THE TRANSMISSION CYCLE OF *BORRELIA BURGDORFERI* AND THE ASSOCIATION WITH LYME BORRELIOSIS RISK IN SCOTLAND, UK

M C James[1], L Gilbert[2] and A S Bowman[1]

[1]School of Biological Sciences, Aberdeen, AB24 2TZ, UK, [2]Macaulay Institute, Craigiebuckler, Aberdeen, AB15 8QH, UK • email: m.james@macaulay.ac.uk

BACKGROUND

Lyme borreliosis (LB) is a tick-borne enzootic infection caused by spirochaetes of the *Borrelia burgdorferi* sensu lato species complex. *B. burgdorferi* is carried and transmitted by the bites of Ixodes ticks and is the most prevalent arthropod-borne disease in the Europe and North America. Human cases of LB have increased by 350% over the past decade in the UK, and in Scotland 171 cases were reported in 2006.

Twelve members of the species complex have been isolated and three been shown to be pathogenic (*B. afzelii, B. garinii* and *B. burgdorferi* ss). The genospecies differ phenotypically, not only in their pathogenicity (resulting in different clinical symptoms), but also in their ecology (e.g. host preferences). The transmission cycle is complex, small mammals and birds support pathogen transmission but feed relatively few ticks, whereas deer support huge tick populations but are thought to "cleanse" feeding ticks of *B. burgdorferi*. Habitat and environmental factors may also play a role.

AIMS AND OBJECTIVES

To analyse the risk of acquiring *Lyme borreliosis* in Scotland by examining the transmission cycle of *Borrelia burgdorferi* sensu lato in Ixodes ricinus ticks and their hosts and relating this to human cases.

Fig 1. *Ixodes ricinus* ticks.
Image courtesy of Alan Smith

In particular we need to address the following questions:

1. How do *B. burgdorferi* genospecies and patient cases vary geographically and with habitat?
2. Which hosts and habitats are associated with LB risk?

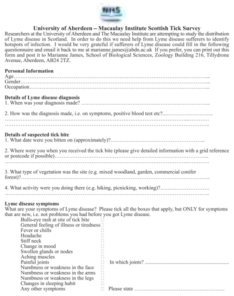
Fig 2. Transmission cycle of *Ixodes ricinus*
Image courtesy of EUCLAB

MATERIAL AND METHODS

Methods 1: Patient survey

LB patients are questioned regarding where they received the infective tick bite and on the clinical manifestations of their disease in order to associate this with the genospecies of *B. burgdorferi* (e.g. dermatological, rheumatological, neurological symptoms etc).

Fig 3. *Erythema migrans* rash (occurs in 85% of cases).
Image courtesy of T. Castell



University of Aberdeen – Macaulay Institute Scottish Tick Survey
Researchers at the University of Aberdeen and The Macaulay Institute are attempting to study the distribution of Lyme disease in Scotland. In order to do this we need help from Lyme disease sufferers to identify hotspots of infection. I would be very grateful if sufferers of Lyme disease could fill in the following questionnaire and email it back to me at marianne.james@abdn.ac.uk If you prefer, you can print out this form and post it to Marianne James, School of Biological Sciences, Zoology Building 216, Tillydrone Avenue, Aberdeen, AB24 2TZ.

Personal Information
Age……
Gender……
Occupation……

Details of Lyme disease diagnosis
1. When was your diagnosis made? ……
2. How was the diagnosis made, i.e. on symptoms, positive blood test etc?……

Details of suspected tick bite
1. What date were you bitten on (approximately)?……
2. Where were you when you received the tick bite (please give detailed information with a grid reference or postcode if possible)……
3. What type of vegetation was the site (e.g. mixed woodland, garden, commercial conifer forest)?……
4. What activity were you doing there (e.g. hiking, picnicking, working)?……

Lyme disease symptoms
What are your symptoms of Lyme disease? Please tick all the boxes that apply, but ONLY for symptoms that are new, i.e. not problems you had before you got Lyme disease.
- Bulls-eye rash at site of tick bite ☐
- General feeling of illness or tiredness ☐
- Fever or chills ☐
- Headache ☐
- Stiff neck ☐
- Change in mood ☐
- Swollen glands or nodes ☐
- Aching muscles ☐
- Painful joints ☐ In which joints? ……
- Numbness or weakness in the face ☐
- Numbness or weakness in the arms ☐
- Numbness or weakness in the legs ☐
- Changes in sleeping habit ☐
- Any other symptoms ☐ Please state ……

Fig 4. Patient Questionnaire

Methods 2: Environmental survey

- Sites which have yielded infected tick bites were identified through patient questionnaires.
- 16 paired woodland sites were sampled twice a year (once during spring and once in summer).
- Eight main sites were blanket dragged to collect questing ticks and small mammals trapped to collect feeding ticks. Eight minor sites were blanket dragged for questing ticks.
- All sites had surveys of the vegetation and large mammals including deer (dung counts).
- Ticks have also been collected from samples of the inguinal skin of deer shot at the study sites.

Fig 5. Removing a tick from a woodmouse (*Apodemus sylvaticus*).
Image courtesy of R. Ross

Fig 6. Blanket dragging for ticks.
Image courtesy of R. Ross

Other host species

Additional ticks are being collected from birds in a collaboration with Glasgow University.

RESULTS

Results 1: Patient survey

More than 50 questionnaires have been returned by LB patients who were bitten in a variety of locations across Scotland (Fig 7). The majority of patients received their infective tick bite in a wooded area (68%) and were working or enjoying a recreational activity whilst bitten (Fig 8).

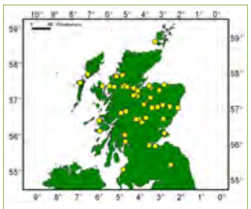


Fig 7. Location of infected tick bite

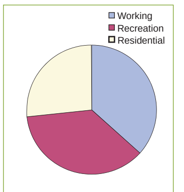


Fig 8. Primary activity of patient when bitten.

Results 2: Environmental survey

Over 30,000 ticks were collected between April-September 2007. Of these, 100% of blanket collected ticks were identified as Ixodes ricinus. Of the ticks collected form small mammals, 91% were *I. ricinus*, 5% *I. trianguliceps* and 4% were unidentifiable.

A series of generalised linear models (GLMs) have been constructed to test the relationships between tick abundance and the variables measured in the field (Figure 9). Nymph abundance increased with increased red deer dung, roe deer dung, higher temperature and higher relative humidity. There was also a difference between the coniferous and deciduous forest types, with significantly higher nymph abundances found in the deciduous forests. No relationship was found between vegetation height and nymph abundance.

Variable	DF	F value	P value
Red Deer	5	5.22	<0.001
Roe Deer	6	5.01	<0.001
Relative Humidity	72	2.36	<0.001
Temperature	185	1.23	0.028
Forest Type	1	45.34	<0.001
Vegetation Height	21	1.26	0.0189

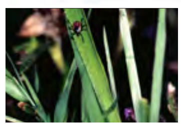
Fig 9. Generalised linear model explaining the relationships between nymph abundance (logN+1) and field variables measured.

Laboratory analysis

A nested PCR of the spacer region between 5S and 23S rRNA gene has been used to test the ticks for *B. burgdorferi* sl. Between 0-12% of ticks tested have been found to be positive. Melting point analysis of the PCR products will be used to determine the genospecies of the positive ticks.

CONCLUSION

It is possible to acquire Lyme borreliosis in many different locations and habitats across Scotland, however, the risk is not uniform. Survey data indicates that most human cases of LB originate in woodland and in the woodlands surveyed, red deer presence is the strongest predictor of tick numbers. However, these woodlands do not necessarily have a high prevalence of *B. burgdorferi*. Ongoing molecular analysis will identify which sites do so and the factors which may determine this will be explored. The relationships between clinical symptoms, location of tick bite and the genospecies present and ecology at that site will also be investigated. It is anticipated that the results of this work will provide a detailed understanding of the transmission cycle of *B. burgdorferi* in Scotland. It is possible that the information gathered can be used to implement prevention and protection measures.



Acknowledgements: We would like to thank the numerous land owners who have given permission to use their land as study sites and in particular the stalkers, managers and residents who helped me in the field. MCJ is extremely grateful for the help of two field assistants, Antoinette Wiebe and Elaine O'Gara. We would also like to thank the Clinicians at the microbiology unit of Aberdeen Royal Infirmary for their help and advice and the Lyme disease patients who have answered the questionnaires. MCJ is in receipt of a BBSRC-DTG studentship, EO'G of a Welcome trust scholarship and LG of a Nuffield Science Bursary.