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Original Article

Serological and PCR evidence of Infection in 105 Patients with SPPT

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Abstract Introduction

The main aim of this study is to determine the nature of the exposure of patients presenting with polymorphic signs and symptoms to the parasite *Babesia*, through the study of serology. The secondary aim is to report the different serological or PCR results observed in these patients.

Material and methods

The following serologies were performed in all patients looking for: *Babesia divergens, Borrelia, Bartonella, Coxiella burnetii, Anaplasma phagocytophilum*. The following PCRs were performed looking for: *Borrelia spp, Babesia spp, Bartonella (Bartonella spp, B. quintana, B. Henselae,) Coxiella spp, Anaplasma spp, Ehrlichia spp, Rickettsia spp*, most often on several matrices (venous blood, capillary blood, urine and saliva).

Results

In this study, 105 patients were included, 62 females and 43 males, sex ratio F/M was 62/43 = 1.44; mean age was 45.5 year old (range; 5 years, 79 years old). Of the 105 serologies for *B. divergens*, 41 % were found to be positive. Of the 104 serologies for *Borrelia*, 19.2 % were found to be positive. Of the 104 serologies for *Borrelia*, 19.2 % were found to be positive. Of the 95 serologies for *Anaplasma*, 27,3 % were found to be positive. *Borrelia spp, Babesia spp, Bartonella spp, Coxiella spp, Anaplasma spp, Ehrlichia spp, Rickettsia spp* were found by using rtPCR.

Conclusion

Our study has shown that patients with SPPT/PTLDS, a syndrome close to fibromyalgia, could harbor several tick borne microorganisms. Microbiologic analyses should thus not be merely limited to *Borrelia*'s research alone.

Keywords: Lyme; Borrelia; Babesia; PCR; PTLDS; SPPT; Serology; PCR

Introduction

Lyme disease is a tick-borne infectious disease caused by the bacterium *Borrelia burgdorferi*. The prevalence seems to be increasing in many countries around the world, particularly in France. Interestingly, ticks transmit many other pathogenic bacteria (*Bartonella spp, Ehrlichia spp, Anaplasma spp, Rickettsia spp*...), parasites (*Babesia spp*) and viruses, the so-called co-infections [1-4]. The main aim of this study is to determine the nature of the exposure of patients presenting with polymorphic signs and symptoms to the parasite *Babesia*, through the study of serology. The secondary aim is to report the different serological or PCR results observed in these patients.

Patients and Methods

This is a retrospective observational study including patients with persistent polymorphic syndrome possibly due to a tick bite (SPPT). SPPT is a clinical syndrome close to post-treatment Lyme disease syndrome (PTLDS), which is officially recognized by the French High Authority for Health (HAS).

The SPPT is defined by a clinical triad persisting for a continuous period of at least 6 months, associating several times a week, a polyalgic syndrome (musculoskeletal pain and/or neuropathic pain and/or headaches); persistent fatigue with reduced physical capacities; cognitive complaints. The difference between SPPT and PTLDS is that the diagnosis of Lyme disease has not to be proven and patients may have not been treated [5, 6].

1. Patients:

Over a reference period from October 2016 to April 2020, patient's inclusion criteria clustered SPPT patients of all gender who were tested positive for Babesia serology, who presented with the following clinical symptoms persisting for a continuous period of at least 6 months, without prior diagnosis:

1) Cognitive disorders

2) More than two of the following chronic symptoms including : myalgia, arthritis or arthralgia, facial paralysis, central or peripheral involvement, myelitis, root pain, paresthesias, dysesthesias, radiculopathy

3) Abnormal asthenia

2. microorganisms that were sought

The following serologies were performed in all patients looking for: *Babesia divergens, Borrelia, Bartonella, (B. quintana and B. henselae) Coxiella burnetii, Anaplasma phagocytophilum*. The following PCRs were performed looking for: *Borrelia spp, Babesia spp, Bartonella (Bartonella spp, B. quintana, B. Henselae,) Coxiella spp, Anaplasma spp, Ehrlichia spp, Rickettsia spp*, most

often on several matrices (venous blood, capillary blood, urine and saliva). Most of the PCRs (in 51 out of 59 patients) were performed in the Adnucleis laboratory using rt-PCR (Table 1, 2).

This retrospective observational study was approved by the "Comité de protection des personnes" CPP SUD 9EST VI Clermont Ferrand, France. All patients and control persons signed an informed consent in accordance with the Declaration of Helsinki.

Results

In this study, 105 patients were included, 62 females and 43 males, sex ratio F/M was 62/43 = 1.44; mean age was 45.5 year old (range; 5 years, 79 years old). Results are summarized in (Table 3).

Table 3: PCR and serological results in 105 patients presenting with a persistent polymorphic syndrome after a possible tick-bite (SPPT)

	Number of patients tested	Positive	Negative
Babesia serology*	105	43 (41%)	62 (59%)
PCR	50	6 (12%) 3/6: negative serology	44 (88%)
Borrelia serology	104	20 (19.2%)	84 (80.8%)
PCR	55	9 (16.4%) 8/9: negative serology	46 (54%)
Bartonella serology	97	3 (3.1%)	94 (96.9%)
PCR	47	9 (19.1%) 9/9: negative serology	38 (80.9%)
Coxiella serology	93	3 (3.2%)	91 (96.8%)
PCR	39	2 (5.1%) 2/2: negative serology	37 (94.9%)
Anaplasma serology	95	26 (27.3%)	69 (72.7%)
PCR	39	3 (7.7%) 2/3: negative serology	36 (92.3%)
Ehrlichia PCR	39	4 (10.3%)	35 (89.7%)
Rickettsia PCR	38	19 (50%)	19 (50%)

**Babesia divergens*

1. *Babesia divergens* serology

Of the 105 serologies for *B. divergens*, 92 were performed in the Biomnis laboratory, 6 at the Strasbourg laboratory (National Reference Center for Borreliosis), 6 at the Institut hospitalo-universitaire (IHU) laboratory in Marseille, and one at Sylab laboratory. Biomnis laboratory gave two levels of results, weakly positive or strongly positive. Results were the following (Figure 1). (i) At the Biomnis laboratory, 39 out of 92 (42.4%) blood tests were found to be positive, 30 (32.6%) weakly positive and 9 (9.8%) strongly positive. (ii) At the Strasbourg laboratory, 2 out of 6 were positive (both quantified at 1/120). (iii) At the IHU of Marseille, 2 out of 6 were positive (respectively quantified at 1/60 and 1/30). (iv) At Sylab laboratory, the only patient was negative. Among the patients who presented with positive results for

B. divergens serology, 4 had had previous negative serology (2 patients two months before and 2 patients 12 months before). Among patients who were tested positive for *B. divergens* serology, 3 out of 31 (9.7%) were *Babesia* positive by PCR, 5 out of 30 (16.7%) were *Borrelia* positive by PCR, 9 out of 43 (20.9%) had a positive *Borrelia* serology (Figure 2), 5 patients had *Borrelia, B. divergens* and *Anaplasma phagocytophilum* positive serology.

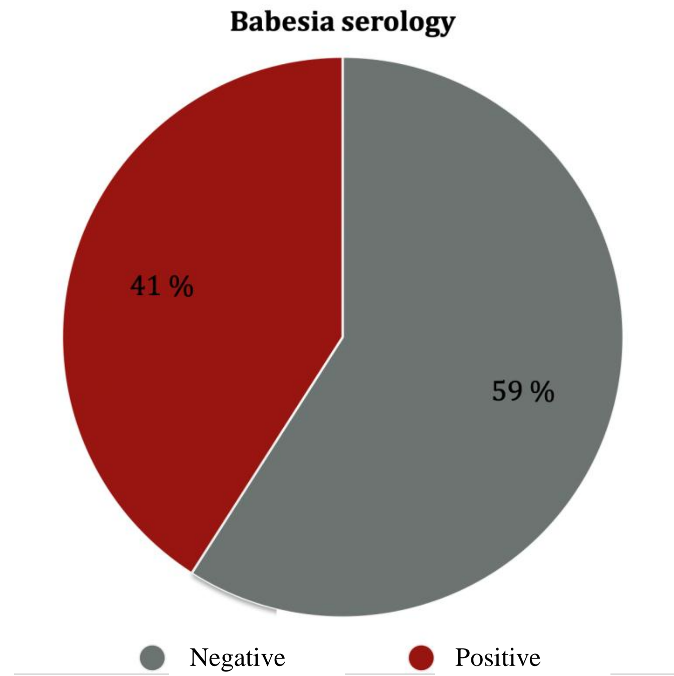

Figure 1: Babesia serology

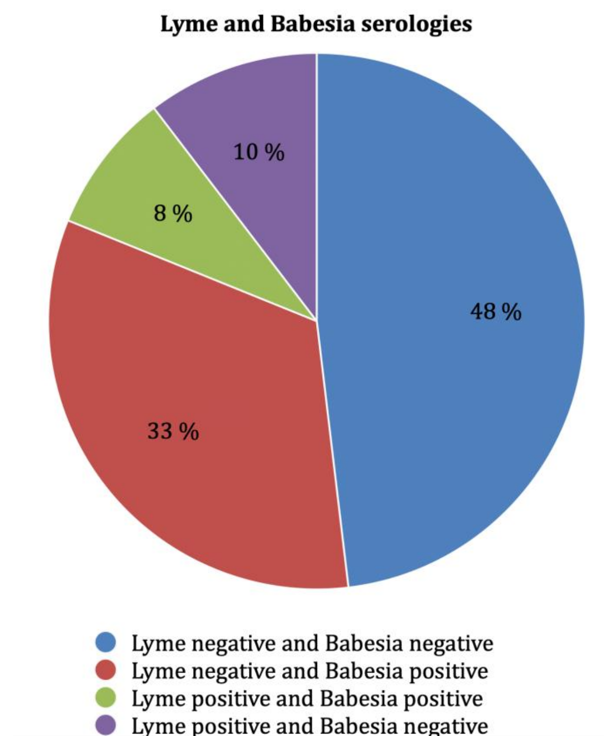

Figure 2: Babesia and Borrelia serologies

2. *Babesia spp* PCR

Babesia spp PCRs were performed in 50 patients (venous blood sampling, n = 19, venous blood and urine sampling; n =3, venous blood, urine and saliva; n = 23, venous blood, urine, saliva and capillary blood sampling; n = 5). *Babesia* PCRs were found to be positive in 6 (12%) patients, (4 in venous blood, 1 in capillary blood and 1 in saliva samples). Among patients presenting with a positive *Babesia* PCR, 3 out of 6 (50%) had a *Babesia* positive serology (3 strongly positive and 1 weakly positive), none out of 5 tested had a *Borrelia* positive serology, one out of 6 (16.7%) tested patients had a positive *Borrelia* PCR result.

3. *Borrelia* serology

Among the 20 (19.2%) patients who had a positive *Borrelia* serology, 5 were positive in IgM and 15 in IgG (Figure 3). Among the patients presenting with a positive *Borrelia* serology, 1 out of 9 had a positive *Borrelia* PCR , 9 out of 20 (45 %) had a positive *Babesia* serology, none out of 10 had a positive *Babesia* PCR (Figure 2).

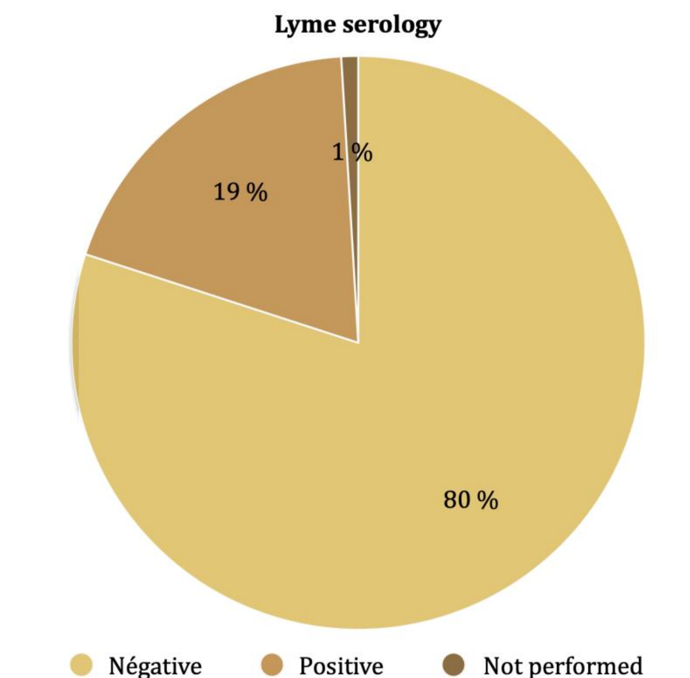

Figure 3: Borrelia serology

4. *Borrelia spp* PCR

Borrelia spp PCRs were performed in 55 patients (venous blood sample; n = 23, blood/urine mixture sample; n = 1, 3 venous blood and urine sample; n = 3, venous blood, urine and saliva, sample; n = 23; venous blood, urine, saliva and capillary blood; n = 5 samples). Nine patients were found to be positive with (16.4%) *Borrelia* PCRs (venous blood sample; n = 5, urine sample; n = 2, blood/urine mixture sample; n =1, saliva sample; n = 1).

Among the 9 patients who were tested positive for *Borrelia* by PCR, 1 patient had a positive *Borrelia* serology (in IgG), 5 patients had a positive *Babesia* serology (3 strongly positive, 2 weakly positive), 1 patient out of 6 tested was tested positive for *Babesia* by PCR. Among the 46 patients presenting with a negative *Borrelia* result by PCR, 8 patients out of 45 tested (17.8%) had a positive *Borrelia* serology (3 specific IgM and 5 IgG), 37 patients out of 45 tested (82.2%) had a negative *Borrelia* serology.

5. *Bartonella* serology

Among the 3 (3.1%) patients with a positive serology for *Bartonella*, one had a positive serology for *Babesia* and *Anaplasma phagocytophilum* (weakly IgG positive result), and one had a positive serology for *Anaplasma phagocytophilum* (weakly IgM positive result).

6. *Bartonella* PCR

Nine (19.1%) patients had a positive PCR for *Bartonella*: *Bartonella spp in 3*, *B. quintana in 2*, and *B. henselae in 4*. Serologies for *Bartonella* were negative in all these cases.

7. *Coxiella burnetii* serology

Three (3.2%) patients had a positive serology for *C. burnetii*. One patient was serologically positive to *Borrelia*, and one patient was positive to *Borrelia* and *Babesia*.

8. *Coxiella spp* PCR

Two (5.1%) patients had a positive PCR for *C. burnetii*. Serologies for *C. burnetii* were negative in these cases.

9. *Anaplasma phagocytophilum* serology

Among the 26 patients with a serology positive for *Anaplasma phagocytophilum,* 18 were IgM positive (of which 16 were weakly positive), 3 were IgG and IgM positive (of which 2 were weakly positive in IgM and strongly positive in IgG, and 1 was weakly positive in IgM and IgG), 5 were IgG weakly positive. Among them, 13 patients had positive *Babesia diverens* serology, 7 patients had positive *Borrelia* serology and 5 patients had positive *Borrelia* and *Babesia divergens* serology.

10. *Anaplasma spp* PCR

Among the 3 (7.7%) patients with a positive PCR for *Anaplasma spp,* one had a low positive IgM serology level.

11. Some patients had a positive PCR for *Ehrlichia spp* or *Rickettsia spp* (Table 1)

Table 1: Real Time Multiplex PCR (ADNucleis laboratory)

Samples	Urine and saliva were collected in dry bottles, five milliliters of blood were collected by venous puncture and around 500µl of capillary blood were collected by finger prick in tubes with EDTA as anti-coagulant, before any antibiotic treatment and were sent in Vacutainer® K2 tubes. Samples (venous blood, urine, saliva, capillary blood) were drawn twice at Day 0 (D0) and Day 2 (D2).
Selection of Primers	To allow the detection of bacteria and parasites, primers targeting specific genes of each microorganism were used to amplify DNA by qPCR. Details of qPCR kits used is listed in Table 2.
Robustness of PCR Mixes	The portion of target genes were synthesized and introduced into a plasmid to obtain a control DNA and facilitate its multiplication. This control DNA was used to validate the amplification mixes. Serial dilution of the plasmid was performed and amplified to determine the robustness parameters of each PCR kit: the limit of detection (LOD), the limit of quantification (LOQ), the repeatability and the reproducibility.
DNA Extraction and Purification	The DNA was extracted without any prior treatment using 300 µl of whole blood with an equal volume of ADNucleis extraction buffer (5 M guanidium thiocyanate, 500 mM TrisHCL, 50 mM EDTA, 20% Tween 20, 20% Triton X-100, 750 µg proteinase K). After incubation for 20 min at 56°C and 15 min at 80°C, the extracted DNA was purified by means of silica magnetic beads and eluted in 250 µl of elution buffer (10 mM TrisHCl, pH 8.5).
Control of the Extraction	Human glyceraldehyde-3-phosphate dehydrogenase (hGAPDH) was used as a housekeeping gene as an internal control for PCR extraction and inhibition. The extracted samples were first checked with a PCR targeting the GAPDH gene. If the results of this PCR were consistent (Ct of GAPDH below 32), the samples were then analyzed for the other pathogens. The sequence of interest of GAPDH was inserted into a plasmid and this plasmid was used as a positive DNA for the validation of GAPDH primers and PCR mix as well as a positive control for subsequent PCRs. The primers used for GAPDH are described in Table 2.
Real-Time PCR (rt PCR)	Real-time PCR was carried out in a total volume of 50 µl with a PCR mix containing ADNucleis PCR buffer (20 mM Tris-HCl, 10 mM NH_4SO_4, 10 mM KCl, 2 mM Mg2+, 0.1% TritonX-100, pH 8.8), 2 mM of each dNTP, 600 nM of each primer, 1 µl of Evagreen and 5 units of ***Taq*** polymerase ADNucleis. Twelve µl of extracted samples were amplified. An initial denaturation step of 5 min at 95°C was followed by 42 cycles of 15 s at 95°C and 40 s at 60°C (hybridization-elongation). The dissociation curves were generated by a last step of 10 min with temperature increments from 75 to 95°C for qPCR kits using Sybr green technology.
Quantification	Positive samples were quantified using a standard curve obtained by amplifying known and calibrated concentrations of control DNA of the desired targets. Quantification was obtained using the standard curve equation (Ct = a (Log10 [DNA]) + b) where “a” is the slope and “b” the intercept of the curve. The results were expressed in genome units (UG) per ml of sample.

Table 2: List of desired targets and details of PCR kits (ADNucleis laboratory)

Microorganisms	Species	Gènes/name	Technology	Primers F	Primers R	Probe	Dye	LOD	LOQ
Borrelia burgdorferi	*sensus lato*	Flagelline	Tqm	CAAAYCAAGATGAAGCDATTGCWGTA	CTTCYTSTTGARCWCCYTCTTGAA	TGCAGYCTGAGCAGYYTGAGCT	FAM	5,7	2280
Borrelia	*miyamotoi*	glpQ	Sybr	TGCACAATTATTTCCCAATCGA	TTCACTGAGACTTAGTGATTTAAGTTCAGTT			12,5	18,8
Borrelia	*hermsii*	flaB	Sybr	AGCTGGATCACAAGCTTCATGGACA	CCCTCTATCTTTGCAAGTGACA			12,5	125
Borrelia	*afzelii*	CP009058.1	Sybr	AATTGCTTGTAGAGTTAA	AAGTTGCTGTTAGTATAG			63,6	636,2
Bartonella	*spp*	rpoB	Sybr	CARGATTTRATTAAYGCRAA	ACRTCRCGMACTTCAAAR			2,57	12,8
Bartonella	*henselae*	ribC	Sybr	GATATCGGTTGTGTTGAAGA	AATAAAAGGTATAAAACGCT			19	125
Bartonella	*quintana*	ribE	Sybr	GATATCGGTTGTGTTGAAGA	AAAGGGCGTGAATTTTG			2,5	125
Babesia	*spp*	18S	Sybr	ACCTGCTAACTAGTDBCC	CACAGACCTGTTATTGCC			5,7	5,7
Rickettsia	*spp*	ARN 23S NR_076610.1	Sybr	ACCGATAGTGAACAAGTA	GGGTCTAATTYATCTAACTAAA			35,6	1780
Ehrlichia	*spp*	16S	Sybr	GAGGATTTTATCTTTGTATTGTAGCTAAC	TGTAAGGTCCAGCCGAACTGACT			6	6
Anaplasma	*spp*	Gène MSP4	Sybr	TTGTTTACAGGGGGCCTGTC	CTTGCCTAGCCTCTAACGTATGAG			25	25
Coxiella	*burnetii*	is111a	Tqm	AATTTCATCGTTCCCGGCAG	GCCGCGTTTACTAATCCCCA	TGTCGGCGTTTATTGGGTTGGTCCC	FAM	2,28	114
Mycoplasma	*spp*	ARN 16S	Tqm	CACACTGGGACTGAGATA	TTCGCCCATTGTGGAATA	CCCTACTGCTGCCTCCCGTA	FAM	5,65	283
Brucella	*spp*	IS711	Sybr	CAATCTCGGAACTGGCCATCTCGAACGGTAT	ATGTTATAGATGAGGTCGTCCGGCTGCTTGG			48,4	48,4
Francisella	*tularensis*	fopA	Tqm	AACAATGGCACCTAGTAATATTTCTGG	CCACCAAAGAACCATGTTAAACC	TGGCAGAGCGGGTACTAACATGAT TGGT	FAM	11,4	114
Theileria	*spp*	ARN 18S	Tqm	ACCTCTTCCAGAGTATCA	GCAGAAATTCAACTACGAG	CAAGTCTGGTGCCAGCAGCC	FAM	11,7	1170
Chlamydia	*spp*	16S	Tqm	TGGCTCTCATGCAAAAGGCA	GATGCCTGGCATTGATAGGCGAWGAAGGA	TGGTTTCAGGTTCTATTTCACTCCC	FAM	48,4	484
hGAPDH		hGAPDH	Tqm	GAAGGTGAAGGTCGGAGT	GAAGATGGTGATGGGATTTC	CAAGCTTCCCGTTCTCAGCC	FAM	22,8	227,9

12. All serology-negative patients:

All serologies (*Borrelia, Babesia divergens, Bartonella, Coxiella* and *Anaplasma phagocytophilum*) were performed in 87 patients. Among them, 32 (36.8%) were negative for all these serologies. Of these 32 patients, one had a positive PCR for *Babesia* by PCR (capillary blood), one for *Borrelia* (venous blood), and one for both *Bartonella quintana* (saliva) and *Ehrlichia* (saliva).

Discussion

The hypothesis of our study is that Lyme disease could not be the only causative factor to explain the persistent polymorphic syndrome possibly due to a tick bite (SPPT), a syndrome close to the post-treatment Lyme disease syndrome (PTLDS) and fibromyalgia syndromes [5-8]. Indeed, A large number of bacteria (other than *Borrelia*), parasites (*Babesia*) and viruses are transmitted by tick bites and could cause different signs and symptoms in patients [5]. Indeed the clinical triptych associates a disabling fatigue, neuro-psychic disorders (memory, sleep, concentration disorders) and various somatic signs including in the first place pain (articular, muscular, tendinous, neurological) [6]. Our retrospective study shows the frequent presence of various different tick-borne infections, first and foremost *Babesia*.

Babesiosis is mainly described as an acute and severe disease in immunocompromised subjects or those with splenectomy. Babesiosis is in fact poorly known, and it is possible that the frequency of infection by these parasites could be underestimated [9]. Some articles report authentic *Babesia spp* infections, as recurrent types in immunocompetent patients, or as a torpid, chronic presentation [10-12]. Diagnosis of babesial infection is usually made by identification of typical intraerythrocytic parasites on a blood smear, *Babesia* DNA by using PCR, and serology test. In our study, exposure to *Babesia divergens* was found in 41% of patients with SPPT and 45% of patients with *Borrelia* positive serology, this result is higher than those reported in previous seroprevalence studies. Studies by Svenson et al performed in patients with a positive serology for *Borrelia*, showed a seroprevalence of respectively: 26.9% for *Babesia microti* (healthy control group: 6.7%) [13] , 16.3% for *B. microti* and *B. divergens* (healthy control group: 2.5%) [14]. Hunfeld et al, found a seroprevalence of 11.5% for *B. microti* and *B. divergens* after tick bite exposures (control group: 1.7%) [15]. Pancewics et al, found a positive *B. microti* serology in 5 out of 144 foresters (4.4%), from the forest inspectorate in Poland. All were also IgG-seropositive for *B. burgdorferi* [16]. In Rigaud et al study, 1 of 810 (0.1%) and 20 of 810 (2.5%) forest workers in France were seropositive for *Babesia. divergens* and *Babesia microti* respectively [17]. While the positivity of a serology test merely discloses a previous exposure, the positivity of *Babesia* by PCR test states that piroplasma was indeed present in 12% of the patients. It could thus be important to diagnose such infections, which can be responsible for the symptomatology and require anti-infectious treatments with anti-malaria drugs. According to the large number of coinfections that were depicted in our study patients, Lyme disease turns out to be the tip of an iceberg, as testing was indeed positive in 19.2% by serology and in 16.4% by PCR of all the tested patients.

No studies have estimated the seroprevalence of *Borrelia* in patients with SPPT yet. In De Kekeureire, et al study, sixty-seven of 310 (21.6%) forest workers were seropositive for Borrelia (18). In Rigaud et al study, 419 of 2975 (14.1%) forest workers were seropositive for *Borrelia* [17]. In Finland, seroprevalence in the general population was estimated at 3.9% [19]. We thus postulate that the patients’clinical symptoms are partly related to the presence of other concomitant microorganisms than *Borrelia*. The sensitivity of *Borrelia* serology

is controversial and some meta-analyses show insufficient sensitivity. In our study, serology was negative in 8 of the 9 patients with a positive PCR for *Borrelia*. However, PCR is still an imperfect technique: PCR sensitivities and specificities are heterogeneous and/or under-evaluated. Some studies used PCR to identify *Borrelia burgdorferi* in early Lyme disease (at a early stage of the disease, thus different from the one in our study) . In Eshoo et al's study, the sensitivity was 62% and the specificity was 100% [20]. Liveris, et al reported a sensitivity of 40.6% [21]. In these studies on early Lyme disease, the direct detection sensitivity was lower than that of the two-tier serology. However, in Bil-Lula's study, 3% negative ELISA IgM results, 2.8% negative results of Line blot IgM, 3.1% and 2.7% of negative ELISA IgG and Line blot IgG results, respectively, were positive in rt PCR [22]. Few studies looked for *Borrelia* in urine by PCR. In one study, detection rate was 91% in patients with Lyme disease skin lesions [23]. In another study, results were disappointing [24]. Our study thus may have underestimated the number of patients infected by *Borrelia.*

Anaplasma phagocytophilum is the second most frequent germ found in serology (27.3%) ahead of Borrelia (19.2%); this microorganism was found in 3 out of 39 PCRs (10.3%). Together with Ehrlichia, found in PCR in 2 cases out of 39 (5.1%), these bacteria are responsible for summer pseudo-flu syndromes, including hepatitis, leukopenia and thrombocytopenia. However, this infection may frequently be subclinical. Indeed, cross-sectional seroprevalence studies have demonstrated that up to 15% of the population in northwest Wisconsin, 1% Connecticut habitants and US military personnel, 17% of Slovenians, and 12% of the population of Sweden's Koster Islands have positive antibodies for *Anaplasma phagocytophilum* without a history of clinical manifestations [25].

Bartonella and *Coxiella* were poorly detected by serology tests during our study. *Bartonella* serology only looks for *B. quintana* and *B. henselae* whereas many other species are described; and *Bartonellae* usually infecting animals have already been observed in humans [26-28]. This study showed that most patients with a combination of signs and symptoms that are consistent with the diagnosis of SPPT have a history of exposure (demonstrated by serology test) or presence of microorganisms (bacteria and parasites) (demonstrated by PCR). In our study, only 36.8% of patients were serologically negative for all micro-organisms tested, and of these, some were PCR positive. Some patients were serologically positive for *Borrelia* and *Babesia divergens*, or for *Borrelia*, *Babesia divergens* and *Anaplasma phagocytophilum*. The clinical signs observed in SPPT may thus have an infectious origin (even if dysimmunitary phenomenons can play a role), and not a psychiatric origin as it has been previously hypothetized [29].

This makes sense, given the number of bacteria and parasites transmitted by ticks, the possibility of multiple tick bites and the fact that ticks themselves can be poly-infected. In addition, some microorganisms are not transmitted by ticks. This study shows a clear limitation of different serologies, which should be put into perspective and the sensitivity reevaluated. Indeed, among patients with positive PCR, serologies were frequently negative: especially for *Borrelia*, *Babesia divergens*, Anaplasma phagocytophilum, *Bartonella* or *Coxiella*. Overall 83% of these serologies were negative when the PCR showed the presence of the micro-organism. One explanation could be that the serologies only look for some species (e.g. only *Babesia divergens* while there are other *Babesia* species as *B. microti* for example [13]. All those results need to be confirmed and further evaluated in larger studies.=This study did

not look for viruses, which are potentially transmitted by ticks. As all patients suffered from signs and symptoms, it is probable that the isolated microorganisms were actually responsible for the disease. However, further studies on larger populations, including healthy control persons, should look at the possibility of asymptomatic carriage.

In conclusion, our study has shown that patients with SPPT/PTLDS, a syndrome close to fibromyalgia, could harbor several tick borne microorganisms. Microbiologic analyses should thus not be merely limited to *Borrelia*'s research alone. The other concomitant pathogens were found by serology and/or PCR. *Babesia* seems to be the most frequent, followed by *Anaplasma phagocytophilum* and *Borrelia*. Future prospective studies are needed by using systematic serology and PCR testings in all patients.

Disclosure: Michel Franck is CEO of ADNucleis; The others authors do not declare any conflict of interest

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