; *Oesophagostomum*, ~6.3%	~56%
Calf 1	TD, ~30,000 larvae; ~89% ostertagids, ~11% *Oesophagostomum*	5	–	–	21	2845	60	0	0	0	Ostertagids, ~10.9%; *Oesophagostomum*, 0%	~7%
Calf 2	TD, ~30,000 larvae;~89% ostertagids,~11% *Oesophagostomum*	5	25	0	62	100	0	0	0	0	Ostertagids, ~0.4%; *Oesophagostomum*, 0%	~60%
Calf 3	OR, ~20,000 larvae; ~84% ostertagids, ~16% *Oesophagostomum*	0	5	–	40	505	0	15	0	0	Ostertagids, ~3.1%; *Oesophagostomum*, 0%	~75%
Lamb 2	AW, ~12,000 larvae; ~91% ostertagids, ~9% *Oesophagostomum*	5	50	–	40	140	570	210[4]	5	70[5]	Ostertagids, ~8.4%; *Oesophagostomum*, ~6.5%	~90%

Explanations: [A]sika deer from: TD – Tullner Donauauen, Austria; OR – Ostrong, Austria; AW – Arnsberger Wald, Germany; [1]FEC – faecal egg count (eggs per gram of faeces); [2]dpi – days post inoculation; [3]all nematodes were immature adults; [4]in addition to the major morphotype (*O. leptospicularis*), males of the minor morphotype (*O. kolchida*) were recovered; [5]female nematodes had eggs in the uteri

interpretation of the results should be within the limitations of the study, considering the use of three lots of larvae for inoculation, originating from sika deer of three separate populations, each collected over a period of several weeks and stored for up to eight months before use for inoculation, as well as the small number of animals inoculated and the limited observations which did not allow to determine parasite dynamics and longevity of the parasites.

Patent infections, confirmed by the shedding of strongyle eggs and the recovery of adult nematodes including females with eggs in the uteri, were observed in all animals. Adult nematodes of three species of ostertagids, *Ostertagia leptospicularis*, *Spiculopteragia boehmi* and *Spiculopteragia houdemeri*, were recovered from both calves and lambs with *S. houdemeri* establishing most frequently and in higher numbers than the two other species. This finding correlates with the proportion of the three ostertagids recovered from the sika deer of the three populations [22,26] which was likely reflected in the species composition of the larvae collectively identified as ostertagid larvae. In addition, five adult *Cooperia pectinata* nematodes were isolated from the small intestine of one lamb, and both lambs were infected with *Oesophagostomum venulosum*.

This is the first report of the establishment of sika deer-origin gastrointestinal nematodes, including the Japanese sika deer-specific ostertagid *S. houdemeri*, in cattle and sheep which suggests a potential for the transmission of *S. houdemeri* when sika deer share grazing areas with livestock. While the authors are unaware of observations of sika deer on livestock pastures in Europe, sika deer have been reported to graze frequently on cattle pastures in Japan [31–33], and the risk of parasite transfer from sika deer to livestock has been discussed there [34]. The shedding of eggs and the observation of eggs in the female nematodes provide evidence of at least one passage including reproductive capability of *S. houdemeri* in domestic bovid hosts. It is, however, not clear whether *S. houdemeri* can sustain itself in these hosts under natural infection conditions compared to or in competition with the well-adapted, cattle- or sheep-specific ostertagid species, i.e. *O. ostertagi* and *Teladorsagia circumcincta*, respectively. The pattern of the egg counts in Calf 1 and Calf 2 relative to the worm counts may suggest host-mediated effects on the infection expressed in an early expulsion of most of the population of a parasite which is largely confined to a non-native cervid. To the best knowledge of the authors, there are no reports of records of *S. houdemeri* in domestic bovids from Japan, but *S. houdemeri* has been detected in the serow (*Capricornis crispus*), a rupicaprine wild bovid in Japan which, interestingly, has not been found to be infected with other ostertagids [35].

In addition to *S. houdemeri*, which formed the predominant component of the gastrointestinal nematode community of the sika deer from the populations whose rectum faeces were cultured to produce the larvae for inoculation in the study, the strongylid species *O. leptospicularis*, *S. boehmi*, *Trichostrongylus askivali*, *Nematodirus roscidus*, *C. pectinata*, *Oesophagostomum sikae* and/or *Oe. venulosum* were recovered from the sika deer [22,26].

The establishment in both calves and lambs of *Ostertagia leptospicularis* and *S. boehmi*, index ostertagids of the native roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*), respectively [4,22], adds to the body of literature indicating these two ostertagids as most often found cervid-associated species in natural infections of both cattle and sheep in Europe, but usually in a limited extent [4,36–52]. However, *O. leptospicularis*, although commonly associated with cervids, may consider attention because there are apparently distinctly cattle-adapted populations [53].

The absence of *T. askivali* from the calves and lambs is likely related to its rare occurrence in very low number in the sika deer whose faeces were cultured for the study [22,26]; however, it may be worth mentioning that this parasite can infect sheep [13].

Nematodirus roscidus and *C. pectinata* are both common parasites of cervids in Central Europe, especially of young red deer and fallow deer (*Dama dama*) [22,54,55]. *Nematodirus roscidus* have been recovered previously from both calves and lambs which acquired the infection by grazing in deer parks or deer farms [11–13], and experimental *N. roscidus* infections established successfully in sheep [56]. However, the coproculture method employed for this study was known to be unsuitable for growing infectious *N. roscidus* larvae which explains their absence from the inoculated animals. Despite no *Cooperia* larvae being identified during the differentiation of the larvae of the inoculation lots, a few *C. pectinata* were isolated from the small

intestine of one lamb. The finding of *C. pectinata* in one of the lambs is in line with occasional records of this species in naturally infected cattle and sheep in Europe [38,41,57,58] and in cross-grazing studies [12,13]. In this context, it should be noted that the identity of nematodes determined as *C. pectinata* in cattle vs. deer is currently under debate [59].

Although *Oe. sikae* outnumbered *Oe. venulosum* in the sika deer in all three populations [22,26], which was likely reflected in the *Oesophagostomum* larval challenge material, no *Oe. sikae* were found in the calves and lambs but *Oe. venulosum* established in the two lambs. The absence of any *Oe. sikae* suggests that cattle and sheep are not suitable hosts for the development of this nematode. This observation is supported by a study which assessed the nematode burden of cross-grazed fallow deer, cattle, sheep and goats and found *Oe. sikae* exclusively in the fallow deer [12]. Within the genus *Oesophagostomum*, *Oe. sikae* and *Oe. radiatum*, the strongly cattle-adapted large intestine species [60], belong to the subgenus *Bosicola* [30], and it may be that *Oe. sikae* exhibits a similar level of adaptation to cervid hosts as does *Oe. radiatum* to cattle. *Oesophagostomum venulosum*, in contrast, is thought to be generally less host specific and known to parasitize readily a range of caprine and cervid species but has been comparatively rarely found in cattle [30,60,61], and experimental studies suggested the existence of sheep-adapted and cattle-adapted populations [61,62]. However, consistent with the recovery of sika deer-derived *Oe. venulosum* in the lambs in this study, induced infection and cross-grazing studies reported the establishment of roe deer origin and fallow deer origin *Oe. venulosum* in sheep [8,12] and, interestingly, also calves grazing in a red deer park or in a fallow deer farm acquired *Oe. venulosum* [11,12].

Besides primarily indicating that sika deer-associated nematodes may not easily establish in cattle and sheep because of inherent host-specificity, as discussed above for cervid-associated gastrointestinal nematodes in general, it is not clear whether the storage for several months of the nematode larvae prior to inoculation may have contributed to the overall low establishment rates observed. Work done with gastrointestinal nematode larvae from domestic ruminants indicated that extended refrigerator-storage of third-stage larvae can be associated with a substantial reduction in infectivity in contrast to larval survival (viability) [63].

In summary, this study has extended the range of cervid-specific nematode species known to be able to mature in domestic ruminants. For the sika deer-specific *S. houdemeri*, which has only been recently identified in Europe, this is the first study providing preliminary data comparable to cross-infections from native deer to cattle and sheep. The results suggest a low susceptibility of infection in bovid (heterologous) hosts but indicate that the transfer of infection from sika deer to livestock cannot be excluded when sharing a common range. From this study, however, infection with *S. houdemeri* of cattle and sheep appears unlikely to be of ecological or epidemiological relevance or to pose a risk to livestock. To better understand the relevance of a potential transmission of sika deer nematodes to livestock, studies monitoring the endoparasite fauna of both livestock and deer under natural shared-grazing conditions would be required.

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