# Risk assessment of transfusion-associated babesiosis in Tyrol: appraisal by seroepidemiology and polymerase chain reaction

*Sissy Therese Sonnleitner,[1] Josef Fritz,[2] Malgorzata Bednarska,[3] Raphaela Baumgartner,[1] Josef Simeoni,[4] Roland Zelger,[5] Harald Schennach,[6] Cornelia Lass-Flörl,[7] Renate Edelhofer,[8] Kurt Pfister,[9] Andrea Milhakov,[9] and Gernot Walder[1,6]*

**BACKGROUND:** After malaria, babesiosis is the second most common transfusion-transmitted parasitic disease in the United States. In Europe, one reported transfusion case, concerning *Babesia microti*, occurred in Germany.

**STUDY DESIGN AND METHODS:** Due to the fact that *Babesia* spp. are present in Tyrolean ticks, the aim of this study is to assess the occurrence of immunoglobulin (Ig)G antibodies against the *Babesia divergens* complex, including *B. divergens* and *Babesia venatorum* (EU1), as well as *B. microti* by screening a representative collective of 988 blood donors from North and East Tyrol (Austria) with indirect immunofluorescence antibody test. Additionally, we investigated 206 local ixodid ticks for the presence of babesial DNA by polymerase chain reaction.

**RESULTS:** Seroprevalence data resulted in rates of 2.1% for IgG antibodies against the *B. divergens* complex and 0.6% against *B. microti* in Tyrolean blood donors. All sera could be confirmed by independent retesting. Our data indicate that cross-reactivity is high between *B. divergens* and *B. venatorum* and lower than 19.8% between *B. divergens* and *B. microti*.

**CONCLUSIONS:** This study shows that *Babesia* spp. are present in the Tyrols, which blood donors come into serologic contact with, and that we have to consider how to sustain blood product safety concerning this new challenge. Additionally, it is the first description of *B. venatorum* in the Tyrols, found in one *Ixodes ricinus* at the Italian border.

*Babesia* spp. are intraerythrocytic protozoan parasites transmitted primarily by ixodid ticks to their vertebrate hosts. Babesial parasites can infect many different vertebrates. Nevertheless, they are reliant on both a competent vertebrate and an invertebrate host for life cycle.[1,2]

In 1957, the first European case of human babesiosis was documented in Croatia.[3] The first human babesiosis in the United States appeared in 1966 in California, but the species of *Babesia* was never definitely characterized.[4] These cases were followed by several hundred cases over a wide geographic range in the United States, Europe,

**ABBREVIATIONS:** IFAT = immunofluorescence antibody test; PRT(s) = pathogen reduction technique(s).

From the [1]Dr Gernot Walder GmbH, Außervillgraten, Austria; the [2]Department of Medical Statistics, Informatics and Health Economics and the [7]Department of Hygiene, Medical Microbiology and Social Medicine, Innsbruck Medical University, Innsbruck, Austria; the [3]Department of Parasitology, Institute of Zoology, Faculty of Biology, University of Warsaw, Warsaw, Poland; the [4]Service for Hygiene and Public Health, Bozen, Italy; the [5]Research Centre for Agriculture and Forestry Laimburg, Pfatten, Italy; the [6]Central Institute for Blood Transfusion and Immunology, University Clinics Innsbruck, Innsbruck, Austria; the [8]Institute of Parasitology, Department of Pathophylsiology, University of Veterinary Medicine, Vienna, Austria; and [9]Veterinary Faculty, Comparative Tropical Medicine and Parasitology, Munich, Germany.

*Address reprint requests to:* Sissy Therese Sonnleitner, Dr Gernot Walder GmbH, Unterwalden 30, A-9931 Ausservillgraten, Austria; e-mail: sissyson@gmx.at.

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Asia, Africa, South America, and Australia.[4-15] Up to the present, approximately 100 species of *Babesia* have been described worldwide, principally based on morphologic characterization.[16]

Babesiosis recently gained in importance as an emerging zoonotic infection in humans. *B. microti, B. divergens, B. venatorum*, and *B. duncani* are the four species of *Babesia* that are generally able to infect people.[4-14]

*B. divergens* is the most human pathogenic *Babesia* in Europe. Approximately 40 cases of human infections have been reported in France, Ireland, and Great Britain, where there is a large cattle industry.[17-19] No infection caused by *B. divergens* has been reported in the United States, although *B. divergens*–like parasites have been identified in three cases by polymerase chain reaction (PCR).[20-22] The entire 18S ribosomal RNA sequences differed by a few base pairs between *B. duncani* and *B. divergens* isolates of European cattle and are now labeled as *B. divergens*-like organisms.[14,23]

The first two infections of *B. venatorum*, formerly known as EU1 *Babesia*, had been reported from Austria and Italy in 2003.[8] In this case, the complete 18S gene was amplified by PCR and compared with 18S ribosomal RNA gene sequences of other *Babesia* spp. In 2007, the third infection of *B. venatorum* had been detected in Germany and was confirmed by PCR. Sequence analysis showed clustering of *B. venatorum* within the *Babesia divergens/odocoilei* complex.[8,24]

*B. microti* commonly appears in the United States[25-28] but also one human case of babesiosis caused by *B. microti* has been reported in Europe, which was confirmed by PCR.[9] *B. duncani*, the previously labeled WA1 *Babesia*, has only appeared in the United States.[29-31]

Besides transmission of *Babesia* spp. by ixodid ticks, transmission via blood transfusion must also be considered. After malaria, babesiosis is the second most common transfusion-transmitted parasitic disease in the United States.[32,33] Since the early 1980s, the number of cases of transfusion-transmitted babesiosis has dramatically increased in the United States. Approximately 120 cases of transfusion-acquired babesiosis caused by *B. microti* and eight fatal cases have been reported from the United States.[34-37] To our knowledge, there was only one reported transfusion case outside North America from Germany that involved *B. microti*.[9]

As a result of this evidence, it would be prudent to consider transfusion-transmitted babesiosis in Europe. Thus, the aim of this study was to assess the occurrence of immunoglobulin (Ig)G antibodies against *B. divergens* and *B. microti* by screening a representative collective of 988 blood donors from North and East Tyrol (Austria) with immunofluorescence antibody test (IFAT) and independent retesting. Additionally, we investigated 206 local ixodid ticks for the presence of babesial DNA by PCR. This study shows that *Babesia* spp. are present in the Tyrols, which blood donors come into serologic contact with, and that we must consider how to sustain blood product safety concerning this new challenge.

## MATERIALS AND METHODS

The area of investigation comprises the Austrian region of the Tyrols with North and East Tyrol and the rivers Drau (East Tyrol), Inn, and Lech (North Tyrol). According to the particular hydrogeographic zones, Tyrol is divided into the valleys Reutte, Upper Inn valley, Central Inn valley, Lower Inn valley, and Drau valley (East Tyrol). The geographical position of the districts is given in Fig. 1. A total of 988 healthy blood donors between 18 and 72 years of age were sampled, representing the total population of the study area regarding sex, profession, and altitude of residency. Participants gave informed consent, answered a questionnaire, and donated 2 mL of blood for study purposes to the Central Institute for Blood Transfusion and Immunology in Innsbruck. A precise description of the donor collective is given in Table 1.

All 988 sera were tested for IgG antibodies against *B. divergens* (a bovine isolate from Hannover, Germany, that had been passaged in jirds) and *B. microti* (strain King's 67) by in-house IFAT. Production of IFAT slides was done as described elsewhere.[8]

Identity of *Babesia* strains on IFATs and screening of ticks were performed by endpoint PCR amplifying a partial segment of the 18S ribosomal RNA gene[38] and subsequent sequencing. IFAT slides were tested by using commercially available hyperimmunesera (SFGP, Fuller Laboratories, Fullerton, CA). Only one serum sample originating from a symptomatic infection with *B. venatorum* was available for testing and yielded a positive result on slides coated with *B. divergens*, provided by the Institute of Parasitology of the University of Veterinary Medicine in Vienna (Austria).

Given the high genetic association between *B. divergens* and *B. venatorum*, the serologic cross-reactivity of IgG antibodies was expected and proven by testing of the only available serum of a confirmed clinical case of babesiosis with the strain EU1.[8] The local cutoff titer for screening against IgG antibodies was set at 1:128 with a well-characterized low-risk collective, consisting of a cohort of 145 blood donors. Blood donors of the low-risk collective were Tyroleans living higher than 1400 m above sea level. None of them had an obvious risk of tick exposure, traveling background, or an employment below 1400 m in altitude.

Sera were assessed positive if the fluorescence signal was strong at a dilution of 1:128 or above and negative if the signal was weak or negative at a titer of 1:128. All sera positive against *B. divergens* were confirmed by independent retesting. Furthermore, the five sera positive for IgG

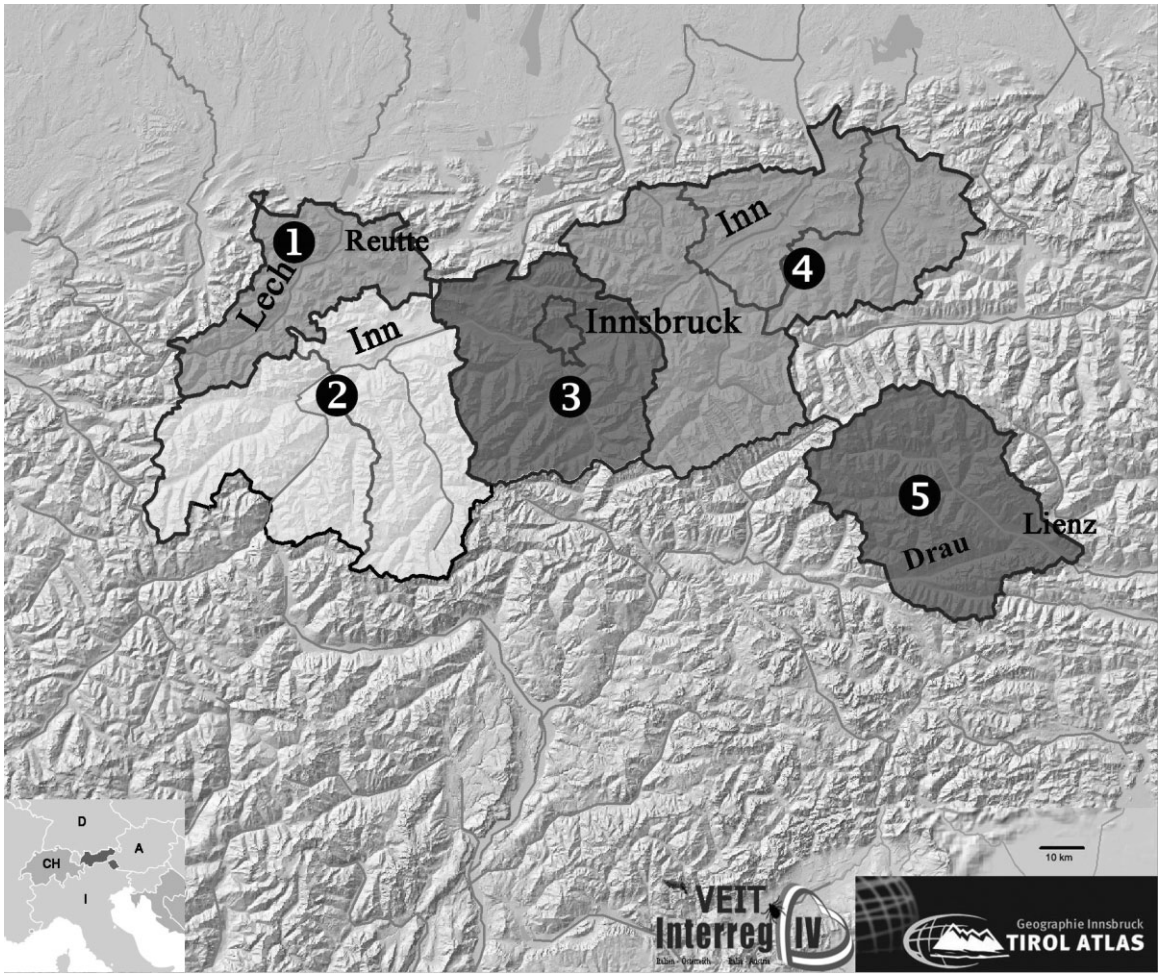


**Fig. 1. Geographic location of the study area in central Europe, the five hydrogeographic zones, and human seroprevalence rates of IgG antibodies against *B. divergens*. Dark gray, more than 2% seroprevalence rate; middle gray, 1% to 2%; light gray, less than 1%.**

**TABLE 1. Overview of the number of inhabitants and the number of tested samples in this study, split into particular river valleys, sex, age groups, urban and rural areas, and the altitude of the river valleys in meters above sea level***

| District | Inhabitants | Samples |
|---|---|---|
| Reutte (1) | 31,584 (2.8) | 58 (0.2) |
| Upper Inn valley (2) | 95,457 (8.4) | 141 (0.2) |
| Central Inn valley (3) | 268,332 (23.6) | 390 (0.1) |
| Lower Inn valley (4) | 227,727 (20) | 317 (0.1) |
| Drau valley (5) | 58,657 (51) | 82 (0.1) |
| Total | 681,757 (60) | 988 (0.1) |
| Male | 333,379 (48.9) | 479 (0.1) |
| Female | 348,378 (51.1) | 509 (0.1) |
| 18-39 years | 374,285 (54.9) | 512 (0.1) |
| 40-72 years | 307,472 (45.1) | 476 (0.2) |
| >10,000 inhabitants | 226,343 (33.2) | 328 (0.1) |
| <10,000 inhabitants | 455,414 (66.8) | 660 (0.1) |
| >1200 m | 47,723 (7) | 69 (0.1) |
| Up to 1200 m | 634,034 (93) | 919 (0.1) |

* Data are reported as number (%).

against *B. microti* were confirmed by using the commercially available IFAT for IgG antibodies against *B. microti* (Fuller Laboratories) at a titer of 1:128. Slides were assessed by two independent examiners.

Collection of questing ticks was performed in the summer of 2009 by approximately 120 volunteer hunters at 25 sampling sites over a period of 3 months by flagging. Ticks were not collected if adhered on deer or other mammals and were identified to species level by morphologic examination[39] under a stereomicroscope as well as by amplifying the six tick-specific reference genes ATP6, COI, COII, COIII, CYTB, and 12S (R. Mitchell, University of Bristol, personal communication, 2012, unpublished). DNA extraction of 206 ticks was performed by alkaline hydrolysis as described elsewhere[40] before screening for *Babesia* spp. by endpoint PCR[38] and subsequent sequencing. Compared data of seroprevalence were tested by a chi-square test. A two-sided significance level of $\alpha = 0.05$ was used for determining significance.

## RESULTS

### Seroepidemiology

Among the low-risk collective of 145 blood donors, 12.4% were positive at a titer of 1:64 and 0 were positive at a titer of 1:128 in IFAT for IgG antibodies against *B. divergens*. Identical results were observed in IFAT for IgG antibodies against *B. microti*. According to criteria of the WHO, the cutoff titer was set at 1:128 for IgG. The same procedure was performed to calculate the cutoff titer for IgM. Of 145 sera, 8.3% were positive at a titer of 1:64 and 0 were positive at 1:128 in IFAT for IgM antibodies against *B. divergens*. Similar results were observed in IFAT for IgM antibodies against *B. microti*, where 11.7% were positive at a titer of 1:64 and 0 were positive at 1:128.

Results of the epidemiologic survey are shown in Table 2. Of 988 sera, 21 (2.1%) were positive in IFAT against the *B. divergens* complex at titers of 1:128 or higher and five (0.5%) were positive in IFAT against *B. microti*. The 95% exact two-sided confidence intervals (CIs) were 1.32 to 3.23 and 0.16 to 1.12, respectively.

All sera could be confirmed by independent retesting. The reaction of IgG antibodies against *B. venatorum* with *B. divergens* in IFAT indicates that these two species are serologically not distinguishable. No serum sample reacted against both *Babesia* spp., indicating that cross-reactivity is lower than 19.1% between *B. divergens* and *B. microti* ($p \leq 0.05$; double-positive samples/all positives + CI of 95%) and specificity lies at 80.9% (actually positives/all positives × 100). The percentage of *B. divergens*–positive blood donors was significantly higher in the older age group from 40 to 72 years (71.4% vs. 28.6%; $\chi^2 = 7.71$; $p < 0.05$). Women were slightly more affected than men (61.9% vs. 38.1%), but this difference was not significant ($\chi^2 = 2.4$; $p > 0.05$). Five of the 21 *B. divergens*–positive blood donors remembered a tick bite (23.8%), and six (28.6%) declared temporary stay abroad outside of Austria. No seropositive cases declared babesiosis-like symptoms like fever, fatigue, headache, myalgia, or erythema. Age patterns and distribution between the sexes were comparable in *B. microti*–positive sera (40% females vs. 60% males; 60% younger than 40% vs. 40% older than 40), but—due to the low number of positive samples (n = 5)—statistically not analyzed. Only two (20%) of those *B. microti*–positive samples remembered a previous tick bite in the past 5 years and only one declared temporary stay abroad outside of Austria. Seropositivity to *B. microti* was not associated with anamnestic clinical symptoms.

**TABLE 2. Seroepidemiologic results of 988 blood donors tested for IgG against *B. divergens* as well as *B. microti* by IFAT at a titer of 1:128, divided into the particular Tyrolean districts***

| District | Number of samples | *B. divergens* | *B. microti* |
|---|---|---|---|
| Reutte (1) | 58 | 1 (1.7) | 0 (0) |
| Upper Inn valley (2) | 141 | 1 (0.7) | 1 (0.7) |
| Central Inn valley (3) | 390 | 11 (2.8) | 1 (0.3) |
| Lower Inn valley (4) | 317 | 6 (1.9) | 2 (0.6) |
| Drau valley (5) | 82 | 2 (2.4) | 1 (1.2) |
| Total | 988 | 21 (2.1) | 5 (0.61) |

* Data are reported as number (%).

### Calculation of the risk assessment

#### *Number of seroconversions per year*

In this study we investigated 988 Tyrolean blood donors with a mean age of 39.3 years, resulting in 38,840 years of life. Among them, we found 21 seropositive samples against *B. divergens*. Under the presumption of a long-lasting immune response we can expect 5.4 (95% CI, 3.4-8.2) seroconversions against *B. divergens* per 10,000 persons per year. For *B. microti* the same calculation results in 1.3 (95% CI, 0.4-2.9) seroconversions per 10,000 persons per year.

#### *Is there a measurable risk for the blood recipient to become infected with* Babesia *spp. in the Tyrols?*

Little literature exists concerning the duration of parasitemia of *Babesia* spp. in humans. First insights into the duration of a human babesiosis is given by Krause and coworkers,[41] detecting *Babesia* spp. in serial measurements of blood samples after months and even years with a mean duration of 82 days. We suppose that a transmission of babesiosis can only occur in the summer months from April to September, when ticks are active (183 days). In this time, the Tyrols record a mean of 25,000 blood donations from approximately 14,000 donors, meaning a crude annual risk of 13.5 donations with seroconversion against *B. divergens* and 3.2 donations with seroconversion against *B. microti* (95% CIs, 8.4-20.5 and 1.0-7.1, respectively).

To calculate the actual risk of transfusion-associated babesiosis in the Tyrols, we used the formula:

Number of seroconversions in the annual blood donations days of parasitemia/Days of tick activity/ Annual number of blood donations × 100,000.

This means for *B. divergens* that the actual risk of a contaminated blood donation can be estimated to be 24.2 per 100,000 blood donations (95% CI, 15.0-36.8). In the case of *B. microti* the actual risk is 5.8 per 100,000 blood donations (95% CI, 1.8-12.8). A summary of the results concerning adjusted incidence rates and actual risk are given in Table 3.

#### *Would* Babesia*-infected blood probably fail another screening test?*

Anamnestic data will not help us identify parasitemic patients. Not one anamnestic factor correlates with

**TABLE 3. Number of seroconversions against the *B. divergens* complex and *B. microti* on a collective of 988 Tyrolean blood donors, adjusted incidence rates of seroconversion, and actual risk at an expected average duration of parasitemia of 82 days**

| Species | Number of seroconversions | Number of person-years | Incidence rate/10,000 person-years (95% CI) | Annual risk/50,000 blood donations (95% CI) | Actual risk/100,000 blood donations (95% CI) |
|---|---|---|---|---|---|
| *B. divergens* | 21 | 38,840 | 5.4 (3.4-8.2) | 27.0 (16.8-41.1) | 24.2 (15.0-36.8) |
| *B. microti* | 5 | 38,840 | 1.3 (0.4-2.9) | 6.4 (2.0-14.2) | 5.8 (1.8-12.8) |

previous babesial infection on the part of the blood donor. A review of the available literature did not yield any clinical symptom or laboratory variable other than serology or eventually direct detection that could contribute to identify persons in the asymptomatic parasitemic stage of disease.

### Investigation of ticks

Of 206 ticks investigated by PCR, none were positive for *Babesia* spp. in North and East Tyrol and the only positive tick was found near the Italian border and could be identified as *B. venatorum*, formerly known as EU1 (Accession Number JX051870). Existing data are limited but support our serologic data on the presence of *Babesia* spp. in the Tyrols.[42]

## DISCUSSION

This study for the first time assesses concomitant seroepidemiology to both *B. divergens* and *B. microti*. To our knowledge, no seroprevalence studies of *B. venatorum* (EU1) have been conducted. It demonstrates that the local population potentially has serologic contact with at least one member of the *B. divergens* complex and, to a lesser extent, *B. microti*. Immunoreactivity is not associated with a history of travels outside of the study area. Direct investigation also supports the occurrence of *Babesia* spp. in local ticks. To our knowledge, it is the first demonstration of *B. venatorum* in the Tyrols. Generally, it is one of only a few seroepidemiologic studies on *Babesia* spp. in European blood donors.[6,7]

Similar studies in Europe revealed rates of 3.6% for *B. divergens* in Midwestern Germany[6] and 5.4 and 1.5% for *B. microti* in Midwestern Germany and Eastern Switzerland, respectively.[7] These rates are comparable to our findings of 2.1 and 0.6% of IgG antibodies against *B. divergens* and *B. microti* in North and East Tyrol.

The age pattern in the 21 *B. divergens*–positive blood donors indicates lasting immunity after serologic contact with *Babesia* spp. Furthermore, our data indicate that cross-reactivity is high between *B. divergens* and *B. venatorum* and lower than 19.8% between *B. divergens* and *B. microti*.

### What are the consequences for blood transfusion medicine?

Symptomatic cases of babesiosis usually affect elderly, asplenic, or immunocompromised patients. Young and healthy blood donors usually experience asymptomatic infections with transient parasitemia.[43,44] Anamnestic data will not help us identify parasitemic patients. Not one anamnestic factor correlates with previous babesial infection on the part of the blood donor.

Austrian blood samples undergo a multistep process to avoid contaminations with infective agents, including tests for neopterin, elevated liver enzymes (still performed only in single blood establishments), anemia, or signs of hemolysis and clinical symptoms consistent with active or recent infections.[45] All of them may single out cases of babesiosis. However, data in literature do not sufficiently support the idea that they would be effective at diminishing the risk of contamination with *Babesia* spp.

Another possibility might be the introduction of pathogen reduction techniques (PRTs), which are highly effective at inactivation of *Babesia* spp.[46] These techniques are available for platelet (PLT) concentrates and plasma but at the moment not for RBCs. Babesiosis is mainly an intraerythrocytic infection and the major amount of blood components transfused are RBCs. However, PLT concentrates, which usually contain a small rest amount of RBCs, might even bear a higher risk of transmitting *Babesia* spp.: PLTs are donated mostly by apheresis, donors with a rather high frequency of donations (average one donation per 2 weeks), and are mostly transfused to immunocompromised patients who bear a high risk to acquire a symptomatic infection of *Babesia* spp. It seems reasonable to start PRT for PLTs and not to wait until PRT is also available for RBCs.

Data on the duration of parasitemia of *Babesia* spp. in humans are rare. Krause and coworkers[41] detected *Babesia* spp. in serial measurements of blood samples after months and even years. The possibility of the asymptomatic persistence of *Babesia* spp. in blood is supported by numerous cases of transfusion-acquired babesiosis.[34-37,47-50] A similar phenomenon of persistent and relapsing infection with *Babesia* spp. was observed by our own work group in a 3-year-old child from North Tyrol (unpublished data).

Parasitemia of a few days (3-7 days) is known for *B. gibsoni*[51] in dogs. Persistent parasitemia for 82 days on average was shown for *B. microti* in humans.[41] However, numerous veterinary data are available on the probability of persistence of diverse *Babesia* spp. in animals for several months or even years at a very low level of parasitemia.[52-65] Chronic infections of *B. microti* have also been shown in primates.[65,66] This chronic carrier state can be indicated by signs of other chronic diseases or stay asymptomatic for many years and only unmask by a relatively high antibody titer.[67] Maybe, just this variable is the most reliable one to identify parasitemic blood preservations, as PCR is more expensive and eventually not sensitive enough to detect the rather low quantum in asymptomatic carriers, especially as parasites can only be detected infrequently, at least in hamsters.[68]

This study shows that *Babesia* spp. is present in the Tyrols, that blood donors come into serologic contact with it, and that we must consider how to sustain blood product safety concerning this new challenge. Therefore, and as vector-borne diseases are subjected to dynamic changes, we recommend reassessment of the risk of transfusion-mediated infections on a regular basis and to introduce PRT for blood components like PLTs.

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#### CONFLICT OF INTEREST

Each author has read the AABB's policy and has no conflicts of interest.

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