

# Competence of Pheasants as Reservoirs for Lyme Disease Spirochetes

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**ABSTRACT** Pheasants, *Phasianus colchicus* L., constitute a major part of the ground-feeding avifauna of England and Wales and are important hosts to immature stages of *Ixodes ricinus* L., the principal tick vector of Lyme borreliosis spirochetes in Europe. Therefore, their competence as hosts for *Borrelia burgdorferi* Johnson, Schmid, Steigerwalt & Brenner sensu lato was investigated. One group of pheasants was inoculated by needle with  $1 \times 10^6$  cultured *B. burgdorferi* s.s. organisms, and a 2nd group of birds was infested with *I. ricinus* nymphs collected from a focus of Lyme borreliosis in southern England. Both bird groups were subjected to xenodiagnoses using uninfected *I. ricinus* nymphs. All recovered engorged ticks, as well as pheasant skin biopsies, were analyzed by a nested polymerase chain reaction targeting the 5S-23S rRNA genes of *B. burgdorferi* s.l. Both groups proved to be infective for ticks. The birds that were infected by tick bites proved to be significantly more infective for ticks (23% of the xenodiagnostic ticks positive) than those infected by needle (5%). The results show that pheasants can be infected experimentally with *B. burgdorferi* s.l., that they can pass the spirochetes to ticks and that their infectivity for ticks may persist as long as 3 mo. We conclude that pheasants are reservoir competent for Lyme borreliosis spirochetes and potentially play an important role in the maintenance of *B. burgdorferi* s.l. in England and Wales.

**KEY WORDS** *Borrelia burgdorferi*, pheasants, ticks, reservoir competence

TRANSMISSION OF THE etiological agent of Lyme borreliosis, *Borrelia burgdorferi* Johnson, Schmidt, Steigerwaldt & Brenner sensu lato, relies on an increasingly widely recognized range of wild vertebrate species, including birds (Anderson et al. 1986; Humair et al. 1993a, b; Olsen et al. 1993; Tällklint and Jaenson 1993, 1994; Craine et al. 1995, 1997; Kurtenbach et al. 1995; Randolph and Craine 1995; Stafford et al. 1995). The risk to humans of infection is subject to fluctuating patterns of interactions between humans and wildlife, often as a result of changes in habitat or wildlife management. For example, in the United States, increased deer populations, especially in areas close to human habitation, have led to increased tick populations and greater contact between humans and ticks (Deblinger et al. 1993). In the United Kingdom, pheasants, *Phasianus colchicus* L., are part of these changing patterns. As significant hosts for the principal European tick vector, *Ixodes ricinus* L. (Randolph and Craine 1995; this study), pheasants potentially

play a role in sylvatic Lyme borreliosis transmission cycles.

Black-necked pheasants, *Phasianus colchicus colchicus* L., were introduced as gamebirds to Britain from Asia many centuries ago. These original stocks have since been largely replaced by ring-necked pheasants, a group of races originating from East Asia (e.g., *P. c. mongolicus* J. F. Brandt, *P. versicolor versicolor* Vieillot). Over the past 30 yr the number of artificially reared pheasants released into British woodlands in early summer to supplement shooting in winter has increased at 4% per annum and now stands at  $\approx 20$  million (Robertson et al. 1993). In terms of biomass, pheasants now constitute the majority of the autumn land-based avifauna in England and Wales, and densities may reach as high as 50/ha in some woodland habitats (A.N.H., unpublished data). As ground-foraging birds, pheasants have frequent contact with *I. ricinus* (Randolph and Craine 1995). Furthermore, they have been implicated as competent amplifying hosts for Lyme disease spirochetes, *B. burgdorferi* s.l., by virtue of the higher infection prevalence of *B. burgdorferi* in engorged larvae removed from a sample of pheasants from Thetford Forest, Norfolk (22%), than in unfed larvae collected from the same site (0.0%) (Craine et al. 1997). This article demonstrates that pheasants can be infected experimentally with *B. burgdorferi* s.l., both by needle inoculation and by naturally infected tick bites, and that they can subsequently pass these spirochetes to *I. ricinus* ticks feeding upon them.

Animal experiments carried out in the current study were authorized by the Home Office Secretary under the terms of the Animals (Scientific Procedures) Act 1986 (Home Office reference number: PPL 30/751).

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### Materials and Methods

**Pheasants.** Two groups of ring-necked pheasants that had been hand-reared by professional breeders at Fordingbridge, Hampshire and Culham, Oxfordshire, free from contact with *I ricinus* were held in the laboratory at 20°C on an antibiotic-free diet. Group 1 was composed of 4 chicks aged 6 wk and group 2 was composed of 5 adult birds aged 6–8 mo.

**Ticks.** *I. ricinus* larvae and nymphs from the tick colony maintained at the Natural Environment Research Council Institute of Virology and Environmental Microbiology, Oxford, were used for xenodiagnosis. The colony was maintained by feeding the ticks on specific pathogen-free hamsters and rabbits and was regularly tested for the absence of spirochete infection. Infected nymphal *I. ricinus* were collected by blanket dragging 1–2 mo before use from the Wimborne St. Giles Estate, Dorset, a known endemic focus of Lyme borreliosis. They were shown by polymerase chain reaction (PCR) diagnosis (see below) to have an infection prevalence of 5% for *B. burgdorferi* s.l.

**Infection of Pheasants and Xenodiagnosis.** Group 1 birds were injected subcutaneously under the wings with  $1 \times 10^6$  *B. burgdorferi* sensu stricto (ZS7 strain, a tick isolate from Freiburg, Germany). Group 2 birds were subjected to an initial xenodiagnosis and 2 wk later exposed to *I. ricinus* nymphs collected in Dorset. From 15 to 80 nymphs per bird fed successfully, resulting in an average of 0.75–4.0 infected tick bites on each bird. Five to 10 wk after infection, all the birds were subjected to xenodiagnoses, using both larvae and nymphs on group 1 birds, but only nymphs on group 2.

To introduce ticks, the feathers on each bird's throat, back of the head, and under the wings were clipped and the skin was intensively degreased using 70% ethanol. Ticks were contained in neoprene cells (1 cm i.d.) glued with latex onto the prepared skin surface and were allowed to feed to engorgement. Ticks that died during feeding were counted and then discarded. Ticks did not feed successfully under the wings of group 1 birds, therefore ticks were introduced to the head and throat only of group 2 birds. Partially or fully engorged ticks were collected, weighed immediately, stored for 10 d in a desiccator over MgSO<sub>4</sub>, and then frozen at –20°C. Twelve weeks after infection, all birds were bled, euthanized, and autopsied; biopsies (2 × 2 mm) were taken from skin sites where the xenodiagnostic ticks had fed. All biopsies were stored at –70°C before processing.

**Polymerase Chain Reaction.** DNA from tick and biopsy samples was extracted using phenol-chloroform (Livesley et al. 1994, Moter et al. 1994). *B. burgdorferi* s.l. specific DNA was detected using a nested PCR targeting the 5S and 23S rRNA genes and, thereby, amplifying the intergenic spacer region between these tandemly duplicated gene clusters (Postic et al. 1994; Rijpkema et al. 1995, 1996a, b). Using cultured spirochetes, this PCR protocol

allowed the detection of DNA equivalent to  $\geq 2$  spirochetes.

To assess whether this PCR detection of *B. burgdorferi* s.l. was inhibited by host tissue, DNA (Cogswell et al. 1996), or tissue derived from engorged ticks, varied numbers of extracted spirochetes were spiked with different dilutions of lysates of host tissues and engorged ticks and subjected to PCR. Host DNA at  $>500$  ng/ $\mu$ l and engorged tick tissue at  $>600$   $\mu$ g/ $50$   $\mu$ l lysate proved to be inhibitory to any PCR amplification of DNA equivalent to  $<100$  spirochetes. Accordingly, all lysates were diluted appropriately before the PCR reaction in order to achieve the desired sensitivity  $\geq 2$  spirochetes per reaction mixture.

### Results

Of the 40 nymphs and 50 larvae introduced to each bird of group 1 for xenodiagnosis, 13–15 nymphs per bird were recovered engorged 5–10 wk after infection (12 larvae had fed successfully on only 1 bird) (Table 1). No ticks introduced under the wings were recovered alive, therefore all the xenodiagnostic ticks that were analyzed for spirochete infection had fed on the head and throat of the birds. Two of 4 birds were infective to *I. ricinus* ticks, and these birds infected 8–13% of the engorged ticks.

The initial xenodiagnosis on group 2 birds (using 20 larvae on each of birds number 5 and 6, and 40 nymphs on each of birds numbers 7–9) yielded few engorged ticks (2 larvae, 11 nymphs), none of which was infected. During the 10-wk period after exposure to infected nymphs, totals of 60–120 uninfected xenodiagnostic nymphs were introduced and removed when engorged. Of the 4 birds (bird number 9 yielded no engorged ticks), 3 birds infected 17–42% of the ticks with *B. burgdorferi* s.l. (Table 1). Overall, group 2 adult birds that had been infected naturally by tick bites transmitted the native strain of *B. burgdorferi* s.l. to  $10/43 = 23.3\%$  of the nymphs that fed on them. This was a significantly higher probability of transmission than from group 1 chicks that had been needle-inoculated with a German strain of *B. burgdorferi* s.s., which on average infected  $4/70 = 5.7\%$  of feeding ticks (Yates corrected  $\chi^2 = 5.843$ ,  $P < 0.025$ ).

Two of group 2 birds and 1 of group 1 birds yielded PCR positive biopsy skin samples (Table 1). During these experiments, the feeding success of larvae on pheasants was low compared with that of nymphs. This was consistent with natural tick infestation levels recorded on pheasants from woodland sites in Dorset (Table 2), which confirmed that pheasants are indeed quantitatively important as hosts for immature stages of *I. ricinus*, especially nymphs.

### Discussion

Our results show that pheasants are competent amplifying hosts both for cultured *B. burgdorferi* s.s.

Table 1. Transmission of *B. burgdorferi* s.l. to ticks from needle and tick-infected pheasants

Bird no.	Treatment	Skin infected <sup>a</sup>	Xenodiagnosis		
			No. ticks introduced	No. ticks recovered	Infected/ examined (%)
Needle infected					
1	1 × 10 <sup>6</sup> sc <sup>b</sup>	–	40N	13N	1/13 (7.7)
			50L	12L	1/12 (8.3)
2	1 × 10 <sup>6</sup> sc	–	40N	15N	0/15 (0.0)
			50L	0L	–
3	1 × 10 <sup>6</sup> sc	–	40N	15N	0/15 (0.0)
			50L	0L	–
4	1 × 10 <sup>6</sup> sc	+	40N	15N	2/15 (13.3)
			50L	0L	–
Subtotal			360	70	4/70 (5.7)
Tick infected					
5	35N <sup>c</sup>	+	120N	13	2/12 (16.7)
6	15N	+	60N	17	5/12 (41.7)
7	80N	–	60N	10	3/10 (30.0)
8	80N	–	60N	9	0/9 (0.0)
9	80N	–	60N	0	–
Subtotal	290		360	49	10/43 (23.3)

L, larvae of *I. ricinus*; N, nymphs of *I. ricinus*.

<sup>a</sup> Skin biopsy taken post mortem 10 d after tick repletion; infected (+) or uninfected (–) as determined by PCR.

<sup>b</sup> Subcutaneous (sc) inoculation of *B. burgdorferi* sensu stricto (strain ZS 7, Freiburg, Germany) under each bird's wing.

<sup>c</sup> Numbers of ticks introduced to the birds to induce infections; ticks were field-collected in February 1995 at Wimborne St. Giles Estate, Dorset, U.K. The infection prevalence for *B. burgdorferi* s.l. was shown to be 5% in nymphs.

introduced by syringe inoculation and for natural infections of *B. burgdorferi* s.l. derived from *I. ricinus* tick bites. Experimental birds remained infective to recipient ticks for at least 10 wk from the time of infection. It remains to be demonstrated whether pheasants can maintain the spirochetes throughout periods when conditions preclude active circulation through the tick population, for example during the winter when tick feeding activity in British woodlands declines (Table 2). Chickens, a laboratory model of an avian host for *B. burgdorferi*, although able to infect *I. scapularis* Say with *B. burgdorferi* s.s., lose their infectivity after a few weeks (Piesman et al. 1996). The recovery of infected xenodiagnostic ticks from pheasants until the termination of the experiment (10 wk after infection) suggests that this seminatural avian host exhibits a rather higher degree of reservoir competence to *B. burgdorferi* s.l. than do chickens.

The detection of spirochetes in the current study was based on the successful amplification of spiro-

chetal DNA, which cannot distinguish between viable and nonviable borreliae. However, because all the engorged ticks were allowed to digest their bloodmeal for 10 d after repletion, it is unlikely that the PCR was detecting only naked DNA from spirochetes in the ticks' midguts, particularly because the target of this PCR is located on the chromosome rather than on a plasmid (Postic et al. 1994).

The finding that the infectivity to ticks of needle-infected birds is much lower than that of tick-infected birds has also been observed in natural rodent reservoir hosts and has been correlated with the quality of the immune response to *B. burgdorferi* (Kurtenbach et al. 1994). For birds, the mechanism underlying this difference in infectivity remains to be determined. The differences observed in the current study may have been as much related to the particular strains or genospecies of *B. burgdorferi* involved or to the different ages of the birds, as to the different modes of infection.

All 3 pheasants that had PCR positive skin samples transmitted *B. burgdorferi* s.l. to xenodiagnostic ticks, indicating the presence of viable borreliae in the birds' skin (see above). However, because 2 of the 5 birds that transmitted infections to ticks yielded negative skin samples, PCR-based xenodiagnosis appears to be much more sensitive in detecting spirochetal infection in birds than direct amplification of *B. burgdorferi* DNA from skin. Therefore, reliance on PCR detection of spirochetes from avian skin biopsies may underestimate the prevalence of *Borrelia* infection in avian reservoirs,

Table 2. Number of *I. ricinus* on pheasants from a woodland site in Dorset

Sex	Winter			Summer		
	No. birds	Larvae	Nymphs	No. birds	Larvae	Nymphs
Male	272	0	1	29	7	27
		(0–3)	(0–14)		(0–64)	(0–123)
Female	263	0	0	22	0	5
		(0–2)	(0–7)		(0–7)	(1–47)

Median followed by range in brackets.

as has also been demonstrated for European rodent reservoir hosts (Petney et al. 1996).

In the course of repeated tick infestations, the experimental pheasants developed strong inflammatory reactions at the tick feeding sites, suggesting the possibility of acquired anti-tick immunity. Similar findings were observed in another natural host, the bank vole, *Clethrionomys glareolus* Schreber (Randolph 1994, Dizij and Kurtenbach 1995). The differential rates of recovery of alive and engorged larvae and nymphs (Table 1) indicates that the feeding success of larvae on this bird species is lower than that of nymphs, possibly caused by inflammatory responses. In fact, most of the introduced larvae died while attempting to feed on the pheasants. Our laboratory-based observations were consistent with (but not necessarily explanatory of) the field observations on natural infestation levels (Table 2). A similar observation on the differential feeding success of larvae and nymphs has been reported for chickens (Piesman et al. 1996).

It is now apparent that many of the major host species for *I. ricinus* in British woodlands contribute to the transmission of *B. burgdorferi* s.l., but they do so in different, complementary ways because they feed different fractions of the tick population. Small rodents (mice and voles) feed mainly larvae, but virtually no nymphs (Humair et al. 1993a, Kurtenbach et al. 1995, Randolph and Craine 1995), whereas pheasants show the reverse pattern, feeding large numbers of nymphs but many fewer larvae. Alongside these abundant rodent and bird hosts in woodlands are grey squirrels, *Sciurus carolinensis* Gmelin, less abundant but feeding large numbers of both larvae and nymphs (Craine et al. 1995) and known to be *B. burgdorferi*-competent hosts (Craine et al. 1997). In addition, roe deer, *Capreolus capreolus* L., play an important role in supporting the tick population by feeding large numbers of all 3 life stages (A.N.H., unpublished data).

In view of the current results we conclude that pheasants, which constitute a major part of the land-based avi-fauna in Britain, play an important role in the transmission dynamics of *B. burgdorferi* s.l.

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