Eur J Wildl Res
DOI 10.1007/s10344-013-0704-0

ORIGINAL PAPER

# Observations on the epidemiology and interactions between myxomatosis, coccidiosis and helminth parasites in a wild rabbit population in Scotland

**Brian Boag · Alexander D. Hernandez · Isabella M. Cattadori**

Received: 7 November 2012 / Revised: 8 February 2013 / Accepted: 8 February 2013


**Abstract** A study of the epidemiology of myxomatosis and the protozoan liver parasite, *Eimeria stiedae*, in a population of wild rabbits in Scotland from 1977 to 2010 is reported. Rabbits were collected on a monthly basis resulting in a total of 5,337 animals examined for the infections. The investigation showed that within any 1 year over the 34 years of the investigation the percentage of rabbits with myxomatosis varied between 0 and 24 %, while for *E. stiedae* infections fluctuated between 3 and 42 %. There were strong seasonal trends in the prevalence of myxomatosis with over 16 % being infected in September and October, and for *E. stiedae*, peaks of over 40 % of livers infected were recorded in July. From 2007 to 2010, faeces were also examined for coccidia oocysts and parasitic nematode eggs. Rabbits infected with the myxoma virus had mean oocyst counts of 73,665 per gram faeces, while rabbits not infected with the myxoma virus had counts of 31,952 oocysts per gram. Comparable figures for nematode faecal egg counts were 911 per gram in myxomatosis-infected animals and 427 per gram in uninfected animals. The elevated nematode faecal egg counts in rabbits with myxomatosis reflects increased worm burdens already reported, but the elevated counts of coccidial oocysts have not previously been reported. This would suggest that myxomatosis could compromise rabbit immunity to nematodes and coccidia.

Communicated by C. Gortazar

B. Boag (✉)
The James Hutton Institute, Invergowrie,
Dundee DD2 5DA, Scotland, UK
e-mail: Brian.Boag@hutton.ac.uk

A. D. Hernandez · I. M. Cattadori
Center for Infectious Disease Dynamics, Millennium Science Complex, The Pennsylvania State University, University Park 16802 PA, USA

**Keywords** European rabbit · Coccidiosis · Parasitic nematodes · Scotland · Myxomatosis · Co-infections

## Introduction

Myxomatosis is a seasonal viral disease of the European rabbit (*Oryctolagus cuniculus*) in most parts of the world where there is an insect vector and can cause high mortality in infected animals (Ross and Tittensor 1986). In Great Britain, an estimated 99 % of infected rabbits died when myxoma virus was first introduced in 1953 (Thompson and Warden 1956), but by 1962, attenuated strains of the virus were detected in 64 % of sick rabbits examined (Fenner and Chapple 1965). From 1960, rabbit survival increased (Thompson 1994) until 1997 when a significant steep decline in numbers was observed, possibly linked to the spread of rabbit haemorrhagic disease in wild rabbits (Aebischer et al. 2011). Mykytowycz (1959) reported that myxomatosis was associated with increased populations of the stomach worm *Graphidium strigosum*, and Boag (1988) showed that myxomatosis-infected wild rabbits had significantly greater populations of the small intestine nematode *Trichostrongylus retortaeformis*, large intestine nematode *Passalurus ambiguus* and the small intestine cestode *Mosgovoyia pectinata*. Further observations by Cattadori et al. (2007) suggested that the myxoma virus interfered with the immune response, which the rabbit naturally develops to the nematode *T. retortaeformis* and was responsible for the elevated worm burdens. Jeklova et al. (2008) characterised the immuno-suppression in rabbits due to the myxoma virus. Immunity to macroparasites, e.g. nematodes, is normally considered to elicit a Th2 response, while immunity to micro-disease organisms, e.g. viruses, usually elicits a Th1 response

Published online: 24 February 2013

(Graham et al. 2003). However, Cattadori et al. (2007) suggested that in the myxomatosis/nematode co-infection model the Th1–Th2 dichotomy was not complete and rabbits can balance the virus nematode co-infection although increased *T. retortaeformis* numbers were observed in myxomatosis-infected rabbits.

Protozoan diseases belonging to the genus *Eimera* can be pathogenic in the European rabbit especially in farmed animals (Chapman 1929; Taylor et al. 2007). The majority of the *Eimeria* species are found in the small and large intestine with the exception being *Eimeria stiedae*, which occurs in the bile ducts and causes symptoms in the liver that are readily recognisable. The impact of *E. stiedae* in laboratory experiments has shown it to be pathogenic (Barriga and Arnoni 1979). In Australia, Mykytowycz (1962) found coccidiosis was the cause of death in 10–18 % of rabbits in two consecutive years, while Hobbs et al. (1999) suggested parasitism and coccidiosis did not appear to be an important mortality affecting wild rabbits. In New Zealand, Henning et al. (2008) diagnosed coccidiosis as the cause of death in only 1 % over a 3-year period, while Bull (1958) came to the conclusion that *E. stiedae* was well suited to control numbers of wild rabbits. In Scotland, over a 23-year period, Lello et al. (2005) indicated that rabbits showing symptoms of *E. stiedae* disease in their liver were 5.6 % lighter than individuals without symptoms. Epidemiological studies into the incidence of coccidiosis in Australia, New Zealand and Britain suggest that the infection occurs more commonly in young animals which may not have developed immunity to the infections (Mykytowycz 1962; Bull 1958; Cowan 1983; Oppelt et al. 2010). It had been considered that protozoan parasites, much like viruses, stimulate a Th1 host immune response, but Yun et al. (2000) showed that coccidiosis produced a combination of Th1 and Th2 responses although the former response was stronger. A recent paper by Berto-Moran et al. (2013) has also investigated the potential interactions and immunity between nematode and protozoan parasites with myxomatosis and rabbit haemorrhagic disease (RHD). Their study found that the size of coccidian and nematode populations helped explain the seasonal antibody prevalence to the myxoma virus but not RHD.

This paper reports the seasonal incidence of myxomatosis and *E. stiedae* and their epidemiology in wild rabbits using data from a long-term study, which also includes data from faecal analysis on the coccidian and nematode infection from 2007 to 2010. This study reports the impact of myxomatosis on protozoan parasites and the faecal output of parasitic nematode eggs in an age-structured wild rabbit population monitored long term.

## Materials and methods

Details on where and how 5,337 rabbit samples were collected from Eastern Scotland from January 1977 until December 2010 are reported in Boag et al. (2001). Briefly, in the laboratory, rabbits were weighed, sexed and for the majority of animals, the incidence and severity of symptoms of the *E. stiedae* infection in the liver (the severity of symptoms being classified into the five categories: no

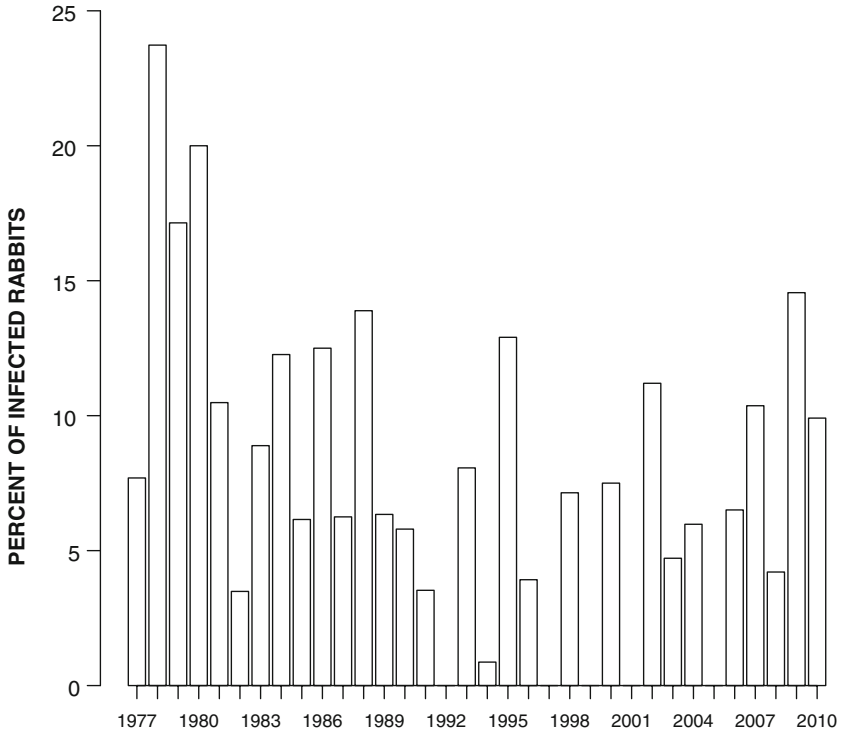


**Fig. 1** Percentage of rabbits infected each year with myxomatosis between 1977 and 2010

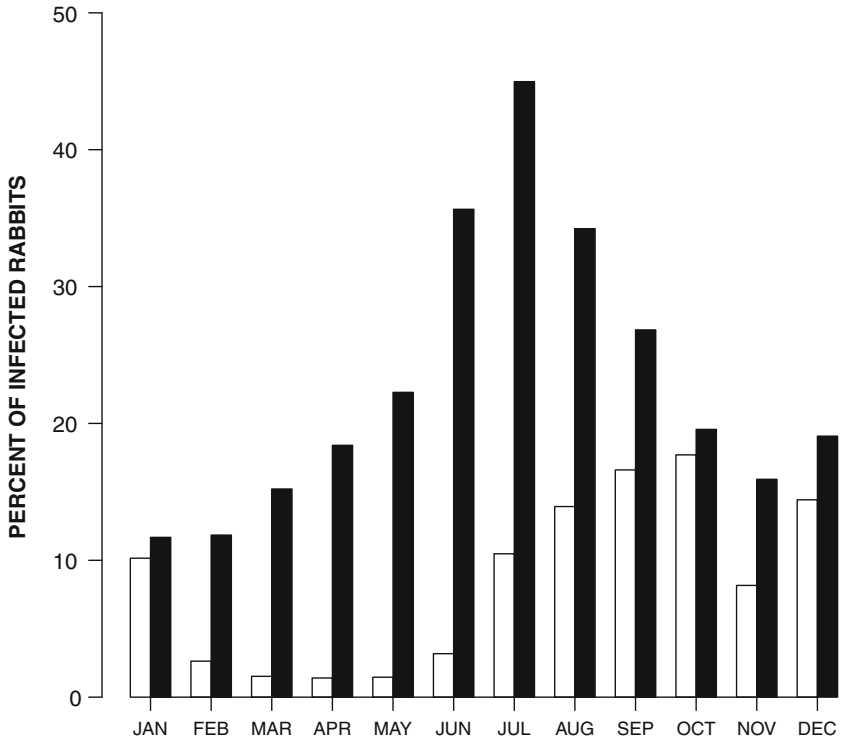


**Fig. 2** Seasonal changes in the percentage of rabbits infected with myxomatosis (*open bars*) and *E. stiedae* in the liver (*black bars*)

symptoms, light infection, moderate infection, heavy infection and very heavy infection), and incidence on myxomatosis was recorded. Myxomatosis was recorded when symptoms of the disease, i.e. swellings or discharges, were seen around the eyes, nose, base of ears and genitalia (Boag et al. 2001). The post-mortem procedure used to collect and identify the nematodes from the intestine followed that described by Boag (1985). From January 2007 to December 2010, 1,093 faecal samples were collected from the rectum of animals, and nematode egg and coccidia oocyst counts were made using McMaster slides (Gordon and Whitlock 1939), and this allowed us to indirectly quantify the size of infection in the rabbits due to nematodes and protozoa.

Statistical analysis of the changes in the myxomatosis and coccidiosis data throughout the years and seasons were undertaken using chi-squared tests. To analyse the impact of myxomatosis on the nematodes, *G. strigosum*, *T. retortaeformis* and *P. ambiguus* eggs shed a log ($x$+1) transformation failed to normalise the egg count data so a Wilcoxon rank sum test was used. Data from February to June were excluded when analyzing the impact of myxomatosis because the incidence of the disease was extremely low during these months (less than 5 %), and any association between the myxomatosis and either nematode or coccidia was likely to be minimal.

## Results

The data from the long-term study of 5,337 rabbits collected between January 1977 and December 2010 showed that the

**Table 1** Effect of age on the percentage of rabbits with different severities of *Eimeria stiedae* infection in the liver

| | Severity of infection | | | | | |
|---|---|---|---|---|---|---|
| Rabbit weight (g) | Number | Uninfected | Light | Moderate | Heavy | Very heavy |
| 0–250 | 58 | 100 | 0 | 0 | 0 | 0 |
| 251–500 | 681 | 90.3 | 6.9 | 1.2 | 0.7 | 0.9 |
| 501–750 | 600 | 43.5 | 42 | 4.8 | 3.3 | 6.3 |
| 751–1,000 | 595 | 40.5 | 45.7 | 5.6 | 3.5 | 4.7 |
| 1,001–1,250 | 668 | 65.3 | 30.8 | 1.5 | 1.2 | 1.2 |
| 1,251–1,500 | 1033 | 81.2 | 17.9 | 0.6 | 0.2 | 0.2 |
| 1,501–1,750 | 1244 | 83.5 | 15.9 | 0.4 | 0.8 | 0.1 |
| 1,751+ | 458 | 88 | 11.8 | 0.2 | 0 | 0 |

**Table 2** Distribution of symptoms of *E. stiedae* in livers of rabbits with and without myxomatosis

| | −Myxomatosis | | +Myxomatosis | |
|---|---|---|---|---|
| Severity of *E. stiedae* infection | Number | Percentage | Number | Percentage |
| Uninfected | 1,724 | 70.7 | 254 | 68.2 |
| Light infection | 632 | 25 | 107 | 28.8 |
| Medium infection | 32 | 1.7 | 8 | 2.2 |
| Heavy infection | 14 | 1.2 | 2 | 0.5 |
| Very heavy infection | 35 | 1.4 | 1 | 0.3 |
| Total | 2,437 | | 372 | |

prevalence of myxomatosis varied from 0 to 24 %, and that in four of the years, it was not detected at all (Fig. 1). Over the 33-year span, there was a strong seasonal trend in the numbers of rabbits infected with myxomatosis, the prevalence being over 16 % in September and October and less than 2 % in March, April and May (Fig. 2).

Infection with *E. stiedae* varied significantly across months and were most prevalent in summer during June, July and August when over 30 % of the rabbits were infected ($\chi^2$= 291, df=11, $p$ <0.0001, Fig. 2). The reason for this is explained by the fact that, overall, a significantly greater number of young animals were infected with *E. stiedae* ($\chi^2$=883, df=7, $p$ < 0.0001). Indeed, young rabbits, many of which were not weaned and weighed below 250 g, showed no signs of infection, but over 9 % of those between 251 and 500 g were infected (Table 1). The level of infection increased significantly to nearly 60 % of rabbits weighing 751–1,000 g, but then, the prevalence declined to 12 % for rabbits over 1,750 g (Table 1).

The results from a subset of the 1977–2010 data (2,809 rabbits, which excluded February, March, April, May and June when myxomatosis was rarely seen) indicated that the prevalence and severity of *E. stiedae* in either myxomatosis-infected and uninfected rabbits was relatively similar ($\chi^2$= 7.05, df=4, $p$ =0.13, Table 2). However, analysis based on faecal coccidial oocysts from rabbits collected between January 2007 and 2010 (again excluding the months of February, March, April, May and June) showed that rabbits infected with myxomatosis had mean oocyst counts of 73,665 per gram compared with 31,952 per gram in uninfected animals (Table 3). This pattern was also reflected in the faecal nematode egg count where comparable differences were seen, i.e. 911 epg in myxomatosis-infected animals compared with 427 epg in uninfected animals. An examination of the post-mortem findings of all the rabbits collected from January 2007 to December 2010, the time when faecal samples were collected, showed that only the *T. retortaeformis* counts were statistically significantly higher (Wilcoxon rank sum test with continuity correction $W$= 34,778, $p$ value <0.001) in the myxomatosis-infected animals and that *G. strigosum* and *P. ambiguus* counts were significantly lower ($W$=17046, $p$ value=0.001 and $W$= 19646, $p$ value=0.039 respectively) (Table 3).

## Discussion

The seasonal epidemiological pattern of myxomatosis reported here shows that it continues to persist, albeit at a low level, even after 57 years of its introduction into the wild rabbit population in the British Isles. However, this pattern is at variance with that reported in Ross and Tittensor (1986) who found a small increase in the incidence of myxomatosis in the spring. The reason for this is unclear but may be due to their data being collected closer to the date of introduction of the disease to rabbits in the British Iles or the fact the data they used was mainly from Southern England.

The epidemiological pattern of *E. stiedae* symptoms in the wild rabbit in Scotland confirm that infections are mainly associated with young rabbits and therefore more prevalent in the summer, which is similar to what is found for

**Table 3** Faecal oocyst and nematode egg counts and *G. strigosum*, *T. retortaeformis* and *P. ambiguus* population in rabbits infected and uninfected with myxomatosis

| | −Myxomatosis | | +Myxomatosis | |
|---|---|---|---|---|
| | Faecal examination | | | |
| | Number | Mean oocyst per gram±SEM | Number | Mean oocyst per gram±SEM |
| Coccidia | 453 | 31,952±4,667 | 88 | 73,665±13,316 |
| | | Mean epg±SEM | | Mean epg±SEM |
| Nematode | 494 | 427±34 | 88 | 911±121 |
| | | Post-mortem examination | | |
| | | Mean nematode count±SEM | | Mean nematode count±SEM |
| *G. strigosum* | | 42±4.9 | | 15±3.3 |
| *T. retortaeformis* | | 941±50 | | 2,819±254 |
| *P. ambiguus* | | 464±124.7 | | 79±43.2 |

coccidiosis in New Zealand (Bull 1958), Australia (Stodart 1968), France (Voza et al. 2003; Gres et al. 2003) and Britain (Cowan 1983). Previous analysis of some of these data by Lello et al. (2005) suggested *E. stiedae* was associated with a loss in weight of 5.6 % but made no estimate of mortality due to this infection. However, if rabbits with *E. stiedae* symptoms in their livers that were classified as being heavy or very heavy were considered likely to die then mortality of rabbits in this study would be 8–9 % in rabbits weighing 501–1,000 g. Bull (1958) considered *E. stiedae* "well adapted as a density-dependent factor in the natural control of rabbit populations" but that the intensity of infection could vary from year to year and by differences in pasture management.

No relationship was found between the prevalence and severity of liver symptoms of *E. stiedae* infections in rabbits that were infected or not infected with myxomatosis. However, myxomatosis-infected rabbits had faecal oocyst count more than twice those of rabbits with no symptoms of myxomatosis. The reason for this anomaly will require further investigation but could be explained by either the presence of myxomatosis increased the number of *E. stiedae* oocysts in infected animals or the fact that the oocysts counted in the faeces may have been from the many other species of coccidiosis which inhabit the rabbit's intestine. The immunity to intestinal coccidiosis was highlighted by Yun et al. (2000), and the immunity to rabbit intestinal *Eimeria* species is known to exist (Pakandl et al. 2008) and may have been compromised by the myxoma virus which is also know to have an effect on *T. retortaeformis* (Boag 1988; Cattadori et al. 2007, 2008). The apparent increase in *G. strigosum* and *P. ambiguus* infections in myxomatosis-free rabbits may be due to an interplay of the immune response against these two nematode parasites with *T. retortaeformis* highlighted by Lello et al. (2004). Cuquerella and Alunda (2009) characterised the immuno-biological response to *G. strigosum* in experimentally infected rabbits, while Murphy et al. (2011) undertook single laboratory experiments with both *G. strigosum* and *T. retortaeformis* and confirmed the field observations stating "that immunity plays a key role in affecting the abundance of these nematodes and different immune mechanisms are involved in regulating the dynamics of each infection." Berto-Moran et al. (2013) found that, contrary to their expectations, there was an inverse relationship between prevalence of antibodies to the myxoma virus and the coccidian load. Our results would support their original hypothesis, which predicted that there would be a positive relationship between myxomatosis and coccidian oocyst counts. The precise reason for the disparity between their and our results is unclear, but one possibility may be that our data were obtained from wild free ranging rabbits of all ages in Scotland, while their results were obtained from adult rabbits constrained to 4-ha plots in a restocking experiment in Spain. Indeed, ecological and environmental factors, which differ between the Mediterranean and temperate regions of their and our study, cannot be discounted and should be considered in future studies (Berto-Moran et al. 2013). A recent review by Demas et al. (2011) has also highlighted the role of the immune system plays in the regulation of disease susceptibility and given rise to the field of eco-immunology. The data presented here highlight the potential differential impact a virus may have on both protozoan and helminth parasites and the complexity which may exist in trying to explain epidemiological patterns in diseases which occur in nature and the need for further laboratory investigations to unravel the mechanisms involved.

**Acknowledgment** We thank H A McLaren, S Wilson, D Allen and J Steele for permission to collect rabbits from the study areas. This study and ADH were supported by an NSF grant (DEB-LTREB 0716885) to IMC.

## References

Aebischer NJ, Davey PD, Kingdon NG (2011). National Gamebag Census: Mammal Trends to 2009. Game & Wildlife Conservation Trust, Fordingbridge (http://www.gwct.org.uk/ngcmammals)

Barriga OO, Arnoni JV (1979) *Eimeria stiedae*: weight, oocyst output and hepatic function of rabbits with graded infections. Exp Parasit 3:407–414

Berto-Moran A, Pacios I, Serrano E, Moreno S, Rouco C (2013) Coccidian and nematode infections influence prevalence of antibody to myxoma and rabbit hemorrhagic disease viruses in European rabbits. J Wild Dis 49:10–17

Boag B (1985) The incidence of helminth parasites from the wild rabbit *Oryctolagus cuniculus* (L.) in Eastern Scotland. J Helminthology 59:61–69

Boag B (1988) Observations on the seasonal incidence of myxomatosis and its interactions with helminth parasites in the European rabbit (*Oryctolagus cuniculus*). J Wild Dis 24:450–455

Boag B, Lello J, Fenton A, Tompkin DM, Hudson PJ (2001) Patterns of parasite aggregation in the wild European rabbit (*Oryctolagus cuniculus*). Int J Para 31:1421–1428

Bull PC (1958) Incidence of coccidian (Sporozoa) in wild rabbits, *Oryctolagus cuniculus* (L.) in Hawk's Bay, New Zealand. N Z J Sci 1:289–329

Cattadori IM, Albert R, Boag B (2007) Variation in host susceptibility and infectiousness generated by co-infection: The myxoma-*Trichostrongylus retortaeformis* case in wild rabbits. J R Soc Interface 4:831–840

Chapman J (1929) A study of coccidiosis in an isolated rabbit colony, the clinical symptoms, pathology, immunology, and attempted therapy of the disease. Amer J Hygiene 9(2):389–429

Cowan DP (1983) Aspects of the behavioural ecology of a population of the European wild rabbit *Oryctolagus cuniculus*, in southern England. Dissertation, University of London

Cuquerella M, Alunda JM (2009) Immunobiological characterization of *Graphidium strigosum* experimentally infection in rabbits (*Oryctolagus cuniculus*). Parasitol Res 104:371–376

Demas GE, Zysling DA, Beechler BR, Muehlenbein MP, French SS (2011) Beyond phytohaemagglutinin: assessing vertebrate immune function across ecological contexts. J Anim Ecol 80:710–730

Fenner F, Chapple PL (1965) Evolutionary changes in the myxoma virus in Britain. J Hyg 63:175–185

Gordon HM, Whitlock HV (1939) A new technique for the counting nematode eggs in sheep faeces. J Coun Sci Industr Res 12:50–52

Graham AL, Cattadori IM, Lloyd-Smith JO, Ferrari MJ, Landau I (2003) Transmission consequences of conifection: cytokines writ large? Trends in Parasit 23:284–291

Gres V, Voza T, Chaubaud A, Landau I (2003) Coccidiosis of the wild rabbit (*Oryctolagus cunniculus*) in France. Parasite 10:51–57

Henning J, Pfeiffer DU, Davies PR (2008) Mortality patterns over 3 years in a sparse population of wild rabbits (*Oryctolagus cuniculus*) in New Zealand, with an emphasis on rabbit haemorrhagic disease (RHD). Eur J Wildl Res 54:619–626

Hobbs RP, Twigg LE, Elliot AD, Wheeler AG (1999) Evaluation of the association of parasitism with mortality of wild European rabbits *Oryctolagus cuniculus* (L.) in southwestern Australia. J Parasitol 85:803–808

Jeklova E, Levaa L, Matiascovica J, Kovarcika K, Kudlackovaa H, Nevorankovaa Z, Psikala I, Faldyna M (2008) Characterisation of immunosuppression in rabbits after infection with the myxoma virus. Vet Microbiol 129:117–130

Lello J, Boag B, Fenton A, Stevenson IR, Hudson PJ (2004) Composition and mutualism among the gut helminths of a mammal host. Nature 428:840–844

Lello J, Boag B, Hudson PJ (2005) The effect of single and concomitant pathogenic infections on the condition and fecundity of the wild rabbit (*Oryctolagus cuniculus* ). Int J Parasit 35:1509–1515

Murphy L, Nalpas N, Stear M, Cattadori IM (2011) Explaining patterns of infection in free-living populations using laboratory immune experiments. Parasite Immunology 33:287–302

Mykytowycz R (1959) Effect of infection with myxomatosis virus on endoparasites of rabbits. Nature 183:555–556

Mykytowycz R (1962) Epidemiology of coccidiosis (*Eimeria* spp.) in an experimental population of the Australian wild rabbit, *Oryctolagus cuniculus* (L.). Parasitology 52:375–395

Oppelt C, Starkloff A, Rausch P, von Holst D, Rodel HG (2010) Major histocomatability complex variation and age-specific endoparasite load in subadult European rabbits. Mol Ecol 19:4155–4157

Pakandl M, Hlaskova L, Poplstein M, Neveceralova M, Vodicka T, Salat J, Mucksova J (2008) Immune response to rabbit coccidiosis: A comparison between infections with *Eimeris flavescens* and *E. intestinalis*. Folisa Parasitol 55:1–6

Ross J, Tittensor AM (1986) Influence of myxomatosis in regulating rabbit numbers. Mammal Rev 16:163–168

Stodart E (1968) Coccidiosis in wild rabbits, *Oryctolagus cuniculus* (L.), at four sites in different climatic regions in eastern Australia 1. Relationship with the age of the rabbit. Aust J Zool 16:69–85

Taylor MA, Coop RL, Wall RL (2007) Veterinary Parasitology. Blackwell, London

Thompson HV (1994) The rabbit in Britain. In: Thompson HV, King CM (eds) The European rabbit. Oxford University Press, Oxford, pp 64–107

Thompson HV, Warden AN (1956) The rabbit. Collins New Naturalist, London

Voza GV, Chabaud T, Landau I (2003) Coccidiosis of the wild rabbit (*Oryctolagus cuniculus*) in France. Parasite 10:51–57

Yun CH, Lillehoj HS, Lillehoj EP (2000) Intestinal immune responses to coccidiosis. Dev and Comp Immunology 24:303–324