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Sensitivity of *Borrelia* genospecies to serum complement from different animals and human: a host–pathogen relationship

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Abstract

Different *Borrelia* species and serotypes were tested for their sensitivity to serum complement from various animals and human. Complement-mediated *Borrelia* killing in cattle, European bison and deer was higher irrespective of the *Borrelia* species whereas in other animals and human it was intermediate and *Borrelia* species-dependent. Activation of the alternative complement pathway by particular *Borrelia* strain was in correlation with its sensitivity or resistance. These results support the incompetent reservoir nature of cattle, European bison, red, roe and fallow deer, at the same time present the probable reservoir nature of mouflon, dog, wolf, cat and lynx. In short, this study reviews *Borrelia*–host relationship and its relevance in reservoir competence nature of animals.

Keywords: *Borrelia burgdorferi*; Complement; C3b; Reservoir competence; Alternative pathway

1. Introduction

Lyme disease is the most common vector-borne disease in North America [1,2] and Eurasia [3]. The causative agent, *Borrelia burgdorferi* is transmitted to a wide range of animal species and human. However, a noteworthy difference in the host specificity and selectivity of different *Borrelia* genospecies exists in nature [4]. The circulation of Lyme spirochete is a perfect model of entangled relationship between host, vector and the *Borrelia* itself. Complement sensitivity or resistance of *Borrelia* genospecies is one of the crucial components governing *Borrelia*–host relationship. Complement-mediated killing of *Borrelia* in hosts has ecological importance as it might determine the reservoir competence of the host [5]. A competent reservoir host for Lyme disease is a vertebrate animal species that gets infected, harbors a particular pathogen and acts as source of infection for other vertebrates via tick vector [6].

The pattern of serum complement sensitivity of different *Borrelia* genospecies matches the known reservoir status of many vertebrate species for *B. burgdorferi* sensu lato [7]. Several studies indicate that *B. garinii* and *B. valaisiana* are mainly transmitted to ticks by avian hosts, whereas, *B. afzelii* is transmitted to tick via rodents. Rodent complement resistance of *B. afzelii* parallels the prime transmission competence of mice, rats and squirrels [8,9]. On the other hand, complement-mediated lysis of *B. garinii* explains why the European rodents are insufficient reservoir for *B. garinii* strains. In case of roe deer lysis of *Borrelia* regardless of its genospecies

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correlates with incompetent reservoir nature of this animal [10].

Borrelia species have developed mechanism of immune evasion and resistance to complement-mediated killing. A newly described mechanism that may contribute to immune evasion is the binding of the complement regulatory protein factor H (fH) to the cell surface [11]. This protein factor is a complement regulatory subunit that serves as a cofactor for the factor I (fI)-mediated cleavage of C3b. By increasing the local concentration of fH at the cell surface, bacteria that bind fH can promote more efficiently the degradation of C3b and thereby decrease the efficiency of complement-mediated killing [4,12–14]. Here we report on the sensitivity of different *Borrelia* genospecies to serum complement from various animals and human detected by a novel flow-cytometric approach.

2. Materials and methods

2.1. Collection of sera and Borrelia strains

Sera from cattle, sheep, dogs and cats were collected from the University Farm, Zemplinska Teplica, and the University Animal House. Sera from wild and game animals were kindly provided by the Zoological Garden, Kosice, Slovak Republic. Human blood samples were collected from healthy persons who had no contact with ticks. All sera were screened to confirm the absence of anti-*Borrelia* antibodies using the methods described earlier [15]. Sera from each animal species were pooled, filtered through 0.22 μm syringe filters (Minisoft) and stored at −70 °C in the form of aliquots. To inhibit the classical pathway of complement activation, some aliquots from each animal species were incubated with 10 mM EGTA, 4 mM $MgCl_2$ in veronal buffer saline (VBS) at 37 °C for 30 min. Some serum aliquots from each animal species were incubated with 10 mM EDTA in VBS at 37 °C for 30 min to inactivate both pathways (classical and alternative) [17].

Borrelia strains (Table 1) were grown by adding 300 μl of deep freeze culture in 10 ml of Barbour–Stoenner–Kelley II (BSK-II) medium (Sigma). Cultures were incubated at 33 °C for three to four days to achieve borrelial cell count up to 10^4 cells/ml. Cell density was adjusted with sterile BSK-II medium. Cell death control of *Borrelia* was prepared by heating at 56 °C for 25 min.

Table 1
Different *Borrelia* genospecies used in complement sensitivity study

Genospecies	Strain	Country of origin	Osp A serotype
B. b. s.s.	SKT-2	Slovakia	1
B. b. s.s.	SKT-8	Slovakia	1
B. b. s.s.	B31	USA	1
B. b. s.s.	NE3480	Switzerland	1
B. afzeli	SKT-4	Slovakia	2
B. afzelii	SKT-5	Slovakia	2
B. afzelii	Pko	Germany	2
B. afzelii	VS461	Switzerland	2
B. garinii	Rio 2	Spain	3
B. garinii	Pbi	Germany	4
B. garinii	PV 6	Spain	5
B. garinii	SKT-1	Slovakia	6
B. garinii	SKT-3	Slovakia	6
B. garinii	SKT-6	Slovakia	6
B. garinii	CL1	Spain	8
B. valaisiana	VS116	Switzerland	NA
B. andersonii	21123	USA	NA
B. lusitaniae	Poti B2	Portugal	NA
B. bissettii	DN 127	USA	NA
B. japonica	HO14	Japan	11

B. b. s.s.: *Borrelia burgdorferi* sensu stricto; NA: Not available.

2.2. Complement sensitivity assay

Complement sensitivity assay (CSA) was performed according to Kurtenbach et al. [7]. Briefly, *Borrelia* suspensions were added to equal amount of serum to give a final volume of 100 μl. Microtiter plates with suspensions were carefully sealed and incubated at 33 °C for 6 h. Complement-inactivated sera were used as a complement control, while for cell living control, *Borrelia* suspensions were incubated without adding any sera or supplements. After incubation, 5–7 μl of suspension from each well was aseptically taken and observed under dark field microscope for borrelial immobilization, bleb formation and bacteriolysis.

Earlier described [16] flow-cytometric analysis for the differentiation of alive and dead spirochetal cells was used simultaneously. In brief, 50 μl of incubated suspension were diluted 1:5 with phosphate buffer saline (PBS 0.01 M, pH 7.2; filtered through a 0.2 μm filter) containing 1 μg of acridine orange/ml, and incubated at room temperature for 5 min. The analysis was done using a FACS single-laser flow cytometer (Becton-Dickinson). Events were acquired in the list mode for 2 min. The sample fluid flow rate was kept low to reduce signal variability. *Borrelia* spirochetes were differentiated from BSK-II and serum particles adjusting the fluorescence intensity and side scatter parameters. Alive and dead cell controls were analyzed first to construct the gates. Constructed non-rectangular gates on the side-scatter vs. FL-1 fluorescence (logarithmically amplified) dot plot were used to differentiate and enumerate alive and dead spirochetes in the samples. Data were analyzed using the WinMDI software (Version 2.8).

2.3. Measurement of complement activation

Complement activation was measured according to van Dam et al., [17]. In brief, *Borrelia* strains were grown in BSK-II medium at 33 °C for one week. Borrelial cells were harvested by centrifugation at 2040*g* for 30

min and resuspended in PBS. The concentration of cells in every *Borrelia* suspension was adjusted to approximately 1×10^7 cells/ml. For cell coating, suspensions (100 μl/well) from each *Borrelia* strain were incubated at 4 °C for 3 h. The wells were emptied and non-specific binding sites were blocked with PiCM buffer (137 mM NaCl, 2.7 mM KCl, 0.8 mM Na_2HPO_4, 13 mM KH_2PO_4, 1.0 mM $MgCl_2$, 0.6 mM $CaCl_2$, 1% Glucose, 2.5% human serum albumin) at 4 °C for 30 min. Afterwards, wells were washed three times with PBS and sera from different animal species and human (100 μl/well) were added to a 50% dilution in PiCM buffer. Microtiter plates were incubated at 37 °C for 1 h and washed once with PBST (PBS + 0.05% Tween 20) and three times with PBS. Polyclonal anti C3c-HRPO conjugate (Dako) having affinity towards C3b from wide range of animals and human was diluted at 1:1000 in PBST and added in each well (100 μl). Plates were incubated at 37 °C for 1 h. Subsequently, wells were washed once with PBST and three times with PBS. A volume of 100 μl of ABTS (2,2′Azino-di-3-ethyl-benzothiazolin-sulfonate; Sigma) was added in each well and plates were incubated at room temperature for 15 min. Absorbance was measured at 405 nm. Serum aliquots treated with EGTA + $MgCl_2$ or EDTA from each animal species were also included in the experiment to compare the activation of the alternative pathway.

2.4. Statistical analysis

Both CSA and measurement of C3b activation were repeated three times and the mean values were taken into account for evaluation. Paired *t*-test was applied to compare the results of dark field microscopy and flow cytometric analysis. The paired *t*-test was also used to compare the *Borrelia* species-dependent complement activation.

3. Results

3.1. Borreliacidal ability of serum complement and sensitivity of different Borrelia genospecies

Serum complement sensitivity of *Borrelia* genospecies measured by flow cytometric analysis (Table 2) in different animals and human did not differ ($P > 0.05$) from the results obtained by dark field microscopy (data not shown). This agreement between dark field microscopy and flow cytometry results indicates that the immobilization and/or bleb formation correlates with spirochetal cell death.

In general, animal species were divided into two groups depending on their serum complement-mediated borreliacidal property. In the first group (cattle, European bison, fallow deer, red deer and roe deer) a high borreliacidal effect was observed killing the maximum numbers of borreliae irrespective of their genospecies (Table 2). In case of cattle and European bison, the borreliacidal effect was more pronounced causing spirochetal cell lysis. Cell lysis was observed under dark field microscopy and successively confirmed by flow cytometric analysis (Fig. 1(b)) by a marked reduction (96%) in the number of events. Distinct lysis effect was not found in fallow, red and roe deer. Maximum borreliacidal effect of roe deer serum (99%) against *B. andersonii*-21123 without distinct reduction in the number of events performed by flow cytometric analysis (Fig. 1(c)), confirms the absence of borrelialysis. The second group was represented by animals (dog, wolf, cat, lynx, mouflons and sheep) with intermediate and *Borrelia* species-dependent killing (Table 2). A representative result of flow cytometric analysis of moderate borreliacidal activity of serum complement (showing two peaks of alive and dead spirochetes) is shown in Fig. 1(d).

Table 2
Percent sensitivity of different *Borrelia* species to serum complement of cattle, European bison, fallow deer, red deer, roe deer, sheep, mouflon, dog, wolf, cat, lynx and human

Borrelia species	Cattle	European bison	Fallow deer	Red deer	Roe deer	Sheep	Mouflons	Dog	Wolf	Cat	Lynx	Human
B. b. s.s. (SKT-2)	99	97	72	80	86	23	30	20	16	20	44	11
B. afzelii (SKT-5)	98	99	60	78	75	48	25	6	68	10	41	23
B. garinii (Rio2)	99	98	88	92	98	25	23	7	6	12	18	50
B. garinii (Pbi)	99	94	92	90	99	21	10	4	10	9	21	41
B. garini (PV6)	98	98	90	96	99	66	42	61	90	59	66	92
B. garinii (SKT-3)	99	96	82	88	98	50	31	68	72	22	35	77
B. garinii (CL1)	99	98	94	96	97	37	10	15	32	28	35	66
B. valaisiana (VS116)	98	96	95	97	97	80	25	43	67	23	29	59
B. andersonii (21123)	99	97	96	94	99	45	43	27	55	34	58	87
B. lusitaniae (Poti B2)	98	98	95	95	99	34	20	80	88	52	71	81
B. bissettii (DN127)	99	99	86	96	98	76	12	11	26	16	39	17
B. japonica (HO14)	97	98	87	97	96	30	10	58	84	9	27	7

All values are in percentage. No significant ($P > 0.05$) variation was observed between the results obtained by SKT -2 and other B.b.s.s. strains. No significant variation in the sensitivity of SKT-5 and other *B. afzelii* strains was also noticed.
SKT-1, SKT-3 and SKT-6 expressed comparatively similar sensitivities.
The results present the mean derived from the values of three experiments.

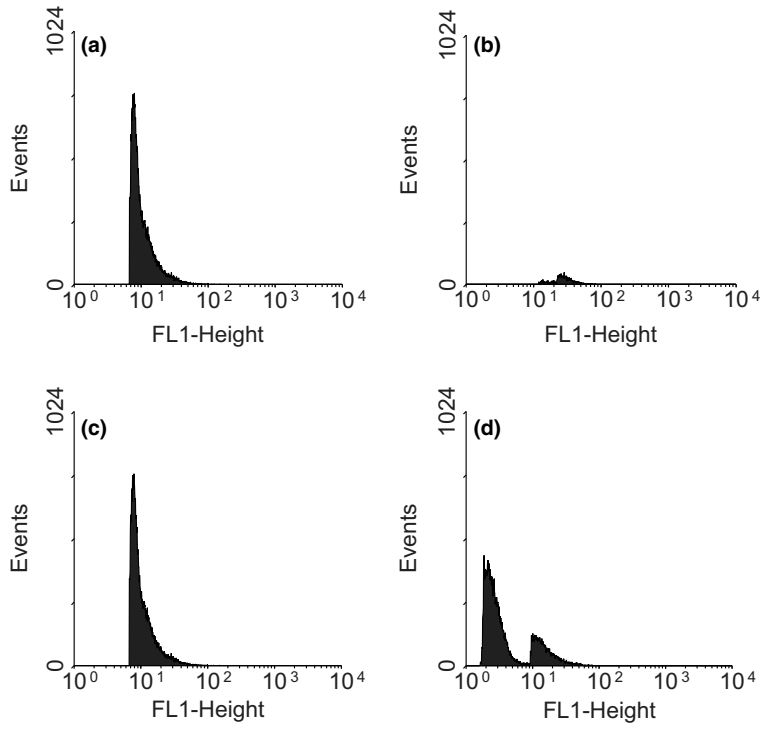

Fig. 1. Serum complement sensitivity of *Borrelia* species estimated by flow cytometric analysis. Equal amounts of alive *Borrelia* and serum complement were incubated for 6 h. Dead and alive cells were differentiated using acridine orange staining and detected in non-rectangular gates on the side-scatter vs. FL-1 fluorescence dot plot. (a) dead cell control; (b) borrelialysis effect of cattle serum; (c) high borreliacidal (*B. andersonii*-21123) ability of roe deer without lysis effect; (d) intermediate killing of *Borrelia* (*B. garinii*, serotype 6- SKT-3) by dog serum complement showing two peaks (larger peak with lower FH1 height represents alive cells while small peak with increase in fluorescence indicates dead cells).

Severance of borrelial genospecies in distinct groups based on their sensitivity or resistance was more difficult than the earlier cited animal group. It was not possible to determine the sensitivity of individual *Borrelia* species in the first group of animals due to their high borreliacidal activity. In the second group of animals, a comparative study among strains was possible because of their moderate and variable borreliacidal nature. A noticeable resistance of *B. japonica*-HO14, *B. bissettii*-DN127 and *B. garinii* (serogroups 4, Pbi & 8, CL1) to mouflon complement was observed. In contrast, *B. valaisian*-VS116 (80% cell death) was the most sensitive species to sheep complement, followed by *B. bissettii*-DN127 (76% cell death) and *B. garinii*-PV6 serotype-5 (66% cell death). *B. afzelii* strains, *B. bissettii*-DN127 and *B. garinii* (serotype 3-Rio2 and serotype 4-Pbi) were more resistant than the other serotypes to dog complement. In case of cat, *B. japonica*-HO14 (9% cell death) was the additional borrelial species recorded as resistant. In wolf, *B. garinii*-PV6 (serotype-5) was found as the most sensitive candidate, followed by *B. lusitaniae*-PotiB2. Borreliacidal ability of human serum varied depending on the *Borrelia* species. In addition to *B. afzelii* strains, *B. japonica*-HO14 and *B. burgdorferi* s.s. strains, the resistance of *B. bissettii*-DN127 to normal human serum was remarkable. Sensitivities of *B. garinii* serotypes (41–92%), *B. valaisiana*-VS116 (59%), *B. andersonii*-21123 (87%) and *B. lusitaniae*-PotiB2 (81%) were evident on the other side (Table 2).

B. garinii is mainly associated with avian hosts. Amongst the different serogroups of *B. garinii*, serogroup 3 – Rio2 and 4 – Pbi were more resistant followed by serogroup 8 – CL1. Higher sensitivity of other *B. garinii* serotypes confirms the non-competence of the majority of the animals used in this study. However, the resistance of Rio2 and Pbi particularly in canines and felines raise the possibility of their transmission to tick vector through blood meal.

Sera treated with EDTA failed to kill the *Borrelia*, whereas no interference ($P > 0.05$) in spirochetal killing was observed in all sera treated with Mg-EGTA, thus indicating the prime role of the alternative pathway in complement-mediated *Borrelia* killing.

3.2. Measurement of C3 activation by different Borrelia genospecies

Sera treated with EDTA failed to deposit C3b on borrelial cells, a finding that is in agreement with the absence of a borreliacidal effect in EDTA-treated sera. The deposition of C3b on borrelial cells was higher when they were incubated with normal animal and human

sera than with the sera treated with EGTA. This indicates that apart from an alternative pathway, the classical pathway was also activated even in the absence of antibodies and gave the additional effect of C3b deposition.

The variation in C3 activation by different *Borrelia* species in normal serum of given animals species was non-significant ($P > 0.05$). However, in normal sera, *Borrelia* genospecies-dependent complement activation was observed in the second group of animal species (Figs. 2–4) as well as in human (Fig. 5) when the sera were treated with Mg-EGTA. This indicates the presence of *Borrelia* species-dependent activation of the alternative pathway in the second group of animals. Conversely, in the first group of animals *Borrelia* species-dependent variation in the activation of the alternative pathway was not found ($P > 0.05$). The narrow range of C3 activation by different *Borrelia* genospecies in Mg-EGTA treated serum of cattle (Optical Density – OD 1.1–1.3), European bison (OD 1.05–1.30), roe deer (OD – 0.87–1.2), red deer (OD – 0.7–1.1) and fallow deer (0.7–1.0) confirms the non-significance of *Borrelia* species in the activation of the alternative pathway in these animals.

A comparison between different animals for the extent of complement activation by given *Borrelia* species was not possible as the polyclonal anti-C3c-HRPO conjugate had different binding affinity in each animal. A positive correlation between complement activation by

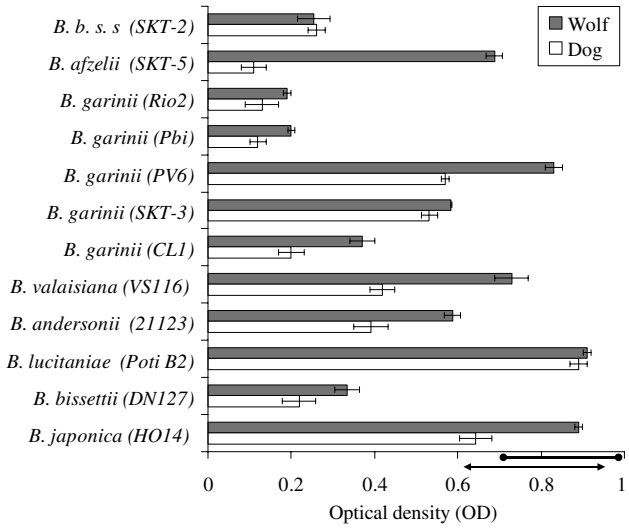

Fig. 3. Activation of the alternative complement pathway by different *Borrelia* species in wolf and dog. Significant variation ($P < 0.05$) in C3 activation by *B. afzelii* strains, *B. garinii* (serotypes 3-Rio2, 4-Pbi and 8-CL1), *B. bissettii*-DN127 on one hand, and *B. japonica*-HO14, *B. lusitaniae*-PotiB2, *B. garinii* (serotypes 5-PV6 and 6- SKT-3) on the other hand was observed in dog serum. The variation in wolf serum was similar with the exception of *B. afzelii* strains where higher activation of C3 was observed. Activation of C3 in normal wolf (↔) and dog (↔) sera by different *Borrelia* genospecies was not significant ($P > 0.05$). The results are the mean derived from the values of three experiments.

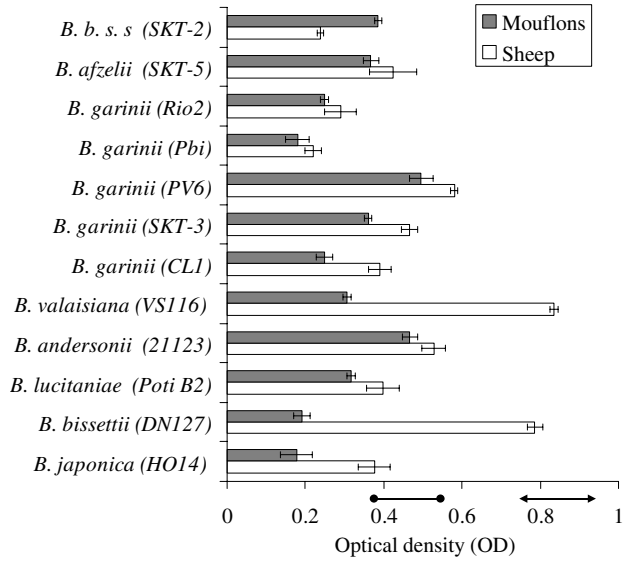

Fig. 2. Activation of the alternative complement pathway by different *Borrelia* species in sheep and mouflon. Significant variation ($P < 0.05$) in C3 activation by *B. garinii* (serotype 4-Pbi & 8-CL1), *B. bissettii*-DN127 and *B. japonica*-HO14 on one side, and *B. andersonii*-21123 and *B. garinii* (serotype 5-PV6) on the other side was observed in mouflon serum. In sheep, significant variation ($P < 0.05$) in C3 activation by *B. garinii* (serotype 3-Rio2 and 4-Pbi) and *B.b.* s.s. isolates on one side, and *B. valaisiana*-VS116 and *B. bissettii*-DN127 on the other side was observed. Activation of C3 in normal mouflon (↔) and sheep (↔) sera by different *Borrelia* genospecies was not significant ($P > 0.05$). The results are the mean derived from the values of three experiments.

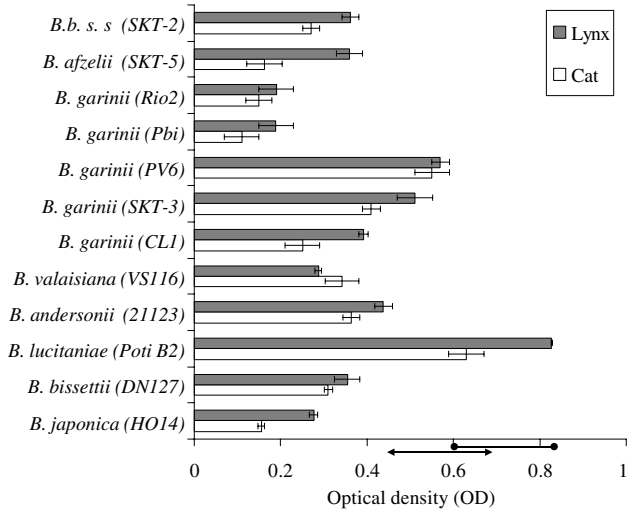

Fig. 4. Activation of the alternative complement pathway by different *Borrelia* species in lynx and cat. Significant variation ($P < 0.05$) in C3 activation by *B. garinii* (serotypes 3-Rio2 and 4-Pbi) and *B. japonica*-HO14 on one side, and *B. lusitaniae*-PotiB2 and *B. garinii* (serotype 5-PV6) on the other side was shown both in cat and lynx. Activation of C3 in normal lynx (↔) and cat (↔) sera by different *Borrelia* genospecies was not significant ($P > 0.05$). The results are the mean derived from the values of three experiments.

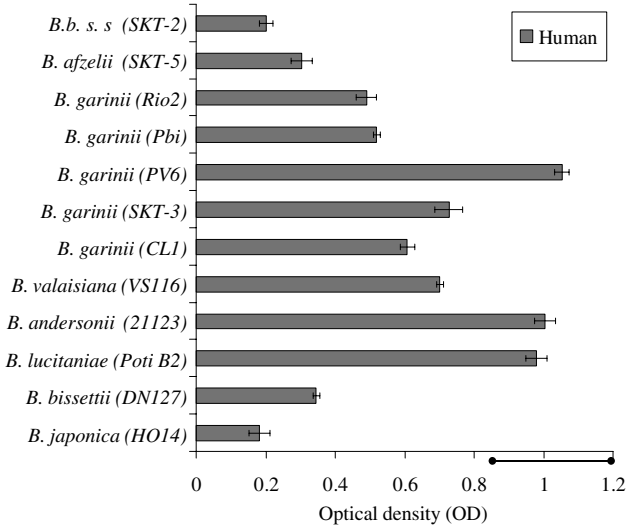

Fig. 5. Activation of complement (C3) by different *Borrelia* species in EGTA-treated human serum. *B. burgdorferi* s.s. strains, *B. afzelii* strains, *B. bissettii*-DN127 and *B. japonica*-HO14 activated C3 significantly lower ($P < 0.05$) than other *Borrelia* species except *B. garinii* (serotype 3-Rio2 and 4-Pbi). The results are the mean derived from the values of three experiments. Activation ($P > 0.05$) of C3 in normal human serum (↔) by different *Borrelia* species.

the alternative pathway and complement-mediated killing was found, i.e. higher activation of complement corresponded with higher borreliacidal effect and vice versa.

4. Discussion

The specific association between *Borrelia* genospecies and various vertebrate hosts is well known. The association is determined by the interaction of host complement and *Borrelia* spirochete [18]. The correlation between serum complement sensitivity of *Borrelia* and reservoir status of animals has been discussed earlier [7]. We found similar correlation in our study. Known reservoir incompetent hosts like fallow, roe and red deer readily killed *Borrelia* spirochetes irrespective of their genospecies. In case of the incompetent reservoir sika deer (*Cervus nippon*) an active killing of *Borrelia* by complement was reported recently [20]. Earlier stated reservoir incompetence of fallow, roe and red deer [6,10,19,21,22] is also supported elsewhere [7] by reviewing their complement-mediated borreliacidal abilities.

In case of European bison, characteristic borrelialysis was recorded with lower intensity than in cattle. The borreliacidal property of cattle serum complement reported earlier [7] was comparable with the results observed in our study. The pronounced borreliacidal ability observed in cattle and European bison indicates their resistance against borrelial infection. Consequently, they can be categorized as non-competent reservoir hosts. Gray et al., [22] have also mentioned the reservoir incompetence of cattle. From a study in Ireland [23], it is evident that cattle act as non-competent reservoir host, wherein authors have documented 0% seroprevalence in Ballina region with abundance of tick population on pastures grazed by cattle.

Borreliacidal property of sheep serum complement against *B. garinii* serotypes and *B. valaisiana*-VS116 observed by us was in disagreement with the earlier reported >95% bacteriolysis [7]. In addition, we found a comparative resistance of *B. garinii* serotype 3 (Rio2) and 4 (Pbi) to sheep complement. Some authors [7,24] have put forth the co-feeding phenomenon on sheep since their complement is partially borreliacidal for *B. afzelii* and *B. burgdorferi* s. s. Authors have further suggested the reservoir competence of sheep. Given the insufficient killing of *Borrelia*, we support the possible transmission of *Borrelia* spirochetes to ticks via co-feeding on sheep and mouflon.

An intermediate borreliacidal effect of carnivore's serum complement (dog, wolf, cat and lynx) was observed against most of the *Borrelia* genospecies. In vitro canine complement sensitivity test [5] against three different *Borrelia* strains showed that *B. burgdorferi* sensu stricto – B31 (4% immobilization) and *B. afzelii* – pKo (6% immobilization) were more resistant than *B. garinii* – A87S (31–95% immobilization). We observed similar resistance of *B. afzelii* strains and sensitivity of *B. garinii* to normal dog serum complement except serotypes 3 (Rio 2), 4 (PBi) and 8 (CL 1). We also found *B. lusitaniae*-PotiB2 and *B. japonica*-HO14 as sensitive genospecies to dog complement, whereas the sensitivities of *B. andersonii*-21123 and *B. bissettii*-DN127 were similar to *B. burgdorferi* s.s. strains. Scanty work is available on xenodiagnosis-based determination of reservoir competence of dog [25]. However, it can be extrapolated that the complement sensitivity may result from a reservoir status of dog [5]. We support this hypothesis in order to identify the dog as reservoir competent host particularly for *B. afzelii* isolates, *B. burgdorferi* s. s. isolates, *B. andersonii*-21123 and *B. bissettii*-DN127.

To date no report is available on the serum complement sensitivity study or xenodiagnosis in cat, wolf, lynx or other wild animals. Except *B. afzelii* strains, the majority of the *Borrelia* species showed a similar sensitivity pattern to wolf and dog sera. Resistance of *Borrelia* genospecies in cat, especially *B. japonica*-HO14, *B. afzelii* strains, *B. garinii* serotype 3 (Rio2) and 4 (Pbi), suggests a potential role of this domestic animal in the circulation (host–vector–host) of these spirochetes. The probable reservoir competence of wolf and lynx is also noteworthy as they can contribute in the circulation of *Borrelia* species in nature. Considering the close relation of dog and cat with human, it is important to know whether co-feeding can take place in these carnivores. In human, studies are available [17,26] on the variable complement sensitivity of *B. burgdorferi* s. s., *B. garinii*

and *B. afzelii*, and its influence on the pathogenic and clinical manifestations. Our study is an extension of previous reports by assessing the complement sensitivities of more genospecies. Resistance of *B. afzelii* and *B. burgdorferi* s. s. strains to human serum complement was in accordance with previous studies [17,27]. Moreover, the resistance of other genospecies like *B. japonica*-HO14 and *B. bissettii*-DN127 suggests the probability of their pathogenic nature [28,29].

Our results and other reports [17,26,30] confirm that *Borrelia* genospecies differ in their ability to activate the alternative complement pathway, thus they differentially resist the killing by serum bactericidal activity. The positive correlation was found between serum complement sensitivity of *Borrelia* strains and the activation of the alternative pathway. Unhindered borreliacidal effect of Mg-EGTA-treated samples proves the importance of the alternative pathway against *Borrelia*. However, the role of the activation of the classical pathway in *Borrelia* killing was not fully understood. Higher deposition of C3b on borrelial cells incubated with normal sera than with EGTA-treated sera indicates the activation of the classical pathway in the absence of specific IgG antibodies. This phenomenon can be explained by the possibility of direct C1q binding to bacterial cell components [31,32] or indirect activation-mediated by natural IgM [33]. The activation of the classical pathway can have an assisting role in the innate immune response. Furthermore, Brown et al., [33] have postulated that the proportion of a population of bacteria bound by C3 depends mainly on the classical pathway, whereas the intensity of C3 binding depends on the alternative pathway.

Borrelial genospecies associated with a specific host are usually resistant to the alternative complement pathway of the given host [4,34]. It is essential to study the molecular basis of differential immune evasion of the given *Borrelia* species in different animals and human. Although some recent publications are available in the human field [13,14,34,35], studies using a wide range of animals are still lacking. In conclusion, our findings on complement sensitivity of *Borrelia* species in different animals and human constitute the basis for further studies at the molecular level to describe the complex host–pathogen (*Borrelia*) relationship.

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