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## An investigation into the possibility of transmission of tick-borne pathogens via blood transfusion

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**BACKGROUND:** Tick-borne illnesses were diagnosed in a group of National Guard members, including some who had donated blood a few days before the onset of symptoms. A voluntary recall of those blood components was issued and a multistate investigation was conducted to determine if transfusion-transmitted illness had occurred.

**STUDY DESIGN AND METHODS:** Donors and recipients were asked to complete questionnaires regarding symptoms and risk factors for infection and to provide blood samples for laboratory analysis.

**RESULTS:** Among National Guard personnel who donated blood, 12 individuals were found to have a confirmed or probable case of Rocky Mountain spotted fever or ehrlichiosis. A total of 320 units (platelets or packed red cells) from 377 donors were transfused into 129 recipients. Although 10 recipients received units from National Guard personnel with confirmed or probable infection, none became ill.

**CONCLUSION:** Transfusion-transmitted illness did not occur. Despite the awareness of the risk for tick-borne diseases and the use of tick-preventive measures, many National Guard personnel reported exposure to ticks. In addition to augmenting current tick-preventive measures, scheduling blood drives before rather than after field exercises could further reduce the potential for transmission of tick-borne pathogens.

**I**atrogenic transmission of tick-borne pathogens by blood transfusion has been well documented for *Babesia* species.<sup>1-5</sup> There has been one documented case of *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever (RMSF), being transmitted by blood transfusion. In that case, the donor developed symptoms 3 days after his donation and subsequently died. After a 6-day incubation period, the recipient also developed symptoms of fever and headache, and his illness was confirmed as RMSF by serologic testing.<sup>6</sup> Other tick-borne pathogens, including *Borrelia* and *Ehrlichia* species, have the same potential, but transmission by transfusion has not yet been documented.<sup>2,7-10</sup>

In the southern United States, the two most serious human diseases resulting from the bite of an infected tick are RMSF and ehrlichiosis. RMSF is an acute febrile illness

**ABBREVIATIONS:** CDC = Centers for Disease Control and Prevention; DOD = Department of Defense; EIA = enzyme immunoassay; FDA = Food and Drug Administration; IFA = immunofluorescent assay; RMSF = Rocky Mountain spotted fever; SFG = spotted fever group.

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characterized by malaise, myalgia, headache, and, in approximately two-thirds of the cases, a petechial rash on the extremities, including the palms and soles. Untreated, the illness lasts 2-3 weeks with up to 30 percent case fatalities.<sup>11,12</sup> There are two clinically similar yet distinct forms of human ehrlichiosis: human monocytic ehrlichiosis, caused by *E. chaffeensis*, and human granulocytic ehrlichiosis, caused by a not-yet-named *Ehrlichia* species closely related to *E. equi* and *E. phagocytophila*. Both agents cause an illness similar to RMSF, usually without the characteristic rash, and frequently with the additional laboratory findings of leukopenia, thrombocytopenia, and elevated hepatic aminotransferases.<sup>13</sup> All three pathogens are transmitted by bites from infected ticks. The antibiotic treatment of choice is doxycycline.

In June 1997, the Iowa State Department of Public Health identified 10 Iowa National Guard members with an acute illness characterized by fever, headache, malaise, and myalgia; six of these individuals also reported a rash. Guard members became ill shortly after returning from their 2-week, annual field training exercises at a military base in Arkansas, during which they experienced extensive tick exposure. In addition, several of the ill guard members reported that they had donated blood 24 to 72 hours before the onset of their symptoms. Preliminary testing of their serum samples by the Centers for Disease Control and Prevention (CDC) and Iowa Department of Public Health laboratories detected antibodies reactive with spotted fever group (SFG) rickettsia in four of the ill guard members.

In response to these reports, a voluntary recall of blood components collected at the base during three previous blood drives between May 17 and June 29 was issued on July 3. The CDC, in collaboration with the National Guard and state health departments, initiated an investigation to determine the number of blood donors who developed a tick-borne illness and ascertain if recipients of transfused blood components developed a tick-borne illness that could be linked to an infected donor.

## MATERIALS AND METHODS

The blood collection agency records were reviewed to identify donors who had participated in blood drives held at the military base during May through June, 1997. Additional donors were identified through the National Guard and state health departments whose personnel attempted to contact all guard members who trained at that military base during the training season. Guard members who participated in the blood drives were asked to complete a self-administered questionnaire that included information about their training experience at the base, illness experienced at, or since returning from, the military base, and any associated medical care, prior medical history of tick-borne disease, tick exposure, and tick-bite prevention measures.

Once a list of all the donors was obtained, the number and type of components prepared from each donation, where the components had been distributed (i.e., consignees), and each component's disposition (e.g., transfused, destroyed) were ascertained. On the basis of the results of the donor's blood tests, physicians of patients who had received potentially infectious, recalled blood components were contacted. These physicians were advised of the signs and symptoms of RMSF and ehrlichiosis and the appropriate treatment if disease were detected in a recipient. If a tick-borne infection was suspected in the recipient, or if a recipient had received components from a donor subsequently shown to have had a confirmed or probable tick-borne disease (see below), then the attending physician was requested to submit a serum specimen and complete a questionnaire detailing the recipient's medical history, recent tick exposure, and their medical condition since receiving the recalled blood component.

Laboratory testing for antibodies reactive with tick-transmitted pathogens and DNA from tick-transmitted pathogens was first performed on retention samples available from most (98%) of the donated units of blood. Because serologic confirmation of a case of tick-borne disease requires paired testing of convalescent- and acute-phase samples, an effort was made to contact all donors through their National Guard units at least 6 weeks after the original donation. This effort was coordinated by state health department and National Guard personnel and relied primarily on collections at regular musters. However, donors not present were repeatedly requested by phone to provide blood through a local health clinic.

Sera were tested by immunofluorescent assay (IFA) for IgG and IgM antibodies reactive with *R. rickettsii*, *E. chaffeensis*, and the human granulocytic ehrlichiosis agent and by an investigational enzyme immunoassay (EIA) for IgG and IgM antibody to SFG rickettsiae.<sup>14,15</sup> An IFA result was considered positive at a titer of  $\geq 64$  and an EIA result at a titer of  $\geq 100$  for these agents. Samples from donors reporting an erythema migrans-like rash were also tested by EIA and, when required, by Western immunoblot, for antibodies to *Borrelia burgdorferi*, the agent of Lyme disease.<sup>16</sup>

Total DNA was extracted from whole blood recovered from the retention samples. Attempts to amplify specific DNA of rickettsial origin by polymerase chain reaction used primers for the citrate synthase gene, *RpCS.877p* and *RpCS.1258n*,<sup>17</sup> for the detection of *R. rickettsii*, and primers HE1 and HE3, targeting the 16S ribosomal RNA gene,<sup>18</sup> for *E. chaffeensis*. The detection sensitivity of the *R. rickettsii* assay, as applied to control samples of spiked whole blood, was between 10 and 100 organisms per 200  $\mu$ L of blood. The detection sensitivity of the *E. chaffeensis* assay, as applied to control samples of spiked whole blood, was 1000 infected cells per 1 mL of blood.

Cases of RMSF and ehrlichiosis were classified as confirmed, probable, asymptomatic seroconversion, or past

infection by using both clinical and laboratory criteria (Table 1). A clinically compatible illness was defined as fever, or rash plus at least one other symptom (e.g., headache, arthralgia, or malaise).

Data collected from the questionnaires were analyzed with the use of statistical software (SPSS for Windows version 7.0).<sup>19</sup> Written informed consent from participating donors and recipients was obtained before collection of questionnaires and whole blood.

## RESULTS

Between May 17 and June 29, 1997, 8984 National Guard members from 10 states (Arkansas, Georgia, Iowa, Illinois, Kansas, Missouri, Nebraska, Oklahoma, Tennessee, and Texas) engaged in annual training exercises at the Arkansas military base. Training exercises typically lasted 2 weeks and principally were conducted away, from the barracks, in the field. Three separate blood drives were held at the military base on May 29-30, June 11-12, and June 25-26, coincident with the final days of each training session. Participants of those blood drives included the visiting National Guard members, as well as a few permanent military and civilian staff members at the military base.

A total of 377 donors were identified who had donated blood at one of the scheduled blood drives at the military base (n = 368), or within 4 weeks after having returned home (n = 9). Of these donors, 306 (81%) completed a questionnaire. The median age of donors was 30 years (range, 19-59 years). Seventy-six percent were male and 1.9 percent were civilian staff at the base. Thirty-seven donors reported fever or rash plus at least one other symptom after donating blood. The convalescent-phase serum samples were collected 2 to 18 weeks (mean, 8 weeks) after the date of blood donation. Twelve of the 37 ill donors also had serologic evidence of recent exposure to either *R. rickettsii* or *E. chaffeensis* (Table 2) and were designated as confirmed or probable cases. The average approximate incubation period until the onset of symptoms was 7 days. Five persons sought medical attention (donors 1, 3, 9, 10, and 11 in Table 2) for their illness and one required hospitalization (donor 10) for 2 days. Sixty (16%) of the 377 donors had evidence

of asymptomatic seroconversion: 54 to *R. rickettsii* and six to *E. chaffeensis*. Overall, 14.3 percent (9 of 63) and 40 percent (4 of 10) of the donors with serologic evidence of recent exposure to *R. rickettsii* and *E. chaffeensis*, respectively, reported having a clinically compatible illness. None of the 10 donors tested were positive for antibodies to *B. burgdorferi*. Polymerase chain reaction testing for pathogen DNA was negative on all blood retention samples from the confirmed and probable cases.

The blood that was collected from the 377 donors was separated into 1020 components, including packed red cells, platelets, and plasma. Of these, 320 units (157 packed red cells and 163 platelets) were transfused into 129 recipients in nine states (Alabama, Arkansas, Illinois, Iowa, Louisiana, Mississippi, Oklahoma, Texas, and Utah). Physicians of all recipients were contacted, and none of the 129 recipients had developed symptoms that could be attributable to a tick-borne illness after receiving one of these blood components. Ten recipients received blood from donors who were confirmed or probable cases (Fig. 1). Physicians of these recipients were recontacted and reported that since receiving their transfusions, none of the 10 recipients developed symptoms consistent with a tick-borne illness. Serum samples were forwarded from six recipients; four recipients had either died because of their preexisting medical conditions or were unwilling to participate. Of the six recipients tested, three had positive serologic results with the investigational EIA for IgG to SFG rickettsia. A recipient from Arkansas submitted paired sera that yielded results consistent with past infection with SFG rickettsia, and recipients from Texas and Mississippi had single positive samples, drawn a minimum of 4 weeks after transfusion, that were consistent with past infection or asymptomatic seroconversion to SFG rickettsia. Corresponding IgM tests and all IFA results were uniformly negative on these three recipients.

Tick exposure and awareness were high among the donors—85 percent (260 of 306) of the questionnaire respondents reported that they had always or sometimes employed at least one tick-bite preventive measure, such as using repellent on their skin or wearing a uniform impregnated with tick repellent. However, 75 percent of the

**TABLE 1. Case definitions for RMSF spotted fever and ehrlichiosis**

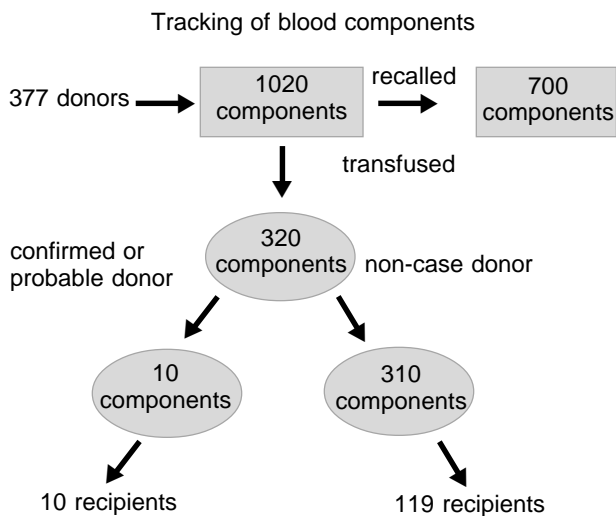
Status	Compatible illness*	Serology
Confirmed case	Yes	≥Fourfold increase or decrease in paired serum IgG titers by IFA
Probable case	Yes	≥Fourfold increase or decrease in paired serum IgM titers by IFA or EIA or ≥Fourfold increase or decrease in paired serum IgG titers by EIA or Single elevated serum IgG or IgM titer by IFA or EIA
Asymptomatic seroconversion	No	≥Fourfold increase or decrease in paired serum IgG or IgM titers by IFA or EIA
Past infection	No	<Fourfold increase or decrease in paired serum IgG or IgM titers by IFA or EIA
Either asymptomatic seroconversion or past infection	No	Single elevated serum IgG or IgM titer by IFA or EIA

\* Fever, or rash plus at least one other symptom.

**TABLE 2. Results of serologic testing and symptoms for confirmed and probable cases of RMSF and ehrlichiosis**

Donor	Antigen	Titers		Fever	Rash	Headache	Myalgia	Arthralgia	Nausea	Saw		Diagnosis
		1st sample	2nd sample							physician	Hospitalized	
1	Ec	<64	1,024	+	-	+	+	-	+	Y	N	Confirmed ehrlichiosis
2	Ec	<64	2,048	+	+	+	-	-	-	N	N	Confirmed ehrlichiosis
3	Ec	<64	512	+	+	+	+	-	+	Y	N	Confirmed ehrlichiosis
4	Ec	<64	64	-	+	-	+	-	-	N	N	Confirmed ehrlichiosis
	Rr	99	12,801									and probable RMSF
5	Rr	99	6,400	+	-	+	+	+	+	N	N	Probable RMSF
6	Rr	99	3,200	+	-	+	+	+	+	N	N	Probable RMSF
7	Rr	99	800	-	+	+	-	+	-	N	N	Probable RMSF
8	Rr	99	6,400	-	+	+	+	-	-	N	N	Probable RMSF
9	Rr	99	1600	+	+	+	-	-	-	Y	N	Probable RMSF
10	Rr	99	12,801	+	+	+	-	-	+	Y	Y	Probable RMSF
11	Rr	99	1,600	+	+	+	+	+	-	Y	N	Probable RMSF
12	Rr	99	12,801	-	+	+	+	+	+	N	N	Probable RMSF

\* Ec = IgG by IFA against *E. chaffeensis* antigen; Rr = IgM by EIA against spotted fever group rickettsiae.



**Fig. 1. Tracking of blood components from 377 donors identified as having been in a tick-infested area within the 4 weeks preceding donation.**

donors reported finding ticks on their body while at the military base, and 33.5 percent reported more than 10 ticks on their body during the training period. Only four donors reported prophylactic use of doxycycline.

## DISCUSSION

In the follow-up of the National Guard blood donations, transfusion-associated illness from a tick-borne pathogen was not demonstrated, although transfusion-associated infection could not be ruled out in all cases. One recipient had static IgG titers against SFG rickettsia that were consistent with past infection (Table 3). In addition, two recipients submitted single serum samples that tested positive for IgG to SFG rickettsia. From these results, it is impossible to determine if these findings represented past infection,

asymptomatic seroconversion, or nonspecific reactivity with the investigational EIA. All of the IFA results were negative, as were IgM assays by EIA. All three individuals were from states where RMSF is endemic, so previous exposure is a possibility. Because no recipient reported the onset of symptoms suggestive of a tick-borne infection after their transfusion, serum samples were not collected from recipients whose donors were asymptomatic seroconverters or from the seronegative donors who reported being ill with symptoms suggestive of infection.

Passive infection with tick-borne pathogens through blood transfusion has been confirmed for one case of RMSF and multiple cases of babesiosis.<sup>2-6</sup> In these cases, there was a period when the donor was asymptomatic and therefore not deferred from donating blood, yet parasitemia was sufficient to result in transfusion-related disease. Other bacteria, such as *B. burgdorferi* and *Orientia tsutsugamushi*, the causative rickettsial agent of scrub typhus, can survive in stored blood products,<sup>7-10,20</sup> but these pathogens have not been linked to transfusion-related disease. Although the pathogenesis of the human ehrlichiosis is incompletely understood, it is likely that an asymptomatic period occurs in which infected individuals have high enough titers of ehrlichiae circulating in their blood that it would be potentially infectious if transfused. Therefore, the potential for transfusion-related disease exists, although the magnitude of the risk is difficult to assess.

There are data to suggest that military training exercises in some locations within the United States place trainees at exceptionally high risk of exposure to ticks<sup>21</sup> and tick-borne diseases.<sup>22,23</sup> In 1989, an outbreak of tick-borne disease coupled with asymptomatic infections occurred among military personnel training at the same base investigated in this report; 38 percent of the training participants were found to have antibodies (single positive titers) against SFG rickettsia (n = 14 cases) or ehrlichial antigens (n = 2 cases).<sup>24</sup> As in this study, the majority (67%) of those persons were asymptomatic or did not have an illness clinically compatible with RMSF or ehrlichiosis. In 1990, a follow up,

**TABLE 3. Characteristics of ill donors and results of serologic testing of recipients' blood**

Donor and status	Recipient	Blood component received	Serology*	Interpretation
1. Confirmed ehrlichiosis	A	Platelets	Rr 1:800, 1:400	Prior RMSF
3. Confirmed ehrlichiosis	B	Platelets	Negative	
	C	Red cells	Unable to test	
4. Confirmed ehrlichiosis and probable RMSF	D	Platelets	Rr 1:400	Prior RMSF or asymptomatic Seroconversion
5. Probable RMSF	E	Platelets	Unable to test	
	F	Red cells	Unable to test	
6. Probable RMSF	G	Platelets	Negative	
9. Probable RMSF	H	Platelets	Negative	
10. Probable RMSF	I	Platelets	Unable to test	
	J	Red cells	Rr 1:100	Prior RMSF or asymptomatic seroconversion

\* Rr = IgG by EIA against spotted fever group rickettsiae (all other IFAs or EIAs for IgM were negative).

prospective, seroepidemiologic study collecting paired pre-exposure and postexposure sera from 1194 military trainees was conducted at the same Arkansas base.<sup>22</sup> Thirty persons (2.5%) seroconverted to *R. rickettsii* and 15 (1.3%) to *E. chaffeensis*, although once again only about 30 percent of the persons seroconverting reported symptoms indicating a possible tick-borne disease.

The current investigation also identified a number of confirmed or probable cases of RMSF and ehrlichiosis among a larger percentage (59 of 71 or 83%) of persons with asymptomatic seroconversions or past infections. Between 1981 and 1992, the average annual incidence of reported cases of RMSF was 0.32 case per 100,000 population in the United States and 1.1 cases per 100,000 in Arkansas.<sup>12</sup> Although not directly comparable, the occurrence of probable RMSF among donors who participated in this investigation and who also sought medical attention was 3 of 306, the equivalent of 980 cases per 100,000 population during a 6-week interval. In assessing the magnitude of the overall situation, one should also consider the potential importance of the large fraction of asymptomatic seroconverting donors. An infection that may be subclinical in a healthy young adult could result in a completely different disease course when transmitted to sick or otherwise compromised transfusion recipients.

Although serum samples were not obtained from all recipients, no one (including those who received components from asymptomatic seroconverting donors) reported becoming ill with symptoms consistent with a tick-borne disease.

At the time of the investigation and the voluntary recall of the blood components, the CDC and the Food and Drug Administration (FDA) advised all individuals who participated in training exercises at the military base during April through June not to donate blood within 4 weeks of their departure. In addition, blood drives there were suspended until September, when the tick season usually is over. On the basis of discussions among representatives from the CDC, FDA, and the Department of Defense (DOD), the FDA has concluded that blood or plasma collection at

this military base during the tick season is safe, provided that no unit is collected more than 48 hours after the potential donor arrives at the base. In addition, the FDA has determined that these trainees will again be advised not to donate blood or plasma for at least 4 weeks after leaving the base.<sup>25</sup> DOD, however, decided not to allow blood collections at this military base in 1998 and 1999.

The potential for transfusion of tick-borne pathogens remains a concern, both within the military and among civilian blood collection agencies. A workshop to review the epidemiology and biology of tick-borne agents with the potential to be transmitted by transfusion was held in Georgia, January 1999, to help agencies responsible for the safety of blood components obtain the background information on these subjects so that appropriate steps can be taken to reduce the risk of transfusion-related infection without mandating unrealistic screening procedures at collection centers.

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