Preplanned Studies

Pilot Surveillance of *Babesia*, *Borrelia burgdorferi*, and *Anaplasma phagocytophilum* Among Chinese Blood Donors — Xinjiang, Inner Mongolia, and Heilongjiang PLADs, China, 2022–2023

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Summary

What is already known about this topic?

Recently, tick-borne pathogens transmitted through blood transfusions have posed new risks to blood safety.

What is added by this report?

We developed a quality control system for nucleic acid testing (NAT) for *Babesia*, *Borrelia burgdorferi*, and *Anaplasma phagocytophilum*, evaluated five Triplex-NAT kits, and conducted external quality assessments of blood centers. This study screened 92,700 blood donors from Heilongjiang, Inner Mongolia, and Xinjiang provincial-level administrative divisions during 2022–2023. A donor in Hegang, Heilongjiang, tested positive for *Borrelia burgdorferi*, marking the first detection of this infection in Chinese blood donors.

What are the implications for public health practice?

Quality control of NAT is vital for managing tick-borne pathogen outbreaks. To ensure blood transfusion safety, screening should be strengthened in high-risk areas outside national borders.

Tick-borne diseases are a growing global concern because of their potential to cause severe illness and geographical spread, which is facilitated by factors such as climate change, human mobility, and ecological disturbances. Tick-borne pathogens like *Babesia*, Colorado tick fever virus, *Anaplasma phagocytophilum* (*A. phagocytophilum*) and others can be transmitted through blood, necessitating vigilant monitoring (*1*). Transfusion-transmitted babesiosis has been reported in the US and Canada (*2*). From May 2020 onwards, a 13-month Babesia nucleic acid pooling testing (16-in-1) program in 14 US states and Washington, D.C. identified 36 positive blood donors with a few subsequent reports of transfusion-transmitted *Babesia* infection (*3*–*4*). China, with its vast territory and diverse climate, hosts over 120 tick species, particularly in provincial-level administratives (PLADs) like Xinjiang, Inner Mongolia, and Heilongjiang (*5*). An examination of blood donors in Mudanjiang City, Heilongjiang Province, in 2016 revealed that 1.3% of individuals tested positive for *Babesia* antibodies (*6*). Additionally, seroprevalence of *B. burgdorferi* in northern Xinjiang PLAD ranges from 9.05% to 16.14% (*7*), highlighting the urgency for targeted surveillance and intervention strategies.

Therefore, the National Health Commission of the People's Republic of China has been conducting nucleic acid testing (NAT) for *Babesia*, *B. burgdorferi*, and *A. phagocytophilum* at border blood centers in Xinjiang, Heilongjiang, and Inner Mongolia PLADs since 2022 to assess the prevalence of tick-borne pathogens in areas with high tick distribution, which provides valuable insights into potential infection risks. Simultaneously, the National Center for Clinical Laboratory (NCCL) has conducted performance evaluations for all screening reagents used. Furthermore, external quality assessments (EQAs) were performed on the blood centers to ensure screening quality.

DNA fragments for detecting *Babesia*, *B. burgdorferi*, and *A. phagocytophilum* in a single assay were synthesized and individually cloned into the pDC316 vector (Shenggong, Shanghai, China) to generate pDC316-*Babesia*, pDC316-*B. burgdorferi*, and pDC316-*A. phagocytophilum* constructs. All three correctly sequenced plasmids were prepared using the thin-film hydration technique to form liposomes (Figure 1A) and were quantified by digital PCR (QIAcuity One 5plex, QIAGEN, Germany). Liposome-based quality control materials were serially diluted in human whole blood to evaluate the limit of detection (LoD) of five domestic blood screening reagents (A, B, C, D, and E), each using its own specific method for blood lysis and nucleic acid extraction. Details on blood volumes for pooled and

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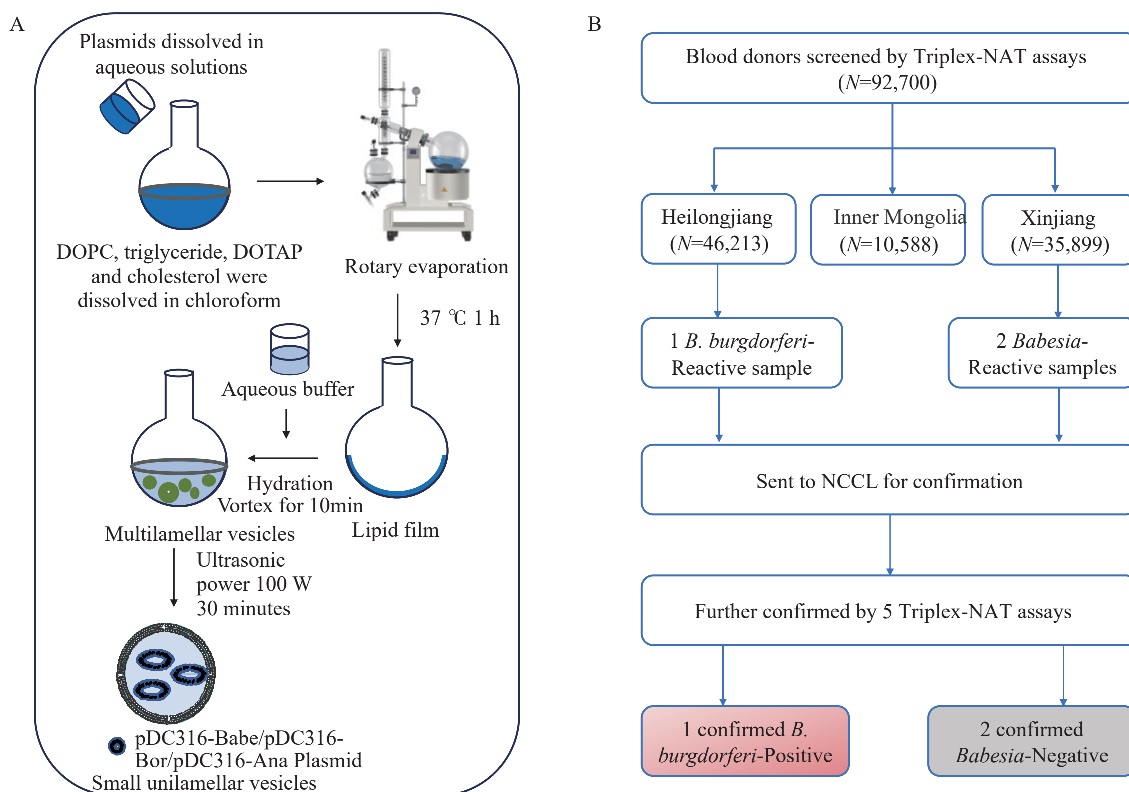

FIGURE 1. Flowchart for the preparation of liposomes and the screening and confirmation of blood donors. (A) Preparation of liposomes by thin-film hydration method. (B) Screening and confirmation algorithm of *Babesia*, *B. burgdorferi*, and *A. phagocytophilum* infection among blood donors.
Abbreviation: DOPC=dioleoyl phosphatidylcholine; DOTAP=1,2-dioleoyl-3-trimethylammonium-propane; NCCL=National Center for Clinical Laboratory ; NAT=nucleic acid testing; *B. burgdorferi=Borrelia burgdorferi*.

individual testing are detailed in Supplementary Table S1 (available at https://weekly.chinacdc.cn/). Each dilution series was tested with approximately seven replicates per lot, totaling around 21 replicates per concentration. Probit analysis was used to determine the LoD of each kit. Within-run and within-laboratory precision were assessed by testing different concentrations (approximately 1–5 LoD and above) five times a day for five consecutive days. EQA was conducted annually at 18 blood centers, involving 10 samples, which covered different concentrations of positive and negative samples. Each participating blood center's results were scored, with a maximum score of 100 points for all correct results. Qualitative results reported by the blood centers are considered acceptable if they score 80 or above.

During the June–July monitoring period for the Inner Mongolia and Heilongjiang PLADs blood centers and the July–August period for Xinjiang PLAD, all blood donors from 18 blood centers were screened for *Babesia*, *B. burgdorferi*, and *A. phagocytophilum* (Figure 1B). Samples with initial reactive results were confirmed at the NCCL using the five Triplex-NAT kits. An initially reactive sample was considered positive when at least two assays yielded positive results.

Five domestic blood screening reagents were used to serially dilute the liposome-based quality control materials to assess the LoDs. Table 1 shows that Reagents A and B had lower LoDs. Reagent A demonstrated the lowest LoDs for both *Babesia* and *B. burgdorferi* in the individual donor (ID)-NAT test, at 33.29 copies/mL and 15.83 copies/mL, respectively. Reagent B had the lowest LoD for *A. phagocytophilum*, at 14.79 copies/mL. Conversely, Reagent B had the lowest LoDs for all three pathogens in the minipool (MP)-NAT test: 178.45, 99.38, and 69.41 copies/mL for *Babesia*, *B. burgdorferi*, and *A. phagocytophilum*, respectively. Reagent D demonstrated the highest LoD for detecting all three pathogens in both test types.

Five screening reagents were used to evaluate the liposome-based quality control materials at different concentrations to determine their precision. All reagents demonstrated good precision, as shown in

TABLE 1. Limit of detection of five Chinese Triplex-NAT reagents (A–E) used to screen for *Babesia* spp., *B. burgdorferi*, and *A. phagocytophilum*.

Tick-borne pathogens	Test types	Reagent manufacturers				
		A	B	C	D	E
Babesia	ID-NAT (copies/mL)	33.29 (23.93, 66.00)	44.9 (34.76, 65.83)	293.37 (236.04, 408.78)	3,819.2	46.55
	MP-NAT (copies/mL)	409.96 (329.39, 567.38)	178.45 (143.46, 241.52)	1,453.67 (908.66, 14,189.50)	35,338.77 (25,412.13, 63,978.22)	238.37 (179.30, 376.17)
B. burgdorferi	ID-NAT (copies/mL)	15.83 (11.83, 26.50)	24.125 (19.80, 31.97)	255.98 (200.80, 372.17)	579.31	445.56
	MP-NAT (copies/mL)	226.93 (177.06, 331.63)	99.38 (80.87, 132.21)	901.42	7,725.61	2,785.42 (2,129.59, 4,199.64)
A. phagocytophilum	ID-NAT (copies/mL)	33.49 (27.22, 45.68)	14.79 (10.80, 26.68)	257.89 (207.96, 356.62	806.19 (614.96, 1215.12)	15.52 (12.71, 20.81)
	MP-NAT (copies/mL)	282.08 (235.03, 363.05)	69.41	1,301.835	19,736.74	237.95 (177.93, 380.22)

Abbreviation: *B. burgdorferi=Borrelia burgdorferi; A. phagocytophilum=Anaplasma phagocytophilum*; ID=individual donor; MP=Minipool; NAT=nucleic acid testing.

Table 2, with -run and within-laboratory coefficients of variation (CVs) of <5%.

From 2022 to 2023, 92,700 blood donors were screened for *Babesia*, *B. burgdorferi*, and *A. phagocytophilum* infections, including 47,172 individuals in 2022 and 45,528 individuals in 2023. In Hegang City, Heilongjiang Province, 4,629 blood donors were screened using reagent A, revealing one *B. burgdorferi*-reactive sample (Ct value of 40.31). This sample was confirmed positive by reagents C and D, with Ct values of 37.6 and 35.29, respectively. The prevalence of *B. burgdorferi* among blood donors in Hegang City was 0.22‰. In 2022, the Kashi and Yili regions of Xinjiang Uygur Autonomous Region each identified one reactive sample during *Babesia* screening; however, both were later confirmed negative. By 2023, no reactive samples were detected in the screened areas.

DISCUSSION

Numerous pathogens capable of blood transmission have been identified. However, most countries, including China, primarily screen for classic blood-borne pathogens such as HBV, HCV, HIV, and syphilis. Routine screening for newly emerging blood-borne pathogens is generally lacking, leaving the global prevalence of infections among blood donors unclear. *Babesia* and *A. phagocytophilum* are usually tick-borne, but are also transmissible by transfusion. *Babesia* was once seen as a significant threat to the US blood supply, with over 250 reported cases of transfusion-transmitted babesiosis, predominantly caused by *B. microti* (98%) (*4*). To date, 11 cases of transfusion-transmitted *A. phagocytophilum* attributed to erythrocytes and platelets, including leukoreduced units, have been reported (*8*–*9*). Although Lyme disease has not been linked to transfusion, death caused by transfusion-associated babesiosis and anaplasmosis has occurred (*9*–*10*). Clinical cases of transfusion-transmitted tick-borne pathogens are rare outside the US and Canada; however, surveillance is crucial in regions with potential risks.

It is important to note that unlike conventional methods that screen for viruses in plasma, tick-borne pathogen identification uses whole blood samples, which can be affected by several factors during extraction. Currently, only Roche and Grifols have FDA-approved commercial reagents. Five domestic blood screening reagents utilize the Triplex-NAT method to detect *Babesia*, *B. burgdorferi*, and *A. phagocytophilum*. To ensure quality monitoring, we prepared quality control materials that mimicked the whole blood matrix used in real samples to assess sensitivity and precision. All five kits demonstrated excellent repeatability and reproducibility with high within-run and within-laboratory precision and a CV of <5%. However, the LoDs differed among reagents. Reagents A, B, and E exhibited lower LoDs and are primarily used in border regions, comprising approximately 82% of the screening applications. In contrast, reagent D exhibited an LoD several orders of

TABLE 2. Precision of five Chinese Triplex-NAT reagents used to screen for *Babesia*, *B. burgdorferi*, and *A. phagocytophilum*.

Test types	Reagent manufacturers		***Babesia* (copies/mL)**				***B. burgdorferi* (copies/mL)**				***A. phagocytophilum* (copies/mL)**			
			100,000	**20,000**	**5,000**	**500**	**100,000**	**20,000**	**5,000**	**500**	**10,0000**	**20,000**	**5,000**	**500**
ID-NAT	A	Average Ct value		28.54	30.66	34.03		27.81	29.9	33.34		28.84	31.03	34.57
		Within-run CV (%)		1.78	1.93	1.82		1.09	1.27	1.61		1.68	1.55	1.19
		Within-laboratory CV (%)		1.92	2.48	3.64		1.86	1.63	3.09		1.83	1.69	2.63
	B	Average Ct value		29.34	30.9	35.29		26.42	27.52	31.71		26.24	27.32	31.43
		Within-run CV (%)		0.38	0.4	0.89		0.27	0.35	0.92		0.17	0.21	0.78
		Within-laboratory CV (%)		0.66	0.42	4.63		0.66	0.41	4.48		0.61	0.37	4.22
	C	Average Ct value	29.28	31.55	34.62		28.81	31.06	33.89		29.23	31.45	34.32	
		Within-run CV (%)	0.62	0.51	1.19		0.57	0.41	0.68		0.83	0.67	0.81	
		Within-laboratory CV (%)	0.66	0.55	1.64		0.64	0.53	1.15		0.99	0.9	0.96	
	D	Average Ct value		25.98				20.11	22.58			22.47	24.92	
		Within-run CV (%)		12.26				0.5	1.85			0.89	1.82	
		Within-laboratory CV (%)		13.15				1.47	2.09			2.06	2.12	
	E	Average Ct value	28.65	31.03	33.44		32.76	34.9	38.77		24.37	26.83	28.79	
		Within-run CV (%)	2.05	1.41	1.3		0.82	1.24	2.14		1.89	1.31	1.59	
		Within-laboratory CV (%)	2.02	2.02	2.16		1.96	1.37	2.83		3.19	2.02	3.21	
MP-NAT	A	Average Ct value		31.38	33.4	36.58		30.75	32.37	35.56		31.62	33.35	37.08
		Within-run CV (%)		2.75	1.94	2.71		1.8	1.63	1.68		2.54	2.07	1.98
		Within-laboratory CV (%)		3.44	3.88	4.02		3.01	2.55	2.61		3.68	3.74	2.93
	B	Average Ct value		30.96	32.78	36.59		28.05	29.39	33.34		27.84	29.24	32.94
		Within-run CV (%)		0.35	0.69	1.8		0.23	0.37	1.16		0.33	0.43	1.29
		Within-laboratory CV (%)		0.61	0.8	2.92		0.6	0.63	3.53		0.95	0.64	3.42
	C	Average Ct value	32.54	34.68	36.71		31.87	33.79	35.86		32.29	34.25	36.65	
		Within-run CV (%)	0.79	0.99	1.93		0.57	0.72	1.26		0.87	0.73	1.35	
		Within-laboratory CV (%)	1.36	1.01	2.01		0.78	1.06	1.3		0.83	0.87	1.62	
	D	Average Ct value	27.72				23.35	24.88			27.13			
		Within-run CV (%)	2.19				5.37	1.39			2.53			
		Within-laboratory CV (%)	2.5				4.91	2.34			4.86			
	E	Average Ct value	31.27	34.29	36.66		34.95	38.03	41.01		26.63	29.48	31.77	
		Within-run CV (%)	1.49	1.55	1.36		1.53	2.71	3.85		1.69	1.54	1.2	
		Within-laboratory CV (%)	2.14	2.25	2.29		1.86	2.65	3.67		2.91	2.55	1.48	

Abbreviations: CV=coefficient of variation; ID=individual donor; MP=Minipool; NAT=nucleic acid testing; *B. burgdorferi=Borrelia burgdorferi; A. phagocytophilum=Anaplasma phagocytophilum*.

magnitude higher than those of reagents A, B, and E, indicating a need for improved detection to prevent viral transmission. Notably, only one laboratory employed reagent D for screening, and all laboratories achieved satisfactory results on EQA.

During the 2-year screening process, a *B. burgdorferi*-positive specimen was found in Hegang, marking the first detection of this infection among Chinese blood donors. This finding highlights the potential presence of tick-borne pathogens in blood donor populations. Conducting pilot surveillance of tick-borne pathogens among blood donors in border regions with developed forestry industries is an efficient strategy to understand the epidemiological status of

these pathogens in China. These regions have a history of pathogen presence and are at higher risk due to their proximity to neighboring countries. They often have limited diagnostic technology, making early pilot surveillance crucial for addressing potential risks and establishing future defense systems. The pilot surveillance employed domestically produced reagents in triplex-NAT mode, using an 8-in-1 or 6-in-1 pooling strategy to enhance screening efficiency significantly. While this study does not cover the entire country, it highlights significant regions where tick-borne diseases may be more prevalent. The findings underscore the need for vigilance in areas with similar ecological conditions, such as Hainan and Guangdong, which are highly favorable for the spread of tick-borne pathogens and experience frequent international trade. Expanding surveillance in these regions will help control tick-borne diseases and establish early warning systems.

This study has some limitations. First, the two initially *Babesia*-reactive samples were sent to the NCCL for confirmation about three months later than scheduled because of the pandemic. This probably resulted in nucleic acid degradation, which made it impossible to confirm the exact infection status of these samples. Second, the two-year monitoring period was limited to border regions, which might not fully reflect the condition across the province. More thorough insights might be obtained by expanding the scope of surveillance and prolonging the observation duration in high-risk locations.

In summary, our study revealed that pilot surveillance in Chinese border regions detected a *B. burgdorferi*-positive sample among donors, highlighting potential pathogen risks. Enhanced screening strategies using reliable quality control systems and highly sensitive assays are crucial for detecting infected blood units, emphasizing preemptive health defenses against tick-borne diseases in high-risk regions.

Conflicts of interest: No conflicts of interest.

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. Details of whole blood extraction methods employed by different reagents.

Reagent manufacturers	Number of pooled samples	Amount of samples required (ID-NAT) (μL)	Amount of samples required (MP-NAT) (μL)	Whole blood volume: lysis buffer volume	Elution volume (μL)	Volume of sample required in PCR (μL)
A	8	400	50	1:3	100	40
B	8	1,200	150	1:3.5	100	20
C	8	1,000	300	1:3	120	5
D	8	400	50	1:2	60	20
E	6	300	50	1:2	70	40

Abbreviation: ID-NAT=individual donor-nucleic acid testing; MP=Minipool; PCR=polymerase chain reaction.