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# The seroepidemiology of Lyme borreliosis in zoo animals in Germany

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## SUMMARY

We conducted the first seroepidemiological study to evaluate the exposure of zoo animals to *Borrelia burgdorferi* s.l. in German zoos and wildlife parks. A total of 1487 individuals representing 148 ungulate and carnivore species belonging to 19 families were examined using a non-species dependent ELISA. Specific antibodies were detected in 154 (10·4%) animals; 168 (11·3%) sera produced borderline results. The percentage of seropositive individuals was related to species and origin (zoo), and increased with age of the animals. Sex and season did not influence seroprevalence. Examination of 600 ticks (*Ixodes ricinus*; caught from vegetation in the zoos) by darkfield microscopy and indirect immunofluorescence technique revealed infection rates within the range typical for Central Europe. The results substantiate that there is an infection risk for zoo animals. A differential diagnosis of Lyme borreliosis should be taken into account in case of suspicious clinical symptoms and possible contact to ticks.

## INTRODUCTION

Emerging infectious diseases do not only involve many wildlife species as reservoirs of pathogens that threaten domestic animal and human health, but pose also a substantial threat to the conservation of global biodiversity [1]. One of these 'new' diseases possibly affecting endangered species is Lyme borreliosis: a zoonosis with world-wide distribution which is now regarded as the most important tick-borne disease in humans [2–4]. Unlike the situation in the USA, no region of endemic occurrence of *Borrelia*-infected ticks has so far been discerned in Germany and neighbouring European countries. The main vector in Europe – the hard tick *Ixodes ricinus* – is indigenous to the entire continent (except Iceland) between sea level and altitudes up to 2000 m [5, 6]. The pathogen *Borrelia burgdorferi* sensu lato (s.l.) of the family *Spirochaetaceae* causes a chronic multisystem disease with a great variety of clinical manifestations [6, 7]. Lameness as a result of either arthralgia or arthritis is the main symptom in all domestic animal species examined so far [8–13]. In contrast to its increasing importance in veterinary medicine, very few reports exist on clinical investigations on Lyme borreliosis in free-ranging animals [14–18]. Although clinical symptoms have been reported in a few wild species only [15, 17, 19], this disease might nevertheless directly affect free-ranging as well as captive wild animals and should therefore be considered as a possible cause of inexplicable population declines in endemic areas [20]. Unfortunately, there exists hardly any information on Lyme borreliosis in the literature on both zoo and wildlife medicine.

A hitherto unique serologic survey in zoo animals was carried out at the St. Louis Zoo [21]. In extension to that investigation the epidemiological study presented here is the first one which evaluates the exposure of a broad range of zoo animals in different

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age classes to *B. burgdorferi* s.l. and assesses the distribution of Lyme borreliosis in a variety of places and settings during different seasons throughout the year.

## METHODS

### Sampling

A total of 1667 blood samples (1241 ungulates and 426 carnivores) were examined originating from 1487 individuals and representing 148 ungulate and carnivore species belonging to 19 mammalian families. In case of two or more sera tested from one animal the mean of all test-values was calculated. The majority of sera came from 11 zoos and wildlife parks all over Germany (Fig. 1), the remaining samples were collected at 13 other German zoos. Sera were taken within a period of almost 30 years (1969–1998), with the majority being collected in the 1990s. Blood specimens were obtained from healthy as well as from diseased animals during various veterinary interventions. Data concerning the sex of the animals was available for 96% and regarding the age for 64% of the samples.

A total of 600 ticks were caught by sweeping low-lying vegetation of all accessible areas at the zoos with a cotton flag (flagging method). At the Serengeti Safaripark Hodenhagen, tick collection was only possible along the fence outside of the park. All ticks were collected between June 1997 and October 1998, identified as unfed stages of *Ixodes ricinus*. Until examination for the presence of *B. burgdorferi* s.l., they were kept cool and dark (refrigerator) in small screw-top tubes equipped with a piece of moist tissue for high humidity.

### Serological test for exposure of zoo animals to *B. burgdorferi* s.l.

Blood samples were tested for *B. burgdorferi* s.l. antibodies by means of a modified non-species dependent enzyme-linked immunosorbent assay (ELISA). Here, specific *B. burgdorferi* s.l. IgG antibodies were detected by peroxidase (PO) conjugated protein A or protein G as an (almost) universal label instead of species-specific secondary antibodies. The test was performed exactly as described elsewhere [22].

### Detection of *B. burgdorferi* s.l. in ticks

The ticks were examined for the presence of spirochetes by darkfield microscopy (DFM) and by the indirect immunofluorescence technique (IFT): nymphs and adult ticks were tested individually and larvae in pools of three. The infection rates of individual larvae ($p$) were calculated with: $p = 1 - \sqrt[4]{1-f}$, where $f$ is the proportion of infected larvae pools [23]. The unfed ticks were homogenized in 50 $\mu$l PBS/$MgCl_2$ (5 mM), and one drop of this suspension was examined under a darkfield microscope at a magnification of 250. For examination by IFT marked microscope slides with 12 patches (medco, Germany) were prepared by coating them with 10 $\mu$l tick suspension as well as with positive (culture of *B. burgdorferi* s.l., strain 1B29 = *B. garinii*) and negative (PBS) control fluid. The preparations were allowed to air dry and subsequently fixed in acetone for 5 min at −20 °C. A polyclonal rabbit anti-*Borrelia burgdorferi* s.l. (strain 1B29)-serum (generated in the laboratory) was added at a dilution of 1:200 in PBS. Subsequently, the slides were incubated in a wet chamber for 30 min at 33 °C, rinsed three times in PBS, and


**Fig. 1.** Main study sites of Germany: 1. Zoological Garden Berlin, 2. Tierpark Berlin-Friedrichsfelde, 3. Zoological Garden Leipzig, 4. Game Park Leipzig, 5. Zoo Dresden, 6. Tierpark Hagenbeck, Hamburg, 7. Serengeti Safaripark Hodenhagen, 8. Zoological Garden Cologne, 9. Zoological Garden Frankfurt, 10. Wilhelma Stuttgart, 11. Tierpark Hellabrunn Munich.

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dried carefully between sheets of filter paper. Finally, fluorescein (DTAF)-conjugated AffiniPure goat anti-rabbit IgG (H + L) (Jackson ImmunoResearch Laboratories, USA) was added at a dilution of 1:200 in PBS/Evans-blue. Final dilution of Evans-blue (Sigma-Aldrich, Germany) in PBS was 1:100 000. The samples were incubated, washed and dried as described above. The slides were coverslipped using glycerol buffer (90% glycerol in PBS) as enhancing fluid and examined under a fluorescence microsope at a magnification of 400.

### Statistics

Blood samples of zoo animals cannot be collected following a pre-set statistical design. Since the animals providing the sera were not chosen randomly from a population, the whole statistical analysis of sera must be regarded as exploratory data analysis. Statistical methods were used to look for patterns in data, but a 'significant' result is to be regarded as a tendency in the sample rather than as a statistical proof.

Stepwise logistic regression [24] was performed to evaluate the contribution of the independent variables origin/zoo (categories 1–11 are explained in Fig. 1, category 12 contains all samples from the 13 other zoos), species (one category per species), age (as continuous variable), sex (male and female) and season (four categories: December–February, March–May, June–August, September–November) to an individual probability of being infected. The polytomous variables (zoo, species, season) were decomposed into binary design variables by the SPSS program (SPSS Germany, München). A polytomous variable with $\langle k \rangle$ categories was replaced by $\langle k-1 \rangle$ design variables. Since the number of species design variables was too high compared with the number of sera, we excluded species with less than 10 individuals from this analysis, thus reducing the number of species to 51 (Table 1a). Interdependencies between pairs of categorical or binary variables were tested by the $\chi^2$ test [25] or, in case of small sample sizes, by its exact version. Pfanzagl's test [25] was used to verify a potentially monotonous trend in a $\langle r \times 2 \rangle$ contingency table. The significance level was generally set to $\alpha = 0{\cdot}05$.

The tick samples can be regarded as randomly collected. The infection rates of ticks in the various zoos were compared using the $\chi^2$ test and are listed with their 95%-confidence intervals (C.I.). The statistical calculations were performed using SPSS 9.0 software and the GraphPad InStat Version 2.

## RESULTS

### Blood samples

A total of 1487 ungulate and carnivore blood samples was examined by means of a modified non-species dependent enzyme-linked immunosorbent assay (ELISA). Specific antibodies were detected in 154 (10·4%) of the animals tested; 11·3% of the ungulates and 7·5% of the carnivores were seropositive. In addition, 168 (11·3%) sera produced borderline results. Seropositive samples came from 10 of the 11 main study sites (with the exception of Hodenhagen) and from 2 additional zoos. Seroprevalence varied from 3·4% (Hamburg) to 22·2% (Cologne). The results of ELISA testing for *B. burgdorferi* s.l. antibodies in sera of captive ungulates and carnivores are listed in Table 1a for all speices from which at least 10 samples were available. Positive sera were found in all families with more than 30 specimens tested, namely *Equidae*, *Camelidae*, *Cervidae*, *Bovidae*, *Ursidae*, and *Felidae*. Positive individuals were found in 57 of the 148 species included in this study (Table 1). In species of which at least 10 samples were accessible, the frequency of antibody response was highest in musk oxen (*Ovibos moschatus*, 72·7%), followed in descending order by mountain goats (*Oreamnos americanus*, 45·0%), Przewalski's horses (*Equus przewalskii*, 22·4%), and lions (*Panthera leo*, 22·4%). The age was known for 951 of the animals examined. The percentage of seropositive individuals ranged in different age classes from 3·7–17·0% (Fig. 2). The sex was known for 1426 animals; 626 being males and 800 being females. In the ELISA a total of 60 sera (9·6%) from males and 91 sera (11·4%) from females were positive. Only minor differences were observed between the percentages of positive individuals in spring (7·4%), summer (12·7%), fall (11·3%), and winter (9·3%).

Stepwise logistic regression for the occurrence of antibodies as dependent binary variable and zoo, species, age, sex, and season as the independent ones generated a final model based on only two predictors: species (log likelihood difference, $P < 0{\cdot}001$) and age (log likelihood difference, $P < 0{\cdot}001$). Because data was incomplete, sample size for logistic regression had to be reduced to 514 individuals. In order to include more test results, the relations were separately tested between seropositivity on one hand and origin (zoo), species, age, sex and seasonal differences on the other. Zoo ($\chi^2$ test, D.F. = 11, $P < 0{\cdot}001$, $n = 1319$), species ($\chi^2$ test, D.F. = 145, $P < 0{\cdot}001$, $n = 1319$), and

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Table 1. *Results of ELISA testing for* B. burgdorferi *s.l. antibodies in sera of captive ungulates and carnivores in German zoos*

| Species | | Sera tested | Number of sera | | |
|---|---|---|---|---|---|
| | | | + | ? | − |
| (a) Species with $n \geqslant 10$ samples | | | | | |
| Equids | Equidae | | | | |
| Domestic ass | *Equus africanus f. asinus* | 13 | 1 | 5 | 17 |
| Grevy's zebra | *Equus grevyi* | 18 | 1 | 3 | 14 |
| Mountain zebra | *Equus zebra* | 25 | 1 | 2 | 22 |
| Kulan | *Equus hemious kulan* | 12 | 2 | 0 | 10 |
| Przewalski's horse | *Equus przewalskii* | 98 | 22 | 15 | 61 |
| Somali wild ass | *Equus africanus somalicus* | 10 | 1 | 0 | 9 |
| Plains zebra | *Equus quagga* | 33 | 9 | 4 | 20 |
| Tapirs | Tapiridae | | | | |
| South American tapir | *Tapirus terrestris* | 10 | 2 | 1 | 7 |
| Camelids | Camelidae | | | | |
| Alpaca | *Lama guanicoe f. pacos* | 22 | 1 | 0 | 21 |
| Llama | *Lama guanicoe f. glama* | 20 | 1 | 0 | 19 |
| Two-humped camel | *Camelus ferus f. bactrianus* | 14 | 1 | 0 | 13 |
| Cervids | Cervidae | | | | |
| Wapiti | *Cervus elaphus bactrianus* | 11 | 0 | 0 | 11 |
| Eld's deer | *Cervus eldi thamin* | 10 | 1 | 0 | 9 |
| Fallow deer | *Dama dama dama* | 20 | 0 | 0 | 20 |
| Moose | *Alces alces alces* | 13 | 2 | 4 | 7 |
| Pere David's deer | *Elaphurus davidianus* | 14 | 0 | 0 | 14 |
| Red deer | *Cervus elaphus hippelaphus* | 37 | 0 | 0 | 37 |
| Reindeer | *Rangifer tarandus* | 13 | 1 | 3 | 9 |
| Sika deer | *Cervus nippon pseudaxis* | 20 | 0 | 1 | 19 |
| Thorold's deer | *Cervus albirostris* | 10 | 1 | 0 | 9 |
| Bovids | Bovidae | | | | |
| African buffalo | *Syncerus caffer caffer* | 17 | 2 | 0 | 15 |
| American bison | *Bison bison* | 14 | 2 | 1 | 11 |
| Banteng | *Bos javanicus* | 23 | 2 | 9 | 12 |
| Bharal, Blue sheep | *Pseudois nayaur* | 11 | 0 | 2 | 9 |
| Bohor reedbuck | *Redunca redunca* | 14 | 0 | 0 | 14 |
| Domestic cattle | *Bos primigenius f. taurus* | 21 | 2 | 3 | 16 |
| Domestic goat | *Capra aegagrus f. hircus* | 17 | 4 | 2 | 11 |
| Domestic sheep | *Ovis ammon f. aries* | 83 | 8 | 12 | 63 |
| European bison | *Bison bonasus* | 17 | 0 | 0 | 17 |
| Greater kudu | *Tragelaphus strepsiceros* | 10 | 0 | 0 | 10 |
| Himalayan tahr | *Hemitragus jemlahicus* | 10 | 0 | 0 | 10 |
| Blackbuck | *Antilope cervicapra* | 16 | 1 | 2 | 13 |
| Barbary sheep | *Ammotragus lervia* | 19 | 1 | 3 | 15 |
| Markhor | *Capra falconeri heptneri* | 12 | 5 | 2 | 5 |
| Mouflon | *Ovis ammon musimon* | 18 | 3 | 0 | 15 |
| Mountain goat | *Oreamnos americanus* | 20 | 9 | 2 | 9 |
| Musk ox | *Ovibos moschatus* | 11 | 8 | 0 | 3 |
| South African oryx antelope, Gemsbok | *Oryx gazella gazella* | 10 | 0 | 1 | 9 |
| Sable antelope | *Hippotragus niger* | 10 | 0 | 0 | 10 |
| Saiga antelope | *Saiga tatarica* | 31 | 1 | 1 | 29 |
| Scimitar-horned oryx | *Oryx gazella dammah* | 19 | 0 | 0 | 19 |
| Takin | *Budorcas taxicolor* | 11 | 3 | 3 | 5 |
| Waterbuck | *Kobus ellipsiprymnus* | 11 | 1 | 1 | 9 |
| Bears | Ursidae | | | | |
| European brown bear | *Ursus arctos arctos* | 11 | 0 | 1 | 10 |
| Polar bear | *Ursus maritimus* | 12 | 0 | 3 | 9 |

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Table 1 (*cont.*)

| Species | | Sera tested | Number of sera | | |
|---|---|---|---|---|---|
| | | | + | ? | − |
| Cats | Felidae | | | | |
| Jaguar | *Panthera onca* | 15 | 1 | 2 | 12 |
| Leopard | *Panthera pardus* | 59 | 8 | 13 | 38 |
| Lion | *Panthera leo* | 49 | 11 | 13 | 25 |
| Puma, couguar | *Puma concolor* | 12 | 0 | 2 | 10 |
| Tiger | *Panthera tigris* | 98 | 2 | 9 | 87 |
| Canids | Canidae | | | | |
| African wild dog | *Lycaon pictus* | 14 | 0 | 1 | 13 |
| (b) Species with $n<10$ samples and at least one positive reacting individual (alphabetical order) | | | | | |
| African wild cat | *Felis lybica* | 4 | 1 | 3 | 0 |
| Asian black bear | *Ursus thibetanus* | 6 | 1 | 0 | 5 |
| Bezoar | *Capra aegagrus cretica* | 9 | 1 | 1 | 7 |
| Bobcat | *Lynx rufus* | 2 | 1 | 0 | 1 |
| Dall's sheep | *Ovis dalli* | 3 | 1 | 2 | 0 |
| Dama gazelle | *Gazella dama* | 9 | 1 | 0 | 8 |
| Domestic horse | *Equus przewalskii f. caballus* | 5 | 2 | 1 | 2 |
| Forest buffalo | *Syncerus caffer nanus* | 9 | 4 | 2 | 3 |
| Gaur | *Bos gaurus* | 8 | 1 | 0 | 7 |
| Gayal | *Bos gaurus f. frontalis* | 4 | 1 | 0 | 3 |
| Guanaco | *Lama guanicoe* | 6 | 1 | 1 | 4 |
| Impala | *Aepyceros melampus* | 6 | 1 | 1 | 4 |
| Musk deer | *Moschus moschiferus* | 4 | 3 | 0 | 1 |
| Nilgai | *Boselaphus tragocamelus* | 7 | 2 | 1 | 4 |
| Nubian ibex | *Capra ibex nubiana* | 6 | 2 | 2 | 2 |
| Red duiker | *Cephalophus natalensis* | 1 | 1 | 0 | 0 |
| Sea lion | *Zalophus californianus* | 1 | 1 | 0 | 0 |
| Serval | *Felis serval* | 3 | 1 | 1 | 1 |
| Snow sheep | *Ovis nivicola* | 1 | 1 | 0 | 0 |
| Sunda sambar | *Cervus timorensis* | 3 | 1 | 0 | 2 |
| Vicuna | *Lama vicugna* | 5 | 1 | 0 | 4 |
| Water buffalo | *Bubalus arnee f. bubalis* | 9 | 2 | 1 | 6 |
| White rhinoceros | *Ceratotherium simum* | 3 | 2 | 1 | 0 |

age class ($\chi^2$ test, D.F. = 5, $P = 0{\cdot}001$, $n = 837$) influenced the serological state, but neither sex ($\chi^2$ test, D.F. = 1, $P = 0{\cdot}295$, $n = 1264$) nor season ($\chi^2$ test, D.F. = 3, $P = 0{\cdot}079$, $n = 1128$). To identify potential trends, six age classes were defined. The percentages of seropositive individuals in these classes displayed a monotonous trend from lower seroprevalences among younger to higher seroprevalences among older animals (Pfanzagl's test, $P < 0{\cdot}001$, $n = 837$; Fig. 2). In the separate comparisons of seropositivity with either origin (zoo), age, sex or season, the sample was composed of individuals of different species, a fact possibly confounding the analysis. Therefore, the set of tests was repeated for the Przewalski's horses only, as this is the species with the largest sub-sample and most of the positive test results. The six age classes were joined to three new classes (classes 1 + 2, 3 + 4, 5 + 6) in order to avoid empty classes. The results confirm the tendencies in the whole data set, according to which zoo ($\chi^2$ test, D.F. = 6, $P = 0{\cdot}027$, $n = 83$) and age class ($\chi^2$ test, D.F. = 2, $P = 0{\cdot}004$, $n = 50$) influenced the serological state, whereas sex ($\chi^2$ test, D.F. = 1, $P < 0{\cdot}313$, $n = 78$) and season ($\chi^2$ test, D.F. = 3, $P = 0{\cdot}617$, $n = 66$) did not, confirming previous findings (35, 37, 40). The monotonous trend of seropositivity increasing with age was also confirmed (Pfanzagl's test, $P < 0{\cdot}001$, $n = 50$).

## Ticks

In order to estimate the exposure of zoo animals, 600 ticks (*Ixodes ricinus*: 172 larvae, 376 nymphs, 27 males and 25 females) caught from vegetation in the zoos were examined for the presence of *Borreliae* both by darkfield microscopy (DFM) and indirect

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Table 2. *Percentages of* Borrelia*-infected ticks collected off the vegetation in German zoos as determined by darkfield microscopy (DFM) and indirect immunofluorescence technique (IFT)*

| Developmental stage | DFM | | | IFT | | |
|---|---|---|---|---|---|---|
| | *n** | % infected | 95% CI† | *n* | % infected | 95% CI |
| Larvae | 172 | 0 | 0–2 | 172 | 6·8 | 3–11 |
| Nymphs | 376 | 12·2 | 9–16 | 376 | 26·6 | 22–31 |
| Males | 27 | 18·5 | 6–38 | 27 | 29·6 | 14–50 |
| Females | 25 | 24·0 | 9–45 | 25 | 36·0 | 18–57 |

* *n*, number of examined ticks.
† CI, confidence interval.

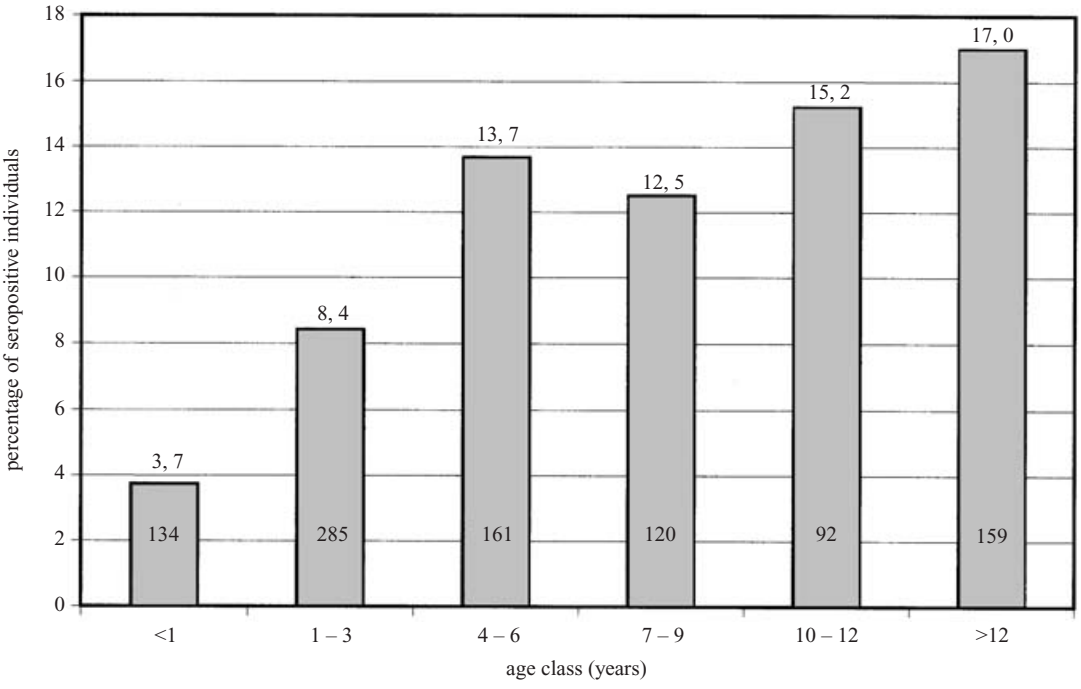


**Fig. 2.** Percentage of seropositive individuals in relation to the age of the animals, number in column indicates the number of animals tested.

immunofluorescence technique (IFT). Both methods produced similar results, though IFT was more sensitive. The rate of infection varied according to the developmental stages of the ticks (Table 2). The calculated infection rates of larvae, nymphs, and adult ticks were within the range typical for Central Europe [5, 6]. In order to assure comparability to already existing data regarding the total tick infection rate, larvae were not taken into account due to their immensely varying proportion within a flagging sample and their usually low infection rate [26, 27]. Excluding larvae, *Borrelia* were detected in 13·3% (DFM) or 27·3% (IFT) of ticks. Furthermore, infested ticks were found in all zoos and wildlife parks where ticks had been caught. There were no significant differences between study sites concerning the percentages of infected ticks (DFM: $\chi^2$ test, D.F. = 11, $P = 0{\cdot}306$, $n = 375$; IFT: $\chi^2$ test, D.F. = 11, $P = 0{\cdot}483$, $n = 375$).

## DISCUSSION

Veterinary care and preventive medicine programmes are fundamental requirements for wildlife in captivity and have to be met according to zoo animal welfare legislation [28]. The recognition of diseases is a basic prerequisite for the implementation of breeding programmes, translocations and reintroduction projects.

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Infectious diseases can seriously endanger the efforts to preserve threatened species [29]. Up to now, information on incidences, distribution, and risks of diseases in wild and captive populations is often inadequate [30]. To close this gap the presented epidemilogical study evaluates for the first time the exposure of zoo animals to *B. burgdorferi* s.l. and assesses the distribution of Lyme borreliosis in German zoos and wildlife parks.

Diagnosis of Lyme borreliosis is usually based on the detection of specific antibodies using the enzyme-linked immunosorbent assay as the favourable screening method [31, 32]. Furthermore, the ELISA is also very suitable for extensive epidemilogical studies and is now more frequently used in wildlife health monitoring. Unfortunately, there are only a few tests available specifically directed at wild animal species [33]. Therefore, in order to meet the time and cost requirements linked to a test applicable in a broad variety of species, the development of a non-species dependent ELISA for the detection of *B. burgdorferi* s.l. antibodies in zoo animals was necessary [22]. However, serological methods to diagnose Lyme borreliosis have not yet been standardized and no sensible cut-offs for antibody titres have been defined for animals. Due to such lack of standardization, both the comparison of results with already reported data and their interpretation should be done with due caution. Specific antibodies were detected in 154 (10·4%) out of 1487 zoo animals tested. Although expecting it to be lower, seroprevalence was similar to that in free-ranging ungulate and carnivore species in Germany. In studies with sample sizes of $n \geqslant 50$ and by using either species-specific or closely related species derived antibodies, 12·0% of fallow deer (*Dama dama*) and wild boar (*Sus scrofa*), 14·0% of red deer (*Cervus elaphus*) and 16·0% of roe deer (*Capreolus capreolus*) [34] as well as 16·3% of red foxes (*Vulpes vulpes*) [35] were seropositive. The actual seroprevalence in the current zoo survey is most likely even higher, because (i) the applied ELISA detects only IgGs (early infections remain undetected), (ii) the examination of exposed animals with a not yet sufficient immunological response or a titre in regression leads to false negatives, (iii) in order to ensure a high specificity of the test, the cut-off was set high with the consequence of a lower sensitivity and thus of an increased number of false negatives; (some borderline animals could actually be positive) and (iv) even a complete detection of all positive sera would only give an incomplete epidemiological survey of Lyme borreliosis due to a considerable number of proven infections lacking seroconversion [36].

Seroprevalences varying among zoos are believed to be based on unequal expositions caused mainly by differences in the occurrence and infection rates of vector-competent ticks. *Ixodes ricinus*, the main vector for *B. burgdorferi* s.l. in Central Europe, is spread all over Germany with relatively uniform infection rates [5], a fact supported by our study where also no significant percentage differences of infected ticks between zoos were found. We would therefore, despite the lack of information about local variation in vegetation and microclimate, not expect much variation among study sites.

The percentage of seropositive individuals among animals tested increased with age. These results are in accordance with findings for roe deer (*Capreolus capreolus*) in France [37], sika deer (*Cervus nippon pseudaxis*) in Japan [38], white-tailed deer (*Odocoileus virginianus*) in Minnesota/USA [39], and red foxes (*Vulpes vulpes*) in Germany [35]. With increasing age, and thus with prolonged exposure time, the chance increases to become infected. Particularly if antibodies persist for months or even years, as suspected for Lyme borreliosis, accumulation of antibodies in an ageing population can be assumed. Possible re-infections and associated booster effects may also lead to higher titres in older animals.

The observed differences in the percentage of seropositive individuals between species can be caused by variation of susceptibility or exposure. Even though the higher susceptibility of a species is problematic to prove, there is some evidence that such differences may exist. For instance, in our study the relative frequency of antibody response was highest in musk oxen (*Ovibos moschatus*) and mountain goats (*Oreamnos americanus*), two species that do not have contact to ticks in their natural habitats and thus support the assumption of being more susceptible to a new agent. The degree of exposure of an animal is also influenced by its housing conditions. In view of the great variety of zoo and enclosure designs, it can be safely assumed that different living and survival conditions for ticks are created. This in mind, one should be aware that with aiming for more natural exhibits conditions for ticks will be also improved resulting in increased tick abundance and density. Furthermore, ticks can be artificially introduced into enclosures with feeding or behavioural enrichment measures/efforts (leaves, branches, green fodder, floor of bark pieces and so on). Furthermore, species specific behaviour may be

of importance; for example, animals which prefer bushy over open habitats are more likely to become infested with ticks than others.

The fact that vector competent ticks (*Ixodes ricinus*) were found in 8 out of the 11 zoos and wildlife parks included in this study, underlines that zoo animals can serve as tick hosts, even if animal keepers do not notice an infestation. In all of the eight zoos, *Borrelia*-infested ticks had infection rates within the range typical for Central Europe (nymphs 10–20% and adult ticks up to 30%) [5, 6]. It is therefore likely that zoos do in general host ticks with a proportion of them being vectors for *B. burgdorferi* s.l. Hence, a potential risk of infection does exist for zoo animals kept there. Therefore, a differential diagnosis of Lyme borreliosis should be taken into account in case of suspicious clinical symptoms and possible contact to ticks. For the examination of ticks (for *Borrelia*) IFT should be preferred to DFM.

Preventive measures should aim at minimising tick infestation of zoo animals and intensifying pest (e.g. mice) control, especially since ticks and mice can also serve as vectors and reservoirs for other important pathogens, respectively. Vaccination seems neither necessary nor feasible since the effectiveness of the only vaccine approved in Germany (for dogs; Merilym®, Merial GmbH, Germany) has not yet been sufficiently demonstrated.

In view of the fact that Lyme borreliosis is a zoonosis, attention of the zoo management should be particularly drawn to the protection of the zoo staff. In contrast to TBE (tick-borne encephalitis, spring–summer encephalitis), for Lyme borreliosis there are still no options available in Europe for active or passive immunization. Therefore, keepers with direct contact to animals and even more gardeners should (i) be aware of possible exposure to *B. burgdorferi*-infested ticks and (ii) know which precautions to take [41]. The risk of infection can be substantially reduced by avoiding habitats with high tick density, such as wooded areas with luxuriant undergrowth and dense vegetation. Further prophylactic measures include wearing of suitable clothing (tightly fitting, with long sleeves and trousers, sturdy shoes), use of insect repellents, thoroughly inspection of clothes and body after venturing tick habitats, and immediate removal of ticks upon discovery [6]. The possible transmission of *Borrelia* via blood or urine of infected animals has also to be considered, but is certainly of low importance. Concluding from the transmission ways of *B. burgdorferi* s.l., visitors do not face an elevated risk to be exposed to the pathogen, at least not more than outside of the zoo.

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