

Longitudinal field study on bovine *Babesia* spp. and *Anaplasma phagocytophilum* infections during a grazing season in Belgium

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Abstract Anaplasmosis and babesiosis are major tick-borne diseases with a high economic impact but are also a public health concern. Blood samples collected in the spring, summer, and autumn of 2010 from 65 cows in seven different farms in Belgium were monitored with an indirect immunofluorescence antibody test to assess seroprevalence against these pathogens. Seroprevalences to *Babesia* spp. were measured as 10.7%, 20%, and 12.3% in spring, summer, and autumn, respectively, whereas seroprevalences to *Anaplasma phagocytophilum* were 30.8%, 77%, and 56.9%, respectively. A total of 805 *Ixodes ricinus* ticks were collected at the same time from both cattle (feeding ticks) and grazed pastures (questing ticks). The infection level of ticks, assessed by PCR assay, for *Babesia*

spp. DNA was 14.6% and 7.9% in feeding and questing ticks, respectively, whereas 21.7% and 3% of feeding and questing ticks were found to be positive for *A. phagocytophilum* cDNA. Fifty-five PCR-positive samples were identified by sequencing as *Babesia* sp. EU1, of which five from feeding ticks were positive for both *A. phagocytophilum* and *Babesia* sp. EU1. The high density of wild cervids in the study area could explain these observations, as deer are considered to be the main hosts for adults of *I. ricinus*. However, the absence of *Babesia divergens* both in feeding and questing ticks is surprising, as the study area is known to be endemic for cattle babesiosis. Increasing cervid populations and comorbidity could play an important role in the epidemiology of these tick-borne diseases.

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Introduction

Anaplasmosis and babesiosis are major tick-borne diseases with a high economic impact on livestock productivity over much of the world. Both diseases are also a public health concern and are caused by intraerythrocytic protozoa of the genus *Babesia* spp. and bacteria within the genus *Anaplasma*, respectively. *Ixodes ricinus*, the most prevalent tick species in northern and western Europe, is an important vector of both these pathogens (Baumgarten et al. 1999; Becker et al. 2009; Bonnet et al. 2009).

In Europe, cattle are known to be the host of *Babesia divergens*, the etiological agent of red water. *B. divergens* has also been found in humans and wild ruminants, roe (*Capreolus capreolus*), and red deer (*Cervus elaphus*), Duh et al. 2005; Zintl et al. 2011), although the designation of red deer as a host is still under discussion. *B. divergens* is endemic in the southern part of Belgium (Losson and Lefevre 1989) but is considered absent from other parts of the country

(Saegerman et al. 2007). To date, as far as we are aware, human babesiosis has never been reported in Belgium.

The availability of new molecular tools has considerably increased knowledge on the epidemiology and taxonomy of *Babesia* spp. A novel species recently described in Europe is *Babesia* sp. EU1, proposed as *Babesia venatorum* (Herwaldt et al. 2003). Roe deer are thought to be the natural host of this potentially zoonotic species. *Babesia* sp. EU1 is closely related to *Babesia odocoilei* (a parasite of white-tailed deer) and based on a 31-nucleotide difference over the complete 18S rRNA gene sequence has been placed phylogenetically in a sister group with *B. divergens* (Herwaldt et al. 2003). Detection of *Babesia* sp. EU1 has been recorded recently in Belgium (Lempereur et al. 2011). *Anaplasma phagocytophilum* is a polymorphic gram-negative bacterium that strictly parasitizes mammals; wild and domestic animals are the main reservoirs (Halos et al. 2010), but infection is potentially zoonotic causing human granulocytic anaplasmosis (HGA). The symptoms of this disease vary from asymptomatic infection to severe disease by way of nonspecific flu-like clinical signs (Bakken and Dumler 2008; Thomas et al. 2009). *A. phagocytophilum* causes hematologic changes characterized by bacteremia, leukopenia, and thrombocytopenia. The loss of white blood cells promotes suppression of the immune response and causes infected humans or animals to be more susceptible to other infectious pathogens (Woldehiwet 2006).

In cattle, *A. phagocytophilum* cause bovine granulocytic anaplasmosis (BGA), also called tick-borne fever (TBF), (Woldehiwet 2006). The symptoms associated with BGA are fever, a drop in milk production, joint swelling and abortion. In addition, many asymptomatic cases of infection are suspected (Pusterla and Braun 1997). In Europe, the majority of clinical cases are observed in June and September, when temperature and humidity are optimal for ticks and cattle are grazing tick-infested pasture (Pusterla et al. 1998). In southern Belgium, the first case was detected in 2005 (Guyot et al. 2011). BGA is thus regarded as an emergent disease in southern Belgium.

Because of numerous suspected cases of bovine anaplasmosis and the proximity of some cattle herds to wildlife habitats, this study was performed to measure seroprevalences for *A. phagocytophilum* and *Babesia* spp. in blood samples of cattle from farms in southern Belgium. The prevalence of *A. phagocytophilum* and *Babesia* spp. infection was also assessed in both feeding and questing ticks collected from cattle and grazed pastures.

Materials and methods

Eight farms in southern Belgium with a known history of anaplasmosis and/or babesiosis and where cattle are likely

to have contact with wildlife were selected (target sampling) (Fig. 1). Participation of the farms in the survey was voluntary.

Serological study

In each farm, ten females (Holstein, Belgian White Blue, or Montbeliard breed) greater than 1 year of age were selected at random. Whole blood was taken into a dry container tube during the spring (May–June), summer (July–August), and autumn (September) of 2010. Centrifugation was performed at 3,000 rpm for 5 min at room temperature, and collected sera were stored at -20°C in 1-ml aliquots, until further use.

Sera were analyzed using an indirect immunofluorescence antibody test (IFAT) developed for *B. divergens* according to Gray and Kaye (1991). A *B. divergens* strain originating from a clinical case in Cherain (province of Luxembourg, Belgium) was used as the antigenic source. A positive control serum was kindly provided by Prof Pfister (Ludwig Maximilians University of Munich). Sera were tested at a dilution of 1:40 and 1:80. *A. phagocytophilum* antibodies were also detected by IFAT, using an IFA detection kit (VMRD, Pullman, USA) at a 1:40 dilution.

Ticks collection and analysis

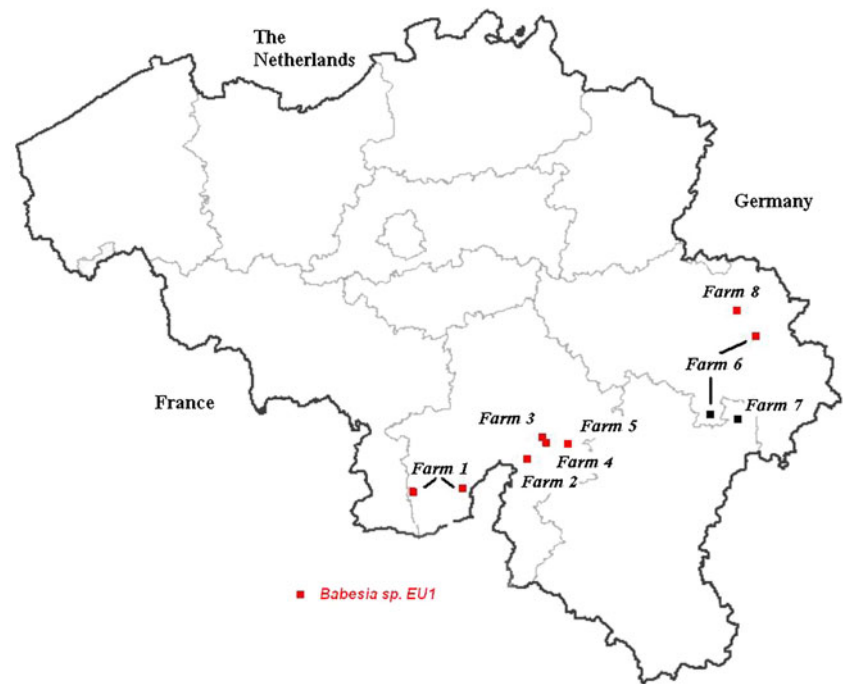
In the participating farms, ticks were collected monthly, between May and September 2010, both from cattle (feeding ticks) and from the environment (questing ticks). In two farms the animals were grazing on two distinct areas (Fig. 1). Ticks were trapped in grazed pastures by flagging with a 100 by 100-cm flannel cloth, performed when conditions were sufficiently dry (Bram 1978). Farmers collected ticks from cattle using forceps, mainly during milking.

Ticks were preserved in ethanol 70% and morphologically identified up to stage and species level using a standard key for morphological identification (Arthur 1963). Sex and level of repletion were also recorded.

Tick DNA extraction was performed on adults and nymphs using proteinase K (20 mg/ml) and the method of Boom et al. (1990). This method is based on lytic activity and nuclease-inactivating properties of proteinase K together with the nucleic acid-binding properties of silica particles following by an ethanol/acetone purification. To remove potential false-negative results due to polymerase chain reaction (PCR) inhibition and to validate the efficiency of the DNA extraction, an initial PCR test targeting the tick 16S rRNA gene was performed using 16S+1 and 16S-2 primers (Baumgarten et al. 1999). PCR-negative samples were subsequently diluted to try and remove any potential inhibition.

A *Babesia* spp. genus-specific PCR based on the 18S rRNA gene was performed on validated DNA extracts, as

Fig. 1 Map showing the selected farms. Farm 1 and farm 6 have two different exploitation sites. In red, *Babesia* sp. EU1 found in ticks collected from cattle and grazed pastures



described by Casati et al. (2006) and adapted by Lempereur et al. (2011) Tick DNA samples positive for *Babesia* spp. were purified using the Qiaquick PCR purification Kit (Qiagen, Germany). Cycle sequencing reactions were then performed in both directions, using a BigDye terminator v3.1 (3730 DNA analyzer; Applied Biosystems) by Giga Genomics Facility (Liège University, Belgium) and by DBS Genomics (Durham University, UK). A consensus sequence representing each PCR amplicon was generated and sequence comparison performed using BLASTn searches in GenBank (www.ncbi.nlm.nih.gov) and alignment with Clustal W software (Thompson et al. 1994).

Detection of *A. phagocytophilum* cDNA was performed by RT-PCR (ABI 7000, Applied Biosystems) using Adiavet® Ana Pha Realtime kit (Adiagene, Saint Brieux,

France). This diagnostic kit can detect to a sensitivity of three biovars of *A. phagocytophilum*.

Results

Serology

Blood samples were collected in spring, summer, and autumn from 65 cows on seven different farms (Table 1). Unfortunately, field conditions during the summer prevented the collection of blood samples from 15 animals of the initial group of 80 selected for sampling.

For *Babesia* spp. seroprevalences of 10.7%, 20%, and 12.3% were recorded in spring, summer, and autumn,

Table 1 Number of seropositive cows for *Babesia* spp. and *A. phagocytophilum*

	<i>Babesia</i> spp.			<i>A. phagocytophilum</i>		
	Spring	Summer	Autumn	Spring	Summer	Autumn
Farm 1	2	7	0	8	9	9
Farm 2	0	0	2	1	6	3
Farm 3	0	0	0	0	5	0
Farm 4	2	0	1	5	6	3
Farm 5	0	6	5	2	4	4
Farm 6	2	0	0	1	10	10
Farm 8	1	0	0	3	10	8
Total	7	13	8	20	50	37
Percentage	10.7	20	12.3	30.8	77	56.9

respectively. Only one farm had no *Babesia* seropositive animals recorded throughout the experimental period. All farms had *A. phagocytophilum*-positive animals. The seroprevalences were 30.8%, 77%, and 56.9% in spring, summer and autumn, respectively. Twelve (18.5%) and 17 (26.2%) cows were found to be either seronegative or seropositive throughout the complete period of the survey. Of the animals 55.3% presented a conversion in their serological status for *A. phagocytophilum*. Eleven cows (16.9%) presented two seroconversions during the study period of 5 months, recorded as positive from the spring and autumn samples but then negative in the summer or only detected as seropositive in sample collected in the summer. Seventeen animals were positive before turn out to pasture in the spring; all of them had been grazed the previous year. Seventeen cows were found to be seropositive for both *Babesia* spp. and *A. phagocytophilum* during the same period of sampling.

Ticks

A total of 805 ticks were collected. All were identified as *I. ricinus*, both from cattle ($n=180$) and pasture ($n=625$), as shown in Table 2. The infection rate for *Babesia* spp. in PCR-validated tick samples was estimated to be 14.6% and 7.9% in attached and questing ticks, respectively, without any seasonal pattern recorded (or obvious). Fifty-five positive samples were identified as *Babesia* sp. EU1 after sequencing (accession no. JF922092-JF922099), on the basis of demonstrating 98% to 100% identity with the GenBank reference sequence (accession no. AY046575). Two *Babesia*-positive samples could not be identified to the species level. *Babesia*-positive samples were found in all seven of the sampled farms (Fig. 1).

The overall tick infection rate for *A. phagocytophilum* was 8%, but strong differences were noticed according to the physiological status and stage of the ticks (feeding/questing; nymph/adult). Thus, 21.7% of the ticks collected from cattle were positive whereas only 3% of questing ticks gave a positive signal. However, almost all ticks collected from cattle were adults, whereas a majority of questing ticks were nymphs. Five female ticks collected from cattle were positive both for *A. phagocytophilum* and *Babesia* sp. EU1.

Discussion

Infection of cattle with the tick-borne pathogens, *Anaplasma* and *Babesia*, can have a serious impact on productivity, with the level of disease threat within a particular geographical region being strongly influenced by the species of these pathogens predominately transmitted by ticks. It is relevant, therefore, to have up-to-date information on the level of infection in cattle and the species of pathogens that are most

Table 2 *I. ricinus* collected between May and September 2010 from cattle and on grazed pastures

	Larvae	Nymphs		Adults	Screened	Validated	<i>A. phagocytophilum</i>		Percentage		<i>Babesia</i> spp.		Percentage
		Nymphs	Adults				Nymphs	Adults	Nymphs	Adults			
Cattle	May	0	3	96	99	84	0	17	20.2	0	6	7.1	
	June	0	0	24	24	21	0	13	61.9	0	0	0	
	July	0	1	9	10	10	0	0	0	0	0	0	
	August	0	0	44	44	39	0	4	10.3	0	17	43.6	
	September	0	1	2	3	3	0	0	0	0	0	0	
Total	0	5	175	180	157	0	34	21.7	0	23	14.6		
Pasture	May	0	229	27	256	239	3	3	2.5	8	1	3.8	
	June	77	157	1	158	151	3	0	2	5	0	3.3	
	July	0	0	0	0	0	0	0	0	0	0	0	
	August	56	42	1	43	25	4	0	16	10	1	44	
	September	15	20	1	21	14	0	0	0	0	1	64.3	
Total	148	448	30	478	429	10	3	3	3	31	3	7.9	
Total	148	453	205	658	586	10	37	8	8	31	26	9.7	

Nymph and adult ticks were validated and screened for *A. phagocytophilum* and *Babesia* spp. specific cDNA and genomic DNA material, respectively

likely to be transmitted by ticks within regions under threat of disease. In the current study, the serological survey for *Babesia* spp. gave a prevalence (14.3%), higher than the prevalence of 7.9% found in the same area by Losson and Lefevre (1989). The seroprevalence to *A. phagocytophilum* was also higher when compared to other previous European publications (Amusatogui et al. 2006; Ebani et al. 2008). The fact that all the participating farms participated on voluntary basis had a confirmed history of tick-borne disease and were located in a known endemic area could explain the high seroprevalences due to targeted sampling.

The high seroprevalence against *A. phagocytophilum* was detected despite evidence that antibodies titer rapidly wanes, as indicated by double seroconversion results. This finding clearly has implications for detection of the true level of pathogen challenge that cattle encounter, and for both for diagnostic and epidemiological studies, the timing of sampling is of critical importance and should be performed close to the peak period of tick activity. Nevertheless, a significant proportion (26%) of cattle were still positive before turn out to pasture suggesting that some cows remain as carriers throughout winter and may provide an overwintering reservoir population of *A. phagocytophilum* for feeding ticks in the spring.

BGA seems to be endemic in southern Belgium, as in a recent survey of 1,197 cattle, 320 tested positive for antibodies against *A. phagocytophilum* (ARSIA, unpublished data). This high level of infection and associated immunity could contribute to the relative lack of acute clinical cases of BGA in monitored herds. However, this may also be linked to *A. phagocytophilum* strain heterogeneity and vertebrate diversity (de la Fuente et al. 2005a; Granquist et al. 2010). Infection rates estimated for *Babesia* spp. in ticks were higher than generally expected for this region (Lempereur et al. 2011). This could well be due to targeted sampling, as mentioned above. However the high density of cervid populations in the study area may also have played a role. The infection rate found in ticks collected from cattle was also higher than that estimated for unfed questing ticks. It should be borne in mind that the ticks collected from cattle were all adult ticks, while the majority of those collected from the vegetation were nymphs. While the infection rates of both adult and nymph ticks will be influenced by the level of *Babesia* infection in the mammalian host, the known amplification of parasite in the tick salivary gland following a blood meal may increase the chances of detection by the PCR test. Furthermore, the adult population will be comprised of ticks that are transmitting and acquiring infection from a definitive host for *Babesia*, while the infection level in the nymph population will be predominately derived from adult female ticks (Zintl et al. 2003) that could, theoretically, have fed on a range of vertebrate hosts.

Babesia sp. EU1 was the only species found in both feeding and questing *I. ricinus*, despite the high number of

ticks that were screened and the selection of farms located in a region previously identified as endemic for *B. divergens*. The same finding was previously described by Becker et al. (2009), who found no evidence for *B. divergens* sporozoites in engorged adult or unfed *I. ricinus* ticks. Cattle and deer are the main hosts of adult *I. ricinus* ticks and are potential carriers of *B. divergens*. One possible explanation for our results could be that the main stage of *I. ricinus* transmitting *B. divergens* in the study area is the larvae after transovarial transmission. This, possibility would need further investigation, as the accepted major transmission route is via feeding adults following transovarian transmission.

As deer can probably be a reservoir host for *B. divergens* (Zintl et al. 2011), it may be that cattle play the same role for *Babesia* sp. EU1 in natural habitats characterized by deciduous forests with large populations of wild ruminants. This hypothesis has yet to proven, indeed experimental validation of cattle acting as a reservoir for a *Babesia* species of deer has only been tested on *Babesia capreoli* (Adam et al. 1976; Gray et al. 1990; Malandrin et al. 2010). If it can be validated that cattle can act as a reservoir for *Babesia* sp. EU1, it would raise implications for the epidemiology and control of this zoonotic parasite. Wild cervids appear to act as a reservoir host for *A. phagocytophilum* (Rosef et al. 2009) as do probably cattle (de la Fuente et al. 2005b; Halos et al. 2010).

Since 1975, wild ruminant populations have doubled in southern Belgium, despite an increase in hunting activity (SPW—DGO3 Agriculture-Ressources naturelles et Environnement-Département de la Nature et des Forêts 2011). Expanding populations of wild cervids that act as important hosts for maintaining tick populations and acting as a reservoir for zoonotic tick-borne pathogens have the potential to alter the balance between the parasite and additional, potentially human, hosts. Therefore, knowledge of common animal reservoirs for transmission of zoonotic *Babesia* sp. will be useful for modelling the risk potential of this infection in humans: the results of this study implicate cattle as well as cervids.

The high seroprevalence to *A. phagocytophilum* in cattle suggest that this pathogen is endemic in southern Belgium. It would be expected, therefore, that ticks and mammalian hosts would be coinfecting with *A. phagocytophilum* and additional tick-borne pathogens. Indeed, 17 cattle and five ticks demonstrated evidence of coinfection. It is known that coinfection of livestock, synchronous or not, with TBD can exacerbate disease morbidity and reduce host fitness (Zintl et al. 2003). Furthermore recent studies have provided evidence that interaction between tick-borne pathogen populations can influence TBD epidemiology. This can be manifest in a positive or negative manner and alter pathogen prevalence, pathogen transmission, and host susceptibility (Ginsberg 2008; Telfer et al. 2010). Therefore, to gain fuller insight

into the factors that influence risk from tick-borne disease, future studies should attempt to capture data on interacting parasite communities rather than individual parasite species.

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References

- Adam KM, Blewett DA, Brocklesby DW, Sharman GA (1976) The isolation and characterization of a *Babesia* from red deer (*Cervus elaphus*). *Parasitology* 73:1–11
- Amusatégui I, Sainz A, Tesouro MA (2006) Serological evaluation of *Anaplasma phagocytophilum* infection in livestock in northwestern Spain. *Ann N Y Acad Sci* 1078:487–490
- Arthur DR (1963) British ticks. Butterworth, London
- Bakken JS, Dumler S (2008) Human granulocytic anaplasmosis. *Infect Dis Clin North Am* 22:433–448, viii
- Baumgarten BU, Rollinghoff M, Bogdan C (1999) Prevalence of *Borrelia burgdorferi* and granulocytic and monocytic ehrlichiae in *Ixodes ricinus* ticks from southern Germany. *J Clin Microbiol* 37:3448–3451
- Becker CA, Bouju-Albert A, Jouglin M, Chauvin A, Malandrin L (2009) Natural transmission of zoonotic *Babesia* spp. by *Ixodes ricinus* ticks. *Emerg Infect Dis* 15:320–322
- Bonnet S, Brisseau N, Hermouet A, Jouglin M, Chauvin A (2009) Experimental in vitro transmission of *Babesia* sp. (EU1) by *Ixodes ricinus*. *Vet Res* 40:21
- Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-van Dillen PM, van der Noordaa J (1990) Rapid and simple method for purification of nucleic acids. *J Clin Microbiol* 28:495–503
- Bram RA (1978) Surveillance and collection of arthropods of veterinary importance. *Agric Handb* 518:108–109
- Casati S, Sager H, Gern L, Piffaretti JC (2006) Presence of potentially pathogenic *Babesia* sp. for human in *Ixodes ricinus* in Switzerland. *Ann Agric Environ Med* 13:65–70
- de la Fuente J, Massung RF, Wong SJ, Chu FK, Lutz H, Meli M, von Loewenich FD, Grzeszczuk A, Torina A, Caracappa S, Mangold AJ, Naranjo V, Stuen S, Kocan KM (2005a) Sequence analysis of the *msp4* gene of *Anaplasma phagocytophilum* strains. *J Clin Microbiol* 43:1309–1317
- de la Fuente J, Naranjo V, Ruiz-Fons F, Hofle U, Fernandez De Mera IG, Villanua D, Almazan C, Torina A, Caracappa S, Kocan KM, Gortazar C (2005b) Potential vertebrate reservoir hosts and invertebrate vectors of *Anaplasma marginale* and *A. phagocytophilum* in central Spain. *Vector Borne Zoonotic Dis* 5:390–401
- Duh D, Petrovec M, Bidovec A, Avsic-Zupanc T (2005) Cervids as Babesiae hosts, Slovenia. *Emerg Infect Dis* 11:1121–1123
- Ebani V, Cerri D, Fratini F, Ampola M, Andreani E (2008) Seroprevalence of *Anaplasma phagocytophilum* in domestic and wild animals from central Italy. *New Microbiol* 31:371–375
- Ginsberg HS (2008) Potential effects of mixed infections in ticks on transmission dynamics of pathogens: comparative analysis of published records. *Exp Appl Acarol* 46:29–41
- Granquist EG, Bardsen K, Bergstrom K, Stuen S (2010) Variant - and individual dependent nature of persistent *Anaplasma phagocytophilum* infection. *Acta Vet Scand* 52:25
- Gray JS, Kaye B (1991) Studies on the use of gerbil-derived *Babesia divergens* antigen for diagnosis of bovine babesiosis. *Vet Parasitol* 39:215–224
- Gray JS, Murphy TM, Taylor SM, Blewett DA, Harrington R (1990) Comparative morphological and cross transmission studies with bovine and deer babesias in Ireland. *Prev Vet Med* 9:193
- Guyot H, Ramery E, O'Grady L, Sandersen C, Rollin F (2011) Emergence of bovine ehrlichiosis in Belgian cattle herds. *Ticks Tick Borne Dis* 2:116–118
- Halos L, Bord S, Cotte V, Gasqui P, Abrial D, Barnouin J, Boulouis HJ, Vayssier-Taussat M, Vourc'h G (2010) Ecological factors characterizing the prevalence of bacterial tick-borne pathogens in *Ixodes ricinus* ticks in pastures and woodlands. *Appl Environ Microbiol* 76:4413–4420
- Herwaldt BL, Caccio S, Gherlinzoni F, Aspöck H, Slemenda SB, Piccaluga P, Martinelli G, Edelhofer R, Hollenstein U, Poletti G, Pampiglione S, Loschenberger K, Tura S, Pieniazek NJ (2003) Molecular characterization of a non-*Babesia divergens* organism causing zoonotic babesiosis in Europe. *Emerg Infect Dis* 9:942–948
- Lempereur L, De Cat A, Caron Y, Madder M, Claerebout E, Saegerman C, Losson B (2011) First molecular evidence of potentially zoonotic *Babesia microti* and *Babesia* sp. EU1 in *Ixodes ricinus* ticks in Belgium. *Vector Borne Zoonotic Dis* 11:125–130
- Losson B, Lefevre F (1989) La babesiose bovine en Belgique. Une enquête sérologique en zone d'endémie. *Ann Med Vet* 133:421–426
- Malandrin L, Jouglin M, Sun Y, Brisseau N, Chauvin A (2010) Redescription of *Babesia capreoli* (Enigk and Friedhoff, 1962) from roe deer (*Capreolus capreolus*): isolation, cultivation, host specificity, molecular characterisation and differentiation from *Babesia divergens*. *Int J Parasitol* 40:277–284
- Pusterla N, Braun U (1997) Clinical findings in cows after experimental infection with *Ehrlichia phagocytophila*. *Zentralbl Veterinarmed A* 44:385–390
- Pusterla N, Pusterla JB, Braun U, Lutz H (1998) Serological, hematologic, and PCR studies of cattle in an area of Switzerland in which tick-borne fever (caused by *Ehrlichia phagocytophila*) is endemic. *Clin Diagn Lab Immunol* 5:325–327
- Rosef O, Paulauskas A, Radzijeuskaja J (2009) Prevalence of *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* in questing *Ixodes ricinus* ticks in relation to the density of wild cervids. *Acta Vet Scand* 51:47
- Saegerman C, Claerebout E, Kalume M, Losson B (2007) Bovine babesiosis in Belgium: preliminary results of postal survey of veterinarians in 2006. *Renc Rech Rumin* 14:220
- SPW—DGO3 Agriculture-Ressources naturelles et Environnement-Département de la Nature et des Forêts, F.c. 2011. Report of Wild ruminants evolution in Belgium
- Telfer S, Lambin X, Birtles R, Beldomenico P, Burthe S, Paterson S, Begon M (2010) Species interactions in a parasite community drive infection risk in a wildlife population. *Science* 330:243–246
- Thomas RJ, Dumler JS, Carlyon JA (2009) Current management of human granulocytic anaplasmosis, human monocytic ehrlichiosis and *Ehrlichia ewingii* ehrlichiosis. *Expert Rev Anti Infect Ther* 7:709–722
- Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22:4673–4680
- Woldehiwet Z (2006) *Anaplasma phagocytophilum* in ruminants in Europe. *Ann N Y Acad Sci* 1078:446–460
- Zintl A, Mulcahy G, Skerrett HE, Taylor SM, Gray JS (2003) *Babesia divergens*, a bovine blood parasite of veterinary and zoonotic importance. *Clin Microbiol Rev* 16:622–636
- Zintl A, Finnerty EJ, Murphy TM, de Waal T, Gray JS (2011) Babesias of red deer (*Cervus elaphus*) in Ireland. *Vet Res* 42:7