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Seasonal breeding drives the incidence of a chronic bacterial infection in a free-living herbivore population

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SUMMARY

Understanding seasonal changes in age-related incidence of infections can be revealing for disentangling how host heterogeneities affect transmission and how to control the spread of infections between social groups. Seasonal forcing has been well documented in human childhood diseases but the mechanisms responsible for age-related transmission in free-living and socially structured animal populations are still poorly known. Here we studied the seasonal dynamics of *Bordetella bronchiseptica* in a free-living rabbit population over 5 years and discuss the possible mechanisms of infection. This bacterium has been isolated in livestock and wildlife where it causes respiratory infections that rapidly spread between individuals and persist as subclinical infections. Sera were collected from rabbits sampled monthly and examined using an ELISA. Findings revealed that *B. bronchiseptica* circulates in the rabbit population with annual prevalence ranging between 88% and 97%. Both seroprevalence and antibody optical density index exhibited 1-year cycles, indicating that disease outbreaks were seasonal and suggesting that long-lasting antibody protection was transient. Intra-annual dynamics showed a strong seasonal signature associated with the recruitment of naive offspring during the breeding period. Infection appeared to be mainly driven by mother-to-litter contacts rather than by interactions with other members of the community. By age 2 months, 65% of the kittens were seropositive.

Key words: Antibody optical density index, *Bordetella bronchiseptica*, European rabbit, host age, mother-to-litter transmission, seasonality, seroprevalence.

INTRODUCTION

Age-related seasonal forcing has been documented in childhood infections and shown to play an important role in driving pathogen dynamics [1, 2]. By definition, childhood diseases have an age-related transmission profile in that individuals are more likely to become infected when they are young and immunologically naive. This results in an inverse relationship between the age of infection and the force of infection [3, 4]. The seasonal force of infection contributes to the infection of susceptible hosts by enhancing individual exposure and facilitating transmission. For instance, the seasonal start of the school term increases the contact rates between children and facilitates measles transmission [5–7]. Human populations do not exhibit a strong seasonal birth pattern, unlike the majority of free-living animal populations, where the birth of susceptible juveniles usually occurs

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during a distinct breeding season. In this instance, the dynamics of infection are often driven by the seasonal breeding and recruitment of naive newborns into the pool of susceptibles [8–11].

Seasonality in the infection of naive offspring generates fundamental questions in disease epidemiology. For example: what is the route of infection of these newborns? and, is there a particular age group or class of individuals that infects the susceptible litter? While theoretical work has been very successful in underpinning how host heterogeneities affect transmission between social groups, applied studies have highlighted the complexity of individual interactions and the challenge of identifying who acquires infection from whom [12–15]. In structured animal populations with seasonal breeding, three non-mutually exclusive processes could be suggested: (i) parents act as a source of infection for their offspring through direct parent–newborn contacts during the pre-weaning/weaning period, (ii) infection occurs in the weaned offspring by contacts with non-breeding adult or sub-adult members of the community, or (iii) naive juveniles get infected by spillover from other host species (including vectors) through direct or indirect contacts. The objective of this study was to examine the first two hypotheses in a free-living rabbit population infected with the respiratory pathogen *Bordetella bronchiseptica* and where distinct seasonal pulses in host births, food availability and climatic conditions were observed.

Infection with *B. bronchiseptica* causes outbreaks in domesticated animals kept in crowded conditions such as kennels, livestock holdings or laboratory colonies [16–19]. In these settings, the bacterium is difficult to control and spreads rapidly between individuals; persistent infections can be life-long with few clinical symptoms or they can prove fatal when associated with other co-infecting pathogens [18, 20–22]. *B. bronchiseptica* has been isolated in a large number of wild animal species but few data are available on the epidemiology of infection in natural animal populations and the processes affecting the pathogen spread between individuals are poorly understood [23, 24].

In this study, rabbits were sampled monthly for 5 years and serologically tested for *B. bronchiseptica* in order to identify whether seroprevalence and antibody levels exhibited inter-annual fluctuations with a seasonal signature and an age-related pattern of infection. The role of host heterogeneities on transmission was further explored to identify the age and status of the individuals responsible for the majority of the infection.

MATERIAL AND METHODS

The host–pathogen system

To understand the dynamics of *B. bronchiseptica* infection in the European rabbit (*Oryctolagus cuniculus*) it is important to consider the social organization in the host population. Rabbits are structured into social groups that live in multi-chamber burrows accessible from different entrances. Each group represents a stable breeding community with both dominant females and males and usually with high reproductive rates [25, 26]. Individuals are highly philopatric and natal dispersal appears to be male biased [25, 27]. Breeding females defend their nest and show a high level of aggression against other females in close proximity or unrelated juveniles; however, their hostility may decrease if community densities are high or few nesting sites are available [28, 29]. The stressful consequences of breeding can be alleviated by the presence of litter sisters with the reproductive females [30]. Newborns emerge from the burrows at age 3–4 weeks after being in close contact with their mother and siblings. Once weaned, the young feed on vegetation close to the burrow. In seasonal climatic regimens, reproduction is concentrated within a few months of the year. For example, in our study population in Scotland the majority of breeding (defined hereafter as breeding season) mainly occurs between April and July [10].

B. bronchiseptica is a Gram-negative bacterium belonging to the genus *Bordetella*, which also includes the aetiological agents of whooping cough in humans, *B. pertussis* and *B. parapertussis*. *B. bronchiseptica* colonizes the respiratory tract causing infections of varying severity, ranging from generally asymptomatic to upper and incidental lower respiratory disease [22, 31]. The majority of studies on the progression of *B. bronchiseptica* infection have focused on the within-host response, particularly during the initial acute phase in laboratory conditions [32–34]. *B. bronchiseptica* develops as a persistent infection that follows an inflammatory phase where the activation of both the innate and acquired immune response is observed [35, 36]. Infection clears from the lungs and trachea by about 90 days post-infection but persists chronically in the nasal cavity up to at least 140 days post-infection in rabbits [36] or 270 days

post-infection in mice [32]. The common route for infection is primarily through oro-nasal transmission (contact or sneezing/aerosol) with chronic carriers potentially acting as a constant source of infection [24]. *B. bronchiseptica* can survive up to 24 weeks in water-based media at 10 °C but indirect transmission appears rare [37].

Data collection

A free-living population of European rabbits was sampled every month from 2005 to 2009 at a study area in Tayside (Scotland), as part of a regular pest control procedure applied by the local landowner under UK regulations. Blood samples collected from each rabbit were centrifuged for 10 min at 13 000 rpm and the serum isolated and stored at −20 °C. Rabbit characteristics, i.e. sex, age, female breeding conditions (enlarged nipples if lactating/nursing or number of foetuses if pregnant) and biometry were collected as described by Cattadori *et al.* [10, 38]. Rabbit age and month of birth were estimated using body and eye lens mass and body measurements [10, 38]. Rabbits were classified in six age groups where each group represents a 1-month age increment, from age 2 months (individuals aged 1 month were not sampled) to $\geqslant 7$ months. For a more general classification, kittens were aged 2–3 months, juveniles aged 4–5 months and adults aged $\geqslant 6$ months [10]. To capture the sexually active and/or dominant males and examine their effect on the newborns, adult male rabbits were classified in two main groups: seropositive adult males (all males in age groups 6 and 7) and seropositive old males (only males in age group 7) with testes mass >2 g, since it was not possible to distinguish the breeding males on clearer characteristics. Significant changes in the testes/body mass ratio of adult males were recorded between months: values were high between January and May, coinciding with the pre- and breeding period, and the lowest during the summer–autumn months [generalized linear model (GLM): D.F. = 11, 290; $P<0{\cdot}001$].

Serological detection of *B. bronchiseptica*

Based on the sequence homology (>95%) and antigenic cross-reactivity between *B. pertussis* and *B. bronchiseptica* proteins, a number of studies have utilized antibodies raised against purified *B. pertussis* proteins to distinguish expression of homologues by *B. bronchiseptica* [39]. To detect antibodies to *B. bronchiseptica* in wild rabbits we used an acellular *B. pertussis* vaccine as the source of antigen [adolescent/adult dose TDaP (Adacel), prepared by Sanofi-Pasteur, USA] in an enzyme-linked immunosorbent assay (ELISA) developed in the current study. This vaccine comprised of purified *B. pertussis* proteins [5 μg/0·5 ml filamentous haemagglutinin (FHA), 3 μg/0·5 ml pertactin (PRN), 5 μg/0·5 ml fimbriae types 2 and 3 and 2·5 μg/0·5 ml detoxified pertussis toxin] in combination with diphtherial and tetanus toxins. The entire contents of an acellular *B. pertussis* vaccine vial (0·5 ml) were mixed with 0·2 M carbonate/bicarbonate coating buffers (9·5 ml, pH 9·6) and 100 μl/well of this antigen mixture was used to coat 96-well ELISA plates (Greiner Bio-One, USA) overnight at 4 °C. Antigen-coated plates were blocked with 5% skimmed milk in PBS-0·1% Tween-20, pH 7·4 (PBS-T) and incubated with either wild or control sera at a dilution of 1:250 in blocking buffer for 1 h at 37 °C, followed by detection with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin antibodies at 37 °C for 1 h (1:5000, Southern Biotechnology, USA). Plates were washed four times with PBS-T between incubations, developed with 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma-Aldrich, USA) for 15 min and read spectrophotometrically at 405 nm. Values were expressed as immunosorbent optical densities (OD). Positive and negative control sera were collected from laboratory-maintained New Zealand White rabbits either positive or naive to *B. bronchiseptica* (our own laboratory experiments and also kindly provided by Covance Inc., USA) and were included on each plate. All sera were tested in duplicate. Negative wild rabbits were inferred as those individuals with OD values equal to or below the negative controls, cases above this cut-off were classified as positive. The specificity and validity of the established cut-off for the ELISA was further confirmed by Western blotting of 86 sera, ranging from negative to positive OD at ELISA, and using the vaccine as antigen source. Briefly, 125 μl of the vaccine solubilized 1:1 in Laemmli's buffer (Bio-Rad, USA) were resolved on 6% SDS–PAGE gels and transferred to Immobilon-P PVDF membranes (Millipore, USA). Membranes were cut into strips and each strip probed with selected sera at a dilution of 1:1000 followed by detection with enhanced chemiluminescence technology (Pierce Biotechnology, USA). The identity of the targeted proteins (FHA and PRN) was independently verified by performing Western blotting of the vaccine antigen

with specific antibodies to FHA and PRN (data not shown).

Data analyses

Initially and to confirm the consistency of the ELISAs in plates, the relationship between OD of positive and negative controls was examined. Plates were repeated if the positive-negative control relationship across all plates showed a Pearson's correlation coefficient (r) $<0{\cdot}70$. For every sample the antibody OD values were then standardized into an OD index as:

$$X_i = \frac{\text{OD test sample} - \text{OD negative control}}{\text{OD positive control} - \text{OD negative control}},$$

where X_i represents a replicate for each individual at every sampling point; the average of the two serum replicates [$X_i = \text{mean}(X_1 + X_2)$] was then estimated for each individual at each sampling point [40]. Data analyses were based on seroprevalence and standardized antibody OD indexes.

To highlight seasonal and long-term changes in *B. bronchiseptica* dynamics a spectral analysis was used on time-series of monthly seroprevalence data and antibody OD indexes from seropositive individuals, between 2005 and 2009. This procedure allowed us to detect the frequency of the most recurrent fluctuations in the time-series, using a fast Fourier transform. The output was visually represented through a spectrogram of the relationship between spectral density and cycle period (1/frequency); the dominant cycle period exhibited the highest spectrum density. To identify the host and environmental variables that mostly affected *B. bronchiseptica* infection in the rabbit population, GLMs were applied where seroprevalence, or OD index, were used as a response variable and host sex, age, breeding status, month and year of sampling were treated as independent factors. Different combinations of independent variables were tested and the most parsimonious models were presented and discussed.

RESULTS

Population data and serological detection of *B. bronchiseptica*

The annual number of rabbits sampled (mean $\pm$ S.D.: $262 \pm 18{\cdot}125$) and their sex ratio (male/female mean ratio $\pm$ S.D.: $1{\cdot}26 \pm 0{\cdot}19$) were consistent over the 5 years of monitoring (χ^2 for both $P > 0{\cdot}05$). In contrast, the juvenile–adult ratio was significantly higher in 2005 and lower in 2007 (juvenile–adult ratio from 2005 to 2009, respectively: 0·38, 0·22, 0·18, 0·27, 0·36; $\chi^2 = 10{\cdot}130$, D.F. $= 4$, $P < 0{\cdot}05$). The recruitment of newborns generally peaked in June, while there were no offspring sampled between September and December and few collected in January and February.

The combination of ELISA and Western blotting based on commercially available multi-component acellular *B. pertussis* vaccine as source of antigen, successfully allowed the detection of serum antibodies to *B. bronchiseptica* in wild rabbits of different age and sex and should thus provide a suitable general approach for identifying seropositive cases in wild populations. Furthermore, the specificity of the procedure was confirmed by Western blotting: a strong reactivity to FHA and PRN was detected in positive but not negative controls (see Supplementary Fig. S1, available online) and matched the samples classified as positive or negative by ELISA.

Annual, seasonal and age-related trends

Monthly seroprevalence and antibody OD indexes were consistently similar between host sexes (female *vs.* male mean $\pm$ S.D., seroprevalence: $94{\cdot}4 \pm 5\%$ *vs.* $93{\cdot}7 \pm 5\%$; OD index on positive cases: $0{\cdot}099 \pm 0{\cdot}029$ *vs.* $0{\cdot}106 \pm 0{\cdot}034$; GLM for all: $P > 0{\cdot}05$) as such, data from both sexes were combined unless specified. Across the sampled population, *B. bronchiseptica* seroprevalence was remarkably high but significantly different among the years: the lowest prevalence was recorded in 2006 and the highest in 2009 (mean annual percentage $\pm$ S.D.: $92 \pm 8\%$, $88 \pm 13\%$, $96 \pm 7\%$, $96 \pm 8\%$, $97 \pm 5\%$ from 2005 to 2009, respectively, $\chi^2 = 147{\cdot}537$, D.F. $= 56$, $P < 0{\cdot}001$; Supplementary Fig. S2, online). Within the year, seroprevalence exhibited a clear seasonal signature; values were at the lowest in May but increased and remained relatively high from July to February (Fig. 1*a*). The antibody OD index from positive cases confirmed this seasonal pattern, mean serological values decreased from April to June and increased thereafter until December (Fig. 1*a*). Overall, both the number of *B. bronchiseptica* cases and associated OD index significantly increased with host age and differed between month of sampling (Figs 1*a*, 2, for all GLM, $P < 0{\cdot}01$; full analysis in Supplementary Table S1, online). Temporal fluctuations in the infection were confirmed by the spectral analysis that showed a strong 1-year cycle for seroprevalence as well as OD index (Fig. 3).

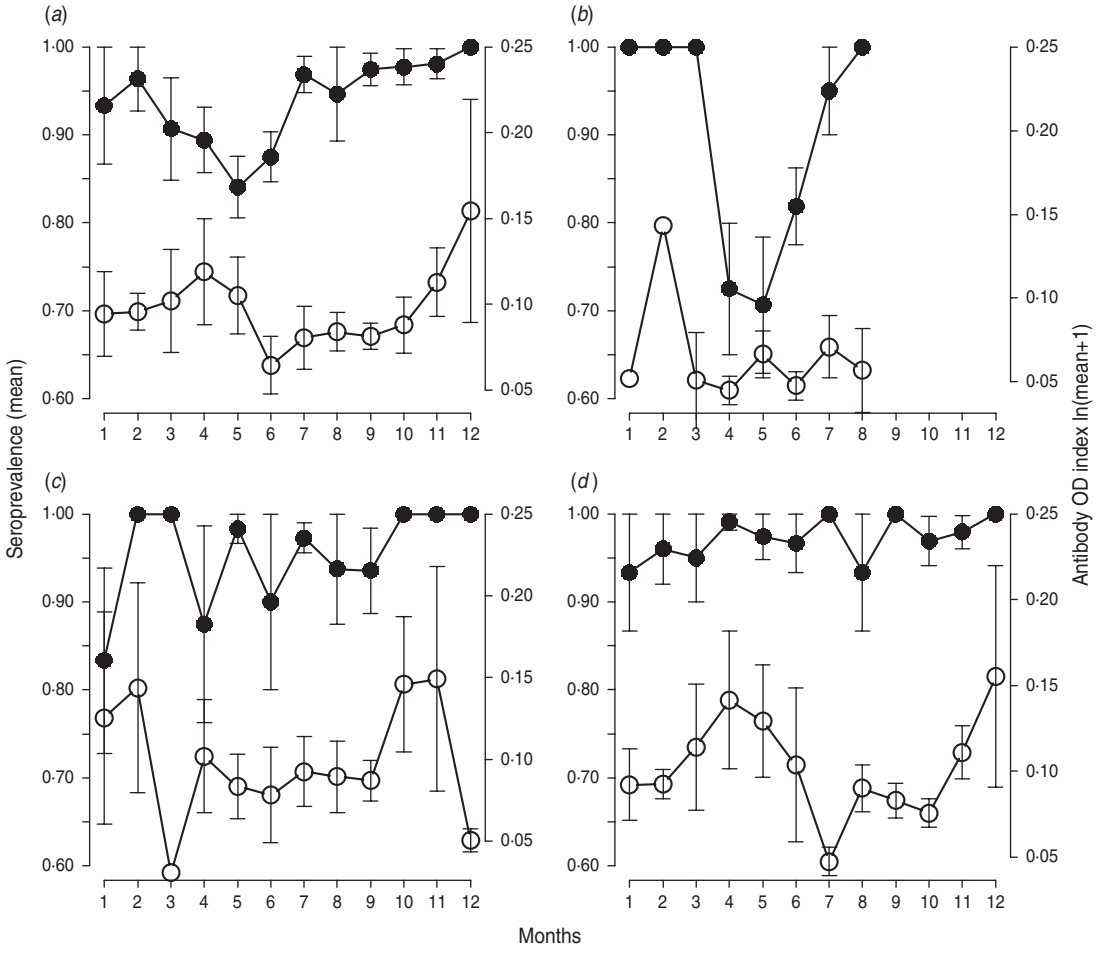

Fig. 1. *B. bronchiseptica* infection in a wild rabbit population based on serological analysis from 2005 to 2009. Monthly (mean ± S.E.) prevalence (●) and optical density (OD) index (○) (only positive cases used) in: (*a*) total sampled population, (*b*) kittens, (*c*) juveniles, (*d*) adults.

To highlight age-related temporal changes in the infection, analyses were repeated using the three main age groups: kittens, juveniles and adults. Prevalence was at the lowest in May for kittens, high and relatively constant in adults and showed a tendency for monthly fluctuations in juveniles (Fig. 1*b–d*). Antibody OD index also showed monthly changes for each age group (Fig. 1*b–d*). Nevertheless, for each age group neither the prevalence nor the OD index were statistically significant among months. The spectral analysis repeated for the three age groups' monthly time-series showed that, over 5 years of monitoring, seroprevalence fluctuated with a 2·5-year cycle in adults and a 1-year cycle in juveniles and kittens, while for the OD index the cycle period was 1 year in adults and kittens and 2·5 years in juveniles (Supplementary Fig. S3, online). All together, these findings suggest that *B. bronchiseptica* incidence increases during the breeding season as newborns become infected but also there may be a decay in the immunological protection of the adults.

Host heterogeneities and time of infection

To explore the hypotheses of pathogen transmission either through parent-to-litter interactions or non-breeding individual-to-litter contacts, monthly changes in the number of seropositive cases, and associated mean antibody level, were examined in the relationship between 2- or 3-month-old kittens and breeding females (nursing/pregnant individuals), sexually active and/or dominant males (adult males in age groups 6 and 7 or only in age group 7) or sexually mature non-breeding groups (adult females and juveniles of both sexes).

The number of 2-month-old positive kittens was positively associated to the number of infected breeding females sampled the previous month (time

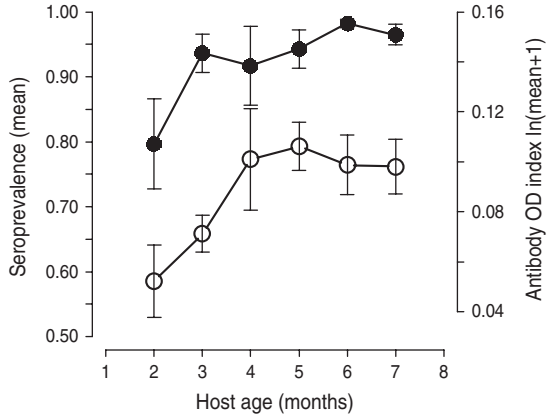

Fig. 2. Changes in *B. bronchiseptica* infection (monthly mean ± S.E.) by host age in a wild rabbit population sampled from 2005 to 2009. ●, Seroprevalence; ○, antibody optical density (OD) index.

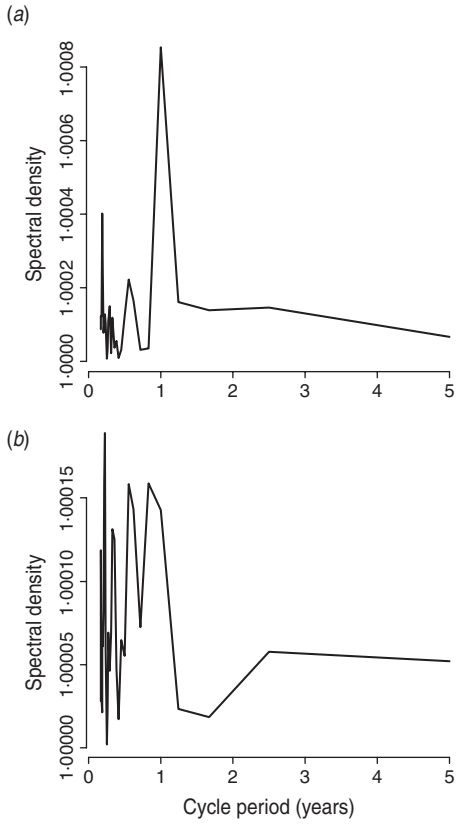

Fig. 3. Spectrogram of spectral density *vs.* cycle period (1/frequency) of *B. bronchiseptica* time-series for (*a*) seroprevalence and (*b*) optical density index data.

lag 1 GLM, coefficient ± S.E.: 0·678 ± 0·227, D.F. = 16, $P < 0{\cdot}003$; Table 1, Fig. 4*a*). This analysis was repeated using the antibody OD index and a similar positive relationship was observed between these two

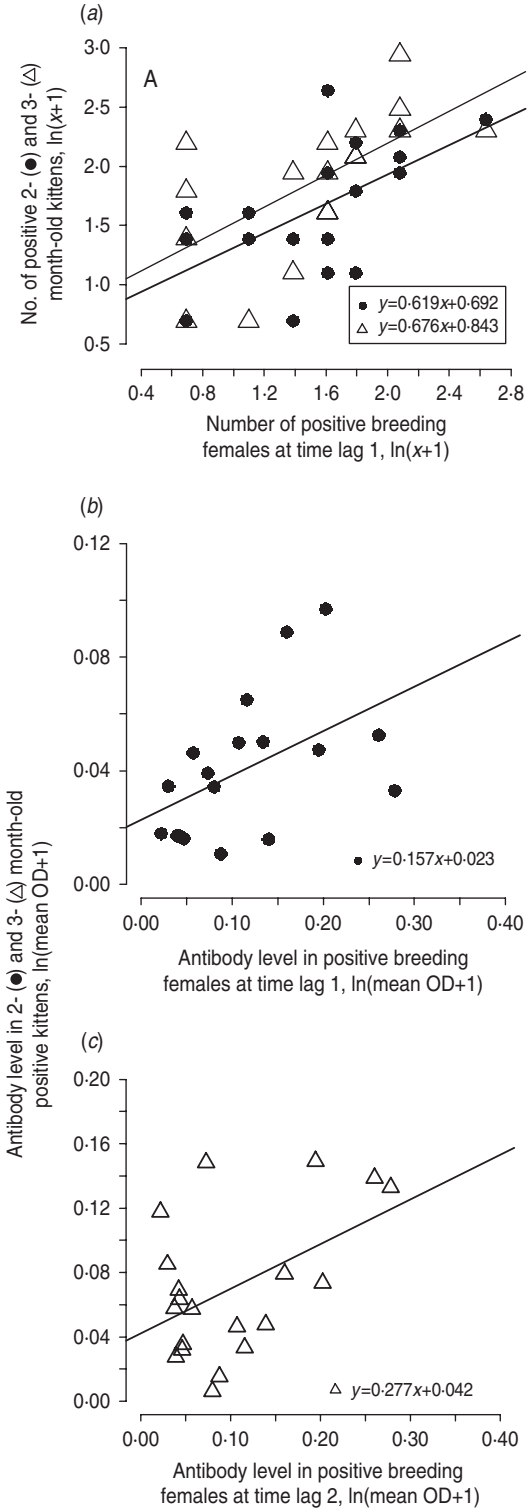

Fig. 4. Relationship between infection of *B. bronchiseptica* in seropositive 2- or 3-month-old kittens and seropositive breeding females. (*a*) Monthly number of positive cases with breeding females sampled the previous month (time lag 1). Monthly mean optical density (OD) index from positive cases with breeding females sampled: (*b*) the previous month (time lag 1) or (*c*) 2 months earlier (time lag 2).

Table 1. *Summary of generalized linear models between 2- or 3-month-old kittens, as a response variable, and other age-related demographic groups as independent variables. Analysis based on number of positive cases, or optical density (OD) index from positive cases. Models with a direct (data collected the same month, i.e. time lag 0) or delayed (data shifted by 1 or 2 months, i.e. time lag 1 or lag 2) relationship between the response and the independent variable were examined (Gaussian errors). The sign of the relationship (+ or −) and the significant relationships [$P(\chi^2)$ value] are reported*

	Positive hosts	Time lag	Breeding females	Non-breeding adult females	Adult males	Old adult males with big testes	Juvenile females	Juvenile males
2-month-old kittens	No. of cases	0 month	+n.s.	−n.s	−n.s.	−n.s.	−0·053	−n.s.
		1 month	+0·003	−n.s.	+n.s.	+n.s.	−n.s.	−0·027
	OD index	0 month	+n.s.	−n.s.	+n.s.	+n.s.	+n.s.	+n.s.
		1 month	+0·029	+n.s.	+n.s.	+n.s.	−n.s.	+n.s.
3-month-old kittens	No. of cases	0 month	+n.s.	−0·048	−0·010	−n.s.	−n.s.	−n.s.
		1 month	+0·003	−n.s.	+n.s.	+n.s.	−0·012	−0·004
		2 month	+n.s.	+n.s.	+0·0003	+n.s.	+n.s.	−0·028
	OD index	0 month	−n.s.	−n.s.	−n.s.	+n.s.	+n.s.	+n.s.
		1 month	+n.s.	−n.s.	+0·0005	−n.s.	+n.s.	−n.s.
		2 month	+0·014	−n.s.	−n.s.	−n.s.	−n.s.	+n.s.

demographic groups (time lag 1 GLM, coefficient ± S.E.: 0·138 ± 0·063, D.F. = 16, $P < 0·029$; Table 1, Fig. 4*b*). The number of *B. bronchiseptica* positive cases and antibody level were also examined between 2-month-old kittens and non-breeding adult females, adult males or old males with large testes as well as juveniles of both sexes, in the same month or at time lag 1. Overall, no significant relationships were found between kittens and these population groups (Table 1). A similar set of analyses was undertaken focusing on 3-month-old kittens. The number of positive 3-month-old kittens was related to the number of positive breeding females sampled the previous month (time lag 1 GLM, coefficient ± S.E.: 0·819 ± 0·274, D.F. = 16, $P < 0·003$; Table 1, Fig. 4*a*). A positive association was also recorded between the antibody levels of positive 3-month-old kittens and infected breeding females sampled 2 months earlier (time lag 2 GLM, coefficient ± S.E.: 0·269 ± 0·110, D.F. = 18, $P < 0·014$; Table 1, Fig. 4*c*). Additionally, a significant positive relationship was observed between the number of positive 3-month-old kittens and the number of positive adult males at time lag 2, as well as between their antibody levels at time lag 1 (Table 1). Both positive non-breeding adult females and adult males showed a consistent negative association with the number of positive 3-month-old kittens (Table 1). These kittens were also negatively related with the number of infected juveniles (Table 1). Collectively, these findings support the hypothesis that seropositive breeding females represented the first source of *B. bronchiseptica* infection for the 2- to 3-month old kittens, while seropositive adult males only partially contributed to this pattern.

DISCUSSION

The high seroprevalence recorded within and between the 5 years of study indicates that *B. bronchiseptica* is a common infection in the natural rabbit population we monitored. The long-term dynamics of infection showed a 1-year cycle in prevalence and antibody level of positive cases, a pattern consistent in the three age groups: kittens, juveniles and adults. The annual signature was mainly driven by the seasonal pulse of susceptible newborns and probably changes in the level of immune protection and the potential for annual re-infections, or infection reactivation, in adults and juveniles. The analysis of intra-annual dynamics confirmed the strong age-related seasonality in the pathogen–rabbit interaction: by age 2 months 65% of

the kittens had already seroconverted and by September 94% of the population was positive.

The social structure of the host population played a major role in affecting pathogen transmission. Number of seropositive kittens was positively associated with an increase in the number of positive breeding females, which supports the hypothesis that mothers represented the first source of infection for their litters. The repeated mother-to-litter oro-nasal contacts in the nest and the stressful conditions associated with breeding may have facilitated or enhanced bacterial transmission even in mothers with an immune response or a low oro-nasal bacterial load. In this respect, our recent study on the immunoepidemiology of *B. bronchiseptica* in rabbits showed that despite a relatively high antibody response (IgG and IgA), individuals shed bacteria periodically through oro-nasal contacts up to 140 days post-infection [36]. Strenuous conditions during post-parturition could also have facilitated the development of disease and the potential for transmission, as observed in cats infected with *B. bronchiseptica* [41]. These results suggest that seropositive breeding females can act as a focal source of infection in natural systems with a strong family structure or parental care of the offspring.

A consistent positive relationship in the antibody level was found between 2- or 3-month-old kittens and breeding females sampled 1 or 2 months earlier (time lags 1 or 2). As newborns grow older maternal immunity, probably involving IgA antibody, wanes and acquired immunity develops [18, 42]. While not conclusive it is possible that kittens' quality (i.e. the ability to mount a strong immune response) is affected by the conditions of breeding females (i.e. health and feeding status) at the month of birth. For example, based on the modelling of this rabbit population, we previously found that the health of breeding female and offspring contributed to the seasonal dynamics of infection of the gastrointestinal nematode *Trichostrongylus retortaeformis* [11]. We did not find strong supportive evidence for an early infection of the litter by other seropositive members of the community. For instance, it is possible that the observed negative relationship between seropositive kittens and non-breeding positive adult females was associated with the territorial behaviour and intra-sexual aggression of mothers against other adult females [26, 28]. Similarly, the negative relationship observed between number of positive kittens and positive juveniles can be explained as an inverse density-dependent relationship between the number of newborns and the dispersal of juveniles, mainly males [43, 44]. We were not able to distinguish between males in breeding and non-breeding status, moreover, the old males with large testes did not appear to play a major role in kitten infections. We did find a positive effect of the total number of seropositive adult males on 3-month-old positive kittens suggesting that there may be some potential infection of kittens by adult males. Previous studies found that dominant males defend the burrow, fight other males for hierarchy and breed with females [28, 29]. Therefore, and as in our system, while they are probably important for pathogen transmission among the older members of the group, adult males appear only to marginally contribute to the infection of individuals aged a few weeks' old.

This study described the epidemiology of infection of *B. bronchiseptica* in a free-living rabbit population monitored for 5 years using serological analyses. Both seroprevalence and OD index generated trends similar to the dynamics of a persistent infection but with annual outbreaks. The combination of ELISA and Western blot represented a reliable measure of relative changes in the systemic immune response of the rabbit to a pathogen infection. We have proposed a possible mechanism of transmission for *B. bronchiseptica*; however, more detailed studies are needed to disentangle the relative contribution of different social groups and how they vary across time and with changes in individual status. In particular, we need to understand the movements and contact rates between mothers and litter as well as the timing of development of social interactions as individuals become older and, ultimately, the long-term dynamics of infection and/or re-infection. More broadly, further studies are needed to understand the dynamics of transmission in socially structured wild animal populations if we aim to apply successful control measures to prevent disease spread across different social groups.

NOTE

Supplementary material accompanies this paper on the Journal's website (http://journals.cambridge.org/hyg).

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DECLARATION OF INTEREST

None.

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