

# An epidemiological survey of HBV infection and low-level HBsAg in military camps in eastern China

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## Abstract

This study aimed to investigate hepatitis B virus (HBV) infection in military personnel in eastern China, which will provide a basis for the prevention of HBV infection.

A total of 15,508 soldiers and 2386 officers were recruited from military camps in eastern China. The markers, deoxyribonucleic acid, serotypes, and genotypes of HBV in serum were detected and analyzed.

Hepatitis B surface antigen (HBsAg) positive rate was 0.44% in soldiers, in whom the low-level HBsAg accounted for 88.24%. The HBsAg positive rate was 1.72% in officers in whom the low-level HBsAg accounted for 12.20%. There were significant differences in the prevalence of high-level and low-level HBsAg, HBV serotypes, HBV DNA positive rate, and mean log HBV DNA between officers and soldiers ( $P < .05$ ). Compared with the conventional method for HBV DNA extraction, the enrichment method for HBV DNA extraction could significantly improve the positive rate and quantification of HBV DNA by real-time fluorescence quantitative polymerase chain reaction ( $P < .05$ ). Sequencing of S gene in HBV was used for the determination of serotype and genotype of HBV. The sequencing success rate was significantly different between soldiers and officers ( $P < .05$ ) as well as between high-level HBsAg group and low-level HBsAg group ( $P < .05$ ). Significant difference was also observed in the genotype distribution between soldiers and officers ( $P < .05$ ).

HBV infection displays a low prevalence and a low epidemic state, and the prevalence of low-level HBsAg is higher in soldiers. We should pay attention to improve the quality of conscription examination as well as emphasize the surveillance, prevention, and protection of HBV infection in military officers.

**Abbreviations:** CMIA = chemiluminescence microparticle immunoassay, ELISA = enzyme-linked immunosorbent assay, HBsAg = hepatitis B surface antigen, HBV = hepatitis B virus, RT-FQ-PCR = real-time fluorescence quantitative polymerase chain reaction.

**Keywords:** hepatitis B surface antigen, hepatitis B virus DNA, hepatitis B virus infection, markers of hepatitis B virus, military camp

## 1. Introduction

Hepatitis B virus (HBV) is one of the most prevalent and most serious viral hepatitis.<sup>[1,2]</sup> There is a high prevalence of HBV in

China, and the high prevalence of chronic HBV infection in China is mainly caused by perinatal or early childhood transmission.<sup>[2]</sup> Since the nationwide HBV vaccination program for neonates was launched by the National Health and Family Planning Commission of People's Republic of China in 1992, remarkable goals have been achieved in controlling the prevalence and propagation of HBV infection. The prevalence of positive hepatitis B surface antigen (HBsAg) reduced to 2.1% in all children and reduced to 1.0% in children born after 1999. The admission of universal HBV vaccination in infants has led to a dramatic decrease in HBV popularity, with HBsAg prevalence declining from 9.75% in 1992 to 7.18% in 2006.<sup>[3]</sup> Among them, people with low-level HBsAg accounts for 6.68% to 23.16% of total HBsAg positive number,<sup>[4,5]</sup> but there is still DNA replication in low-level HBsAg objectives. Especially, the detection and report of low-level HBsAg in clinical laboratories and conscription examination bring new challenges to the clinical examinations, physical examination in conscription, and the prevention and control of HBV in the military camp in China, which has been a concern of clinical, diagnostic, epidemiological and molecular biological experts.<sup>[6-12]</sup> In China, detection of HBsAg has been compulsory in the physical examination of conscription. In order to understand the situation of HBV infection and the characteristics of low-level HBsAg in Chinese military personnel in recent years, and to provide a basis for the prevention of HBV infection, this epidemiological survey was undertaken by stratified cluster sampling in eastern China in 2016, in which chemiluminescence microparticle immunoassay

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(CMIA), real-time fluorescence quantitative polymerase chain reaction (RT-FQ-PCR), and sequencing were used to detect the HBV infection-related parameters.

## 2. Materials and methods

### 2.1. Sample collection

A total of 15,508 soldiers and 2386 officers were recruited by stratified cluster sampling in eastern China in 2016. Among them, there were 15,225 males and 283 females with the age ranging from 18 to 23 years and serving 1 to 3 years as soldiers; there were 2116 males and 270 females with the age ranging from 25 to 55 years and serving 5 to 30 years as officers. Liver-protective, enzyme-lowering, immunoregulatory, and antiviral therapies were not performed within 6 months before study. Serum samples were stored at  $-70^{\circ}\text{C}$ . This study was approved by the Ethics Committee of the 117th Hospital of PLA (PLA-117-20160518).

### 2.2. Reagents

Architect i2000 automatic chemiluminescence immunoassay instrument, kits for the detection of HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc (Abbott Laboratories, Lake Bluff, IL), ABI stepone plus real-time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA), NP968 nucleic acid extraction system, nucleic acid extraction kit (Tianlong Biotechnology Co., Ltd., Suzhou, China), anti-HBs (1000 IU/mL) for HBsAg test, HBV DNA Fluorescence Quantitative Detection Kit [ACON Biotech (Hangzhou) Co., Ltd., Hangzhou, China], BIO-RAD S1000 thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA), and ABI PRISM BigDye sequencing Kit and ABI 3730 Genetic Analyzer (ABI Applied Biosystems, Foster City, CA) were used in the present study.

### 2.3. Measurement of HBV markers and confirmation of HBsAg

CMIA was used to detect the anti-HBs, HBeAg, anti-HBe, and anti-HBc in HBsAg positive serum with Architect i2000 automatic chemiluminescence immunoassay instrument according to the manufacturer's instructions. Positive results were determined if HBsAg was  $>0.05$  IU/mL, anti-HBs was  $>10$  mIU/mL, HBeAg was  $>1.0$  S/N, anti-HBe was  $<1.0$  S/N, and anti-HBc was  $>1.0$  S/N. If serum HBsAg was higher than 250 IU/mL, the sample should be diluted with normal saline to assure the serum HBsAg was  $<250$  IU/mL. The serum HBsAg level was further confirmed by neutralization test. In brief, 100  $\mu\text{L}$  of HBsAg positive serum was independently added into 2 tubes, followed by addition of 100  $\mu\text{L}$  of anti-HBs (1000 IU/mL; measurement value) and normal saline (control value). The mixture was incubated at  $37^{\circ}\text{C}$  for 30 minutes. CMIA was used again to measure HBsAg. True positive result was confirmed if the ratio of (control value – measurement value) to control value was  $\geq 50\%$ , otherwise false positive result was determined.

### 2.4. Extraction of HBV DNA with immunobeads and detection of HBV DNA by RT-FQ-PCR

NP968 nucleic acid extraction system and nucleic acid extraction kit (TianLong) were used to extract HBV DNA from serum samples with slight modifications for routine method and enrichment method. As for the routine method, a 96-well plate was placed into NP968 nucleic acid extraction system; serum samples (200  $\mu\text{L}$ /well) and trypsin (20  $\mu\text{L}$ /well) were added to the

first column; the immunobeads in column 1 were transferred into column 2, followed by lysis at  $90^{\circ}\text{C}$  for 15 minutes; then, the immunobeads were transferred into columns 3 and 4, followed by washing at  $85^{\circ}\text{C}$  for 2 minutes; finally, the immunobeads were transferred into column 5 for elution at  $85^{\circ}\text{C}$  for 5 minutes (elution volume: 100  $\mu\text{L}$ ). The immunobeads were removed, and then 100  $\mu\text{L}$  of eluent (HBV DNA extraction solution) was harvested for the RT-FQ-PCR according to manufacturer's instructions (ABI Applied System and ACON). The remaining HBV DNA extraction solution was stored at  $-70^{\circ}\text{C}$ . HBV DNA positive result was determined if HBV DNA was  $\geq 30$  IU/mL. In addition, enrichment method was used for the extraction of HBV DNA with 500  $\mu\text{L}$  of serum samples and 50  $\mu\text{L}$  of trypsin, and other steps were the same as in the routine method.

### 2.5. HBV "S" gene sequencing

The HBV S gene was amplified by Nest-PCR method,<sup>[13]</sup> amplification products were then purified, recycled and sequenced in Sangon Biotech Co, Ltd. Primers used for amplification in this study were as follows: 1 pair of outer primers I (IF: ACCWTATWCYTGGGAACAA, nucleotide [nt] positions: 1859-1879; IR: TCAGCAAAYACTYGGCA, nt: 1190-1174), 2 pairs of inner primers Ia (IaF: ACCWTATWCYTGGGAACAA, nt: 2819-2837; IaR: GAYGAYGGGATG GGAATACA, nt: 617-598) and Ib (IbF: GACTYGTGGTG-GACTTCTC, nt: 251-269; IbR: TCAGCAAAYACTYGGCA, nt: 1190-1174). For the amplification in PCR, 5  $\mu\text{L}$  of 5 $\times$  KAPA2G buffer A, 5  $\mu\text{L}$  of 5 $\times$  KAPA enhancer, 0.1  $\mu\text{L}$  of KAPA2G Robust HotStart DNA polymerase, 0.5  $\mu\text{L}$  of 10  $\mu\text{M}$  dNTP Mix (Takara, Shiga, Japan), 1  $\mu\text{L}$  of each 10  $\mu\text{M}$  primer, 3  $\mu\text{L}$  of DNA template, and remaining PCR-grade water were included in 25- $\mu\text{L}$  reaction mixture. The PCRs were performed using KAPA2G Robust HotStart PCR kit (KAPA BIO, Boston, MA). PCR was performed using touchdown method consisting of 2 cycles: in the first cycle, the conditions were  $95^{\circ}\text{C}$  for 3 minutes, then 5 cycles of  $95^{\circ}\text{C}$  for 30 seconds,  $57^{\circ}\text{C}$  to  $53^{\circ}\text{C}$  (annealing temperature) for 30 seconds, and  $72^{\circ}\text{C}$  for 30 seconds, followed by 30 cycles of  $95^{\circ}\text{C}$  for 30 seconds, Ta ( $53^{\circ}\text{C}$ ) for 30 seconds,  $72^{\circ}\text{C}$  for 30 seconds, and a final step at  $72^{\circ}\text{C}$  for 2 minutes; in the second cycle, the same conditions were used with the amplification products after the first cycle as the DNA template. The PCR products were sequenced, and the acquired sequences were analyzed and spliced by SeqMan in the Lasergene software (DNASTAR, Inc, Madison, WI). Sequence comparison was performed by using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

### 2.6. Genotyping, serotyping, and phylogenetic tree construction

The HBV S gene sequence was compared by MEGA 6.0<sup>[14]</sup> and a phylogenetic tree was built by using the ortho-abutting method for the obtained sequence to perform genotyping with the representative genotypes (A [AF090842 and X02763], B [AB033554, AF100309, and D00329], C [AB014381, AY123041, and X04615], D [M32138, X65259, and X85254], E [AB032431 and X75657], F [X69798, AB036910, and AF223965], G [AB064310, AF160501, and AF405706], and H [AY090454, AY090457, and AY090460]) recommended by NCBI.<sup>[15]</sup> The presence of S genotype with  $>96\%$  homology is defined as the identical genotype.<sup>[16]</sup> The serotype is determined by the expression of amino acid at the specific site of S gene.<sup>[17]</sup>

**Table 1**  
**HBsAg positive rate and serological patterns of HBV infection in 15,508 soldiers and 2386 officers (%).**

HBsAg grouping	Soldiers (n = 15,508)				Officers (n = 2386)			
	HBsAg positive cases	Serological patterns*			HBsAg positive cases	Serological patterns		
		HBV-M1	HBV-M2	HBV-M3		HBV-M1	HBV-M2	HBV-M3
High-level group	8 (0.05)	5	3	0	36 (1.51)	2	32	2
Low-level group	60 (0.39)	0	57	3	5 (0.21)	0	4	1
$\chi^2$	79.53	40.55	46.88	1.57				
<i>P</i>	<.05	<.05	<.05	>.05				
Total	68 (0.44)**	5	60	3	41 (1.72)	2	36	3

HBsAg = hepatitis B surface antigen, HBV = hepatitis B virus, HBV-M1 = HBsAg/HBeAg/anti-HBc+, HBV-M2 = HBsAg/anti-HBe/anti-HBc+, HBV-M3 = HBsAg/anti-HBc+.

\* *P* > .05 vs officers.

\*\* *P* < .05.

**2.7. Grouping**

Sixty-eight soldiers and forty-one military officers with HBsAg positive serum were divided into high-level HBsAg group and low-level HBsAg group according to the HBsAg threshold at 10 IU/mL.<sup>[4,5,18,19]</sup> According to HBV markers (HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc), subjects with HBV infection were divided into 3 serological patterns: HBV-M1 (HBsAg/HBeAg/anti-HBc+), HBV-M2 (HBsAg/anti-HBe/anti-HBc+), and HBV-M3 (HBsAg/anti-HBc+). Then, the level and the positive rate of HBsAg, the serological patterns of HBV markers, the level and the positive rate of HBV DNA, and serotypes and genotypes of HBV were tested in soldiers and officers.

**2.8. Statistical analysis**

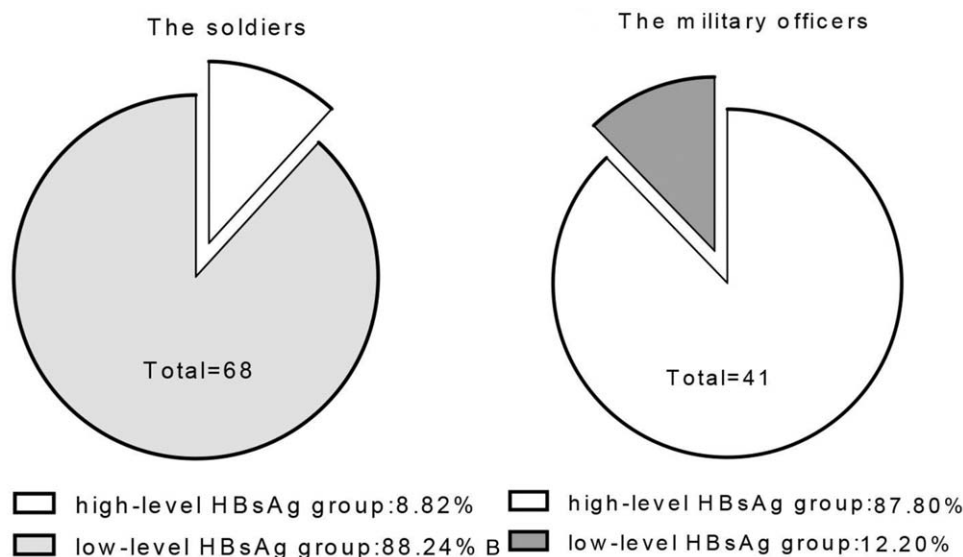
Qualitative data were expressed as frequencies or rates (%). HBV DNA (Log<sub>10</sub>IU/mL) were expressed as mean ± standard deviation; 0 (Log<sub>10</sub>IU/mL) was used when HBV DNA was <30 IU/mL and the actual HBV DNA (Log<sub>10</sub>IU/mL) was used when HBV DNA was ≥30 IU/mL. Comparisons of HBsAg positive rate, HBV DNA positive rate and composition ratio were done with chi-squared test among different groups. Means were compared among different groups with *t* test for equal variances assumed and not assumed. The comparisons between 2 methods (enrichment method and routine method) in the detection of positive rate and mean HBV DNA level were performed with

paired chi-squared test and paired *t* test. Figures were prepared with GraphPad Prism 6 for Windows, and statistical analysis was done with SPSS version 12.01 for Windows. A value of *P* < .05 was considered statistically significant.

**3. Results**

**3.1. Prevalence of HBV infection and low-level HBsAg in military personnel**

HBsAg positive subjects were identified in 68 out of 15,508 soldiers with a positive rate of 0.44% (68/15,508); they were all males, and low-level HBsAg subjects accounted for 88.24% (60/68). In 2386 officers, HBsAg positive serum was observed in 41 subjects with a positive rate of 1.72% (41/2386). Among them, there were 36 males and 5 females, and low-level HBsAg subjects accounted for 12.20% (5/41). The positive rate of HBsAg in soldiers was lower than that in officers (*P* < .05). In soldiers, the HBsAg positive rate of high-HBsAg group was lower than that in low-level HBsAg group (*P* < .05), and the distribution of serological patterns was also significantly different between 2 groups (*P* < .05). In officers, HBsAg positive rate in high-level HBsAg group was higher than that in low-level HBsAg group (*P* < .05), but there was no significant difference of the distribution of serological pattern between them (*P* > .05) (Table 1; Fig. 1).



**Figure 1.** The distribution of high-level HBsAg and low-level HBsAg in the soldiers and the military officers. HBsAg = hepatitis B surface antigen.

**Table 2****Detection of HBV DNA in HBsAg positive soldiers and officers by 2 methods (%).**

Method and HBsAg grouping	Soldiers			Officers		
	n	HBV DNA positive cases	HBV DNA, Log <sub>10</sub> IU/mL	n	HBV DNA positive cases	HBV DNA, Log <sub>10</sub> IU/mL
Routine method						
High-level group	8	7 (87.5)	3.39 ± 2.34	36	30 (83.3)	3.60 ± 2.21
Low-level group	60	16 (26.7)	0.84 ± 1.41	5	1 (20.0)	0.61 ± 1.36
χ <sup>2</sup> /t		10.82	4.40		9.55	2.94
P		<.05	<.05		<.05	<.05
Total	68	23 <sup>***</sup>	1.14 ± 1.73 <sup>***</sup>	41	31 <sup>*</sup>	3.24 ± 2.33 <sup>*</sup>
Enrichment method						
High-level group	8	8 (100)	4.31 ± 2.00	36	36 (100)	4.93 ± 1.65
Low-level group	60	27 (45)	1.41 ± 1.69	5	2 (40.0)	1.37 ± 1.89
χ <sup>2</sup> /t		8.55	4.46		23.31	4.46
P		<.05	<.05		<.05	<0.05
Total	68	35 <sup>**</sup>	1.75 ± 1.96 <sup>**</sup>	41	38	4.49 ± 2.03

HBsAg = hepatitis B surface antigen, HBV = hepatitis B virus.

<sup>\*</sup> P < .05 vs enrichment method.<sup>\*\*</sup> P < .05 vs officers.

### 3.2. Detection of HBV DNA in military personnel with HBV infection

Two methods (enrichment method and routine method) were used for the detection of HBV DNA. Results showed 68 soldiers and 41 officers were HBsAg positive. The positive rate of HBV DNA and mean logarithmic HBV DNA in high-level HBsAg group were higher than that in low-level HBsAg group ( $P < .05$ ). The positive rate of HBV DNA and mean logarithmic HBV DNA, which was extracted with the enrichment method and detected by RT-FQ-PCR, were higher than by the routine method ( $P < .05$ ), meanwhile the positive rate of HBV DNA and mean logarithmic HBV DNA in officers were higher than soldiers ( $P < .05$ ) (Table 2).

### 3.3. The distribution of serotypes and genotypes in military personnel with HBV infection

HBV DNA was extracted by enrichment method from 68 soldiers and 41 officers. After sequencing of S gene, the serotypes and genotypes were determined. The sequencing success rate in soldiers (51.47%, 35/68) was significantly lower than that in officers (92.6%, 38/41) ( $P < .05$ ). Furthermore, sequencing success rate in high-level HBsAg group (100%, 44/44) was significantly higher than that in low-level HBsAg group (44.62%, 29/65) ( $P < .05$ ). The distribution of serotypes was not significant different between soldiers and officers ( $P > .05$ ). For the distributions of serotypes and genotypes, there were no significant differences between high-level

and low-level HBsAg groups ( $P > .05$ ). However, the distribution of genotypes was significantly different between soldiers and officers ( $P < .05$ ) (Table 3).

### 3.4. Evolutionary analysis and phylogenetic tree construction of S gene

Phylogenetic tree was built according to the 44 S genes with success sequencing from high-level group and 29 from low-level HBsAg group with the representative genotypes recommended by NCBI.<sup>[15]</sup> Results showed that the most genotype was B (89.7%, 26/29) in low-level HBsAg group. The homology of representative genotype (genotype B: AB033554, AF100309, and D00329) with S gene was 96.1% to 98.9%, and 61.5% (16/26) of them located at the front part of phylogenetic tree, which meant closer to evolutionary center (Fig. 2, group 1). There were only 3 objectives with genotype C in low-level HBsAg group. The homology of representative genotype (genotype C: AB014381, AY123041, and X04615) with S gene was 98.2% to 99.4%. However, the distribution on phylogenetic tree was not significant due to a small case number (Fig. 2).

## 4. Discussion

This study investigated the status of HBV infection and the epidemiology of low-level HBsAg in military officers and soldiers

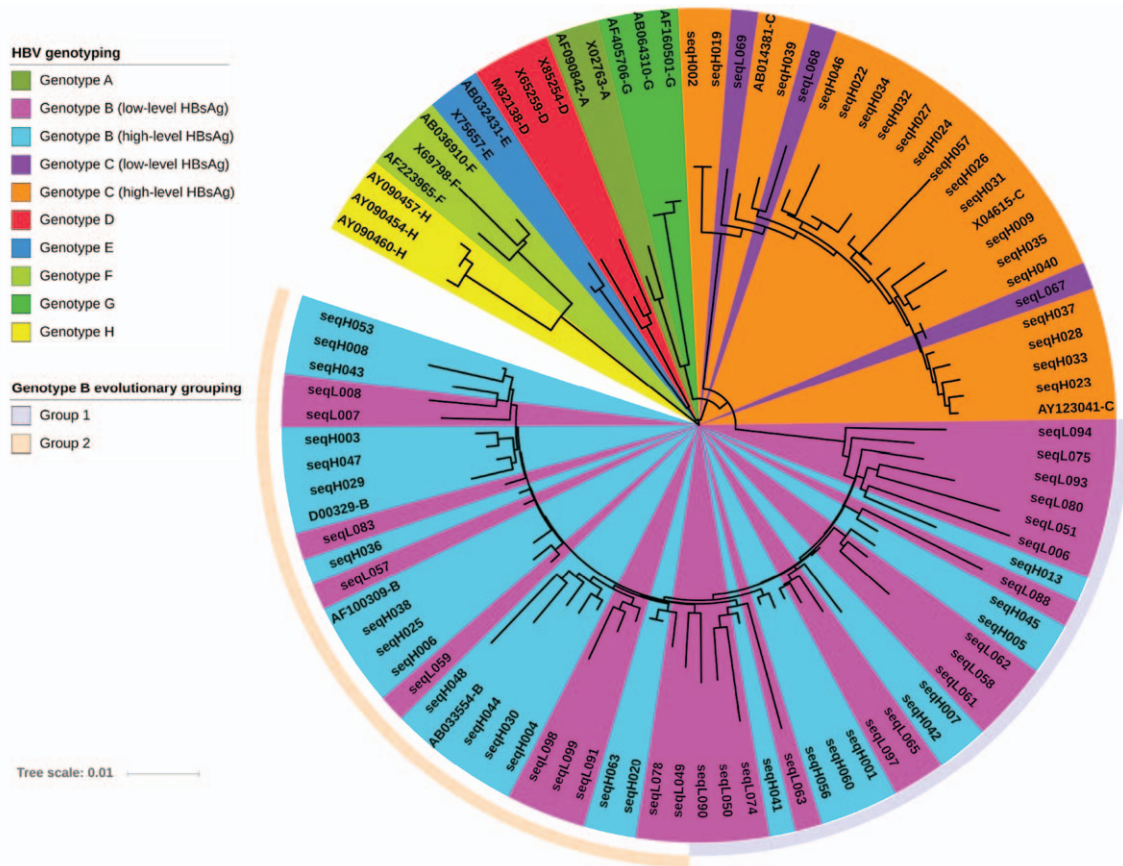
**Table 3****Serotypes and genotypes in HBV DNA positive soldiers and officers (%).**

HBsAg grouping	Sequencing success cases <sup>*</sup>	Soldiers (n = 68)					Officers (n = 41)					
		Serotype <sup>**</sup>			Genotype <sup>*</sup>		Serotype			Genotype		
		adr	adw	ayw	B	C	adr	adw	ayw	B	C	
High-level group	8 (100)	2	5	1	5	3	36 (100)	14	21	1	20	16
Low-level group	27 (45)	3	23	1	24	3	2 (40)	0	2	0	2	0
χ <sup>2</sup>	8.55	2.07	3.03	23.31	1.38	1.54						
P	<.05	>.05	>.05	<.05	>.05	>.05						
Total	35 (51.47)	5	28	2	29	6	38 (92.68)	14	23	1	22	16

HBsAg = hepatitis B surface antigen, HBV = hepatitis B virus.

<sup>\*</sup> P < .05.<sup>\*\*</sup> P > .05 vs officers.





**Figure 2.** The distribution of genotypes B and C on phylogenetic tree in low-level HBsAg group and high-level HBsAg group. HBsAg = hepatitis B surface antigen.

in eastern China. Results showed that HBsAg positive rate in soldiers (0.44%, 68/15,508) was significantly lower than officers (1.72%, 41/2386) ( $P < .05$ ). This may be ascribed to the effective implementation of the immunization program in China in past 20 years<sup>[3]</sup> and the screening for serum HBsAg in conscription by enzyme-linked immunosorbent assay (ELISA). After looking after soldiers' enlist files, it was found that there were 85.71% soldiers (13,292/15,508) had been vaccinated. In this study, CMIA with a higher sensitivity than ELISA was used to detect the serum HBsAg. As shown results from ELISA, missing diagnosis was observed in approximate 0.39% (60/15,508) of soldiers, and among them, 8 had a high-level HBsAg, which might be related to HBV infection after enlistment. For the officers, there was also possibility of infection in daily work and living environment, and the low prevalence of low-level HBsAg was similar to the findings from of nonmilitary personnel in previous studies.<sup>[4,5,18]</sup> Among the soldiers, low-level HBsAg subjects accounted for 88.24% of HBsAg positive subjects, in whom the main serotype was HBV-M2 (HBsAg/anti-HBc/anti-HBc+). In the military officers, HBV infection was mainly with high-level HBsAg (87.8%), and the positive rate of low-level HBsAg among officers (0.21%) was not significantly different from soldiers (0.39%) ( $P > .05$ ). Furthermore, the distribution of serological patterns was compared between high-level and low-level HBsAg groups (Table 1; Fig. 1). The results showed that the different prevalence of HBV infection was mainly in the high-level HBsAg group among the officers and soldiers, while the prevalence of the low-level HBsAg infection in the officers was similar to that of the soldiers, it is due to the

missing diagnosis in the medical examination before service. The positive prevalence of the high-level HBsAg infection population in the officers was higher than that of the soldiers. It may be closely related that the population of high-level HBsAg infection was infected by HBV in the military camp. The service life of the soldiers (1–3 years) was shorter than the military officer (5–30 years), and the officer's risk of HBV infection in the military camp was larger than the soldiers. Therefore, the high-level HBsAg infection prevalence of the officers was higher than the soldiers ( $P < .05$ ).

As for popularity of HBV in military, there were big differences in deferent countries and regions. For example, the positive rate of HBsAg was 10.8% in Senegalese military.<sup>[20]</sup> The seroprevalence of HBV infection was 4.2% among military personnel at Bahir Dar Armed Forces General Hospital, Ethiopia. And higher prevalence of HBV infection (11.3%) was observed in the age group of 40 years old and above.<sup>[21]</sup> The prevalences of HBV infection in these countries or regions were higher than that in Chinese military (0.61%, 109/17,894). However, the positive rate of HBsAg was 1.97% among Lithuanian army soldiers,<sup>[22]</sup> and the prevalence rate of HBV infections was 0.40% in Maroc army soldiers. Sero-prevalence of HBV viral markers among army blood donors at the Blood Donor Center of Mohammed V Military Teaching Hospital of Rabat, Morocco was similar with Chinese soldier.<sup>[23]</sup> These might due to the implementation of HBV vaccine program in these countries or regions<sup>[3]</sup> and HBV screen before enlistment. But these studies only investigated the infection of HBsAg and HBV, which did not further study the

level of HBsAg, and analyze HBV DNA replication, serological typing, and genotyping.

In the detection of serum HBV DNA by RT-FQ-PCR, results showed that both the positive rate of HBV DNA and mean logarithmic HBV DNA in officers were significantly higher than soldiers. The positive rate of HBV DNA and mean logarithmic HBV DNA in high-level HBsAg group were also significantly higher than low-level HBsAg group. Furthermore, the positive rate of HBV DNA and mean logarithmic HBV DNA extracted by enrichment method were significantly higher than those determined by routine method (Table 2). These results indicated that there was low DNA replication (40–50%) in the low-level HBsAg soldiers and officers, the positive rate of HBV DNA was related to the method for nucleic acid extraction (higher efficiency in enrichment method than in routine method). These results were similar to our recently reports on nonmilitary HBV-infected population.<sup>[18]</sup>

In the S gene sequencing for serotyping and genotyping, results showed sequencing success rate in low-level HBsAg group was lower (40–45%) due to the low HBV DNA replication. In addition, the HBV serotype distribution had no relationship with the serum HBsAg level ( $P > .05$ ), and also comparable between soldiers and officers ( $P > .05$ ). However, the genotype distribution was significantly different between high-level and low-level HBsAg groups ( $P < .05$ ): genotype B was dominant in low-level HBsAg group (89.66%, 26/29), while it accounted for 56.82% (25/44) in high-level HBsAg group (Table 3), the genotype distribution of HBV-infected military officers and soldiers was similar to our recent reports on nonmilitary HBV-infected population.<sup>[18]</sup> Evolutionary analysis of S gene in low-level HBsAg group showed that 61.5% (16/26) of them located at the front part of phylogenetic tree, indicating that evolutionary process of S gene in low-level HBsAg was slower than that in high-level HBsAg (Fig. 2).

There is low HBV DNA replication in low-level HBsAg subjects (Table 2),<sup>[5,18,19,24]</sup> and thus the possibility of HBV infection and its transmission cannot be excluded in military camps. Therefore, to effectively lower, control, and prevent the HBV infection in military camps, especially the prevention of missing detection of low-level HBsAg subjects are of great importance. We recommend that detection of all the HBV markers (HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc) was required instead of detection of single HBsAg; CIMA with higher sensitivity was preferred for the detection of HBV markers to reduce the missing diagnosis; the combination of multiple measures such as follow-up, double check, and neutralization test was recommended to avoid the missing detection of low-level HBsAg objectives; and although soldiers and officers have been screened before admission in our country, HBV infection was not only affected by the testing method, but also threatened by HBV infection spreading factors such as life and environment. Therefore, that it was a practical and effective means of prevention and control of HBV infection to conduct regular physical examination and HBV vaccination of the soldiers and officers, to improve hygienic conditions within the camp, and to establish a perfect monitoring and management system for HBV infection.

In conclusion, HBV infection displays a low prevalence and a low epidemic status in military officers and soldiers in eastern China.<sup>[1,2]</sup> Prevalence of low-level HBsAg in soldiers was higher than that in officers and there was still low HBV DNA replication in low-level HBsAg objectives. Measures such as usage of advanced equipment, usage of better HBV marker kits with

higher sensitivity, and improvement of workflow are needed to assure the quality of conscripts. In addition, measures should be taken to monitor and prevent HBV infection in military officers and soldiers in daily work.

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