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Serological detection strategy and prevalence of HIV and Viral Hepatitis B and C in blood donors in Yaoundé Cameroon

Dr Diderot Fopa[1], Claude Tayou Tagny[2], Dora Mbanya[1], Hany Ibrahim Kenawy[3]

1 University Teaching Hospital of Yaounde
2 Université de Yaoundé I
3 Mansoura University

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Abstract

Background. The high prevalence of transfusion-transmissible infections (TTIs) is the most important challenge of safe blood supply in Cameroon. The seroprevalence of Hepatitis B virus (HBV), Hepatitis C virus (HCV), Human immunodeficiency virus (HIV) was determined among prospective blood donors at blood bank Yaoundé University Teaching Hospital (YUTH), Yaoundé, Cameroon.

Material and Methods. Blood donors were consecutively screened for HBV, HIV and HCV infections (Murex HBsAg Version 3, Murex HIV Ag/Ab Combination, and Murex HCV Ag/Ab Combination [DiaSorin]). Additional HBV testing including anti-HBc (Monolisa Anti-HBc PLUS; BIO-RAD) were performed. HIV and HCV serology were confirmed with HIV BLOT 2.2 (Genelabs Diagnostic) and INNO-LIA HCV (Fujirebio), respectively.

Results. In total, 1.162 donors were serially included in the study. Screening for viral infections showed that 91 (7.80%) of total sample donations were reactive for HBsAg+, 14 (1.2%) for HIV+, 11 (0.95%) for HCV+, and 1 (0.08%) for HBsAg+ and /HIV+. Screening samples for total Anti-HBc IgG+IgM revealed that 613(52.75%) samples were reactive. All of the 91 samples positive HBsAg positive were also positive for HBcAb. In the 1071 HBsAg negative participants, the prevalence of HBcAb was 48.7% (n=522). In seronegative participants for HBsAg, HCV and HIV, the prevalence of HBcAb was 48.8% (n=511). Testing of 13 HIV and 9 of HCV reactive samples revealed that 4 and 3 were confirmed positive by western blot, respectively.

Discussion. This study clearly showed a high prevalence of viral infections among Cameroonian blood donors at the YUTH. Strategies to increase voluntary and regular donors should be intensified to improve the medical selection of blood donors and reduce the frequency of TTIs found in donated blood. The confirmatory results of HIV and HCV underline the need to re-evaluate viral infection prevalence in Cameroonian blood donors.

D. Fopa[1], **D. Candotti**[2], **C. T. Tagny**[1,3], **E. L. Murphy**[4], **S. Laperche**[2], **D. Mbanya**[1,5], **F. El Chenawi**[6], **H. I. Kenawy**[7]

[1] *Hematology & Transfusion Service, Yaounde University Teaching Hospital, Yaoundé, Cameroon.*

[2] *Former National Institute of Blood Transfusion/INTS, Department of Blood borne Agents, National reference Center for Infectious risks in blood transfusion, Paris, France.*

[3] *Department of Hematology, Faculty of Medicine & Biomedical Sciences of University of Yaoundé I, Yaoundé, Cameroon.*

[4] *University of California San Francisco and Vitalant Research Institute, San Francisco, California, USA.*

[5] *National Blood Transfusion Service, Yaounde, Cameroon.*

[6] *Faculty of Medicine, Mansoura University, Mansoura, Egypt.*

[7] *Faculty of Pharmacy, Mansoura University, Mansoura, Egypt.*

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Abbreviations

anti-HBc	antibodies against the HBV core antigen
DNA	Deoxyribonucleic acid
anti-HBc	anti-HBc antibodies against the HBV Core antigen
cccDNA:	*covalently closed circular DNA*
EIA:	*Enzyme Immuno-Assay*
HBsAg:	*Hepatitis B Surface Antigen*
HBV cccDNA	Hepatitis B viral convalently-closed-circular DNA
HBV:	*Hepatitis B Virus*
HBV-DNA	Deoxyribonucleic acid of hepatitis B virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HVB:	Hépatite Virale B
INTS:	Institut National de la Transfusion Sanguine
NAT	Nucleic acid testing
OBI	Occult Hepatitis B Infection
PCR	Polymerase Chain Reaction
pgRNA:	*pregenomic RNA*
TTIs	Transfusion Transmitted Infections

Background

In sub-Saharan Africa (SSA) the prevalence of viral infections such as Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) in blood donors is high considering their epidemiology and especially the blood safety process and procedures implemented by blood services [1][2]. Therefore screening for transfusion-transmissible infections (TTIs) to exclude blood donations at risk of transmitting infections from donors to recipients is a critical part of the process of ensuring that transfusion is as safe as possible [3]. According to World Health Organization (WHO) recommendations, screening of all blood donations should be mandatory for the following markers: screening for either a combination of HIV antigen-antibody or HIV antibodies for HIV-1 and HIV-2, screening for hepatitis B surface antigen (HBsAg) for Hepatitis B, screening for either combination of HCV antigen-antibody or HCV antibodies and screening for specific treponemal antibodies [3].

Globally, there are approximately 71 million individuals chronically infected with hepatitis C virus (HCV), more than 257 million with hepatitis B virus (HBV), and 37.9 million human immunodeficiency virus (HIV)-infected people [4][5]. According to WHO reports, the prevalence of *HBV*, *HCV* and *HIV* varies from 0.008% to 6.08%, 0.004% to 1.96%, and 0.0004% to 2% respectively in different parts of the *world* [6][7].

In SSA the incidence of HIV attributable to transfusion is uncertain; but some findings suggested that only 1% of new HIV infections are attributable to transfusion [8]. The prevalence of HBV in blood donors is above 10%, Occult HBV infection remains unaddressed. Also, there is an increase in donor HCV prevalence, despite dedicated donor selection and educational efforts[8].

In Cameroon, there is a National Programme of Blood Transfusion that coordinates blood transfusion activities at the Ministry of Health in collaboration with hospital blood banks. This programme elaborates policies and guidelines in order to harmonize the blood banks practices for blood safety. But there National Centre of Blood Transfusion has been created in 2018 and still yet to function. Despites those measures taken in Cameroon, there are lots of critical challenges to improve supply and safety of blood through voluntary donors [9][8].

Provision of constant and safe blood has been a public health challenge in SSA with high prevalence of TTIs. Monitoring of the magnitude of TTIs in blood donors is important for determining the risk of transmission of infections and optimizing donor recruitment strategies. Therefore, this study aimed to determine seroprevalence of HIV, HBV, HCV and associated factors among blood donors at the Yaoundé University Teaching Hospital Blood Bank.

Materials and Methods

Study setting and population

Blood donors' sera were collected serially at the Blood Bank of the Yaounde University Teaching Hospital (YUTH) (Cameroon) and were screened for HBsAg, HIV and HCV serology. Two aliquots of recruited donor were made and stored at –20°C and later on transported to the Reference Laboratory of Virology of the National Institute of Blood Transfusion in Paris (France) where further investigations were made (screening for HBcAb, western blot for HIV and HCV, retesting of

HBsAg). The study obtained an authorization for research for the General Directorate and the Ethical Clearance from the Regional Ethical Committee for Center Region, Yaounde. Prior to recruitment at the Blood Bank of YUTH, each participant was informed about the study through an information leaflet where they read and signed the consent form. A questionnaire was then answered by each consent participant.

Serological screening and confirmation

Blood donors were screened initially for HBV, HIV and HCV infections (Murex HBsAg Version 3, Murex HIV Ag/Ab Combination, and Murex HCV Ag/Ab Combination [DiaSorin]) at the YUTH.

Further serological investigations of viral infections were done at the Reference Laboratory of Virology of the National Institute of Blood Transfusion in Paris (France). All samples were retested by Murex HBsAg Version 3 and HBsAg positive samples were screened for anti-HBc (Monolisa™ Anti-HBc PLUS; Biorad, Marne la Coquette, France). Reactive samples on Murex HCV Ab/Ag combo were retested by Monolisa HCV Ag/Ab ULTRA and then confirmed by INNO-LIA HCV (Fujirebio). HIV positive samples were confirmed using HIV BLOT 2.2 (Genelabs Diagnostic).

Statistical analysis

The Statistical Package of Social Sciences (IBM SPSS® Statistics, Armonk, NY, USA) version 21 was used in statistical analysis. Cross tabulation and χ^2 test were used to detect the significant differences between serological markers (Hepatitis B surface antigen, HIV antibodies and HCV antibodies) and the donor characteristics (Gender, age groups and donor type). $p<0.05$ was considered statistically significant

Results

A descriptive and cross-sectional study was conducted on 1162 blood donors (1002 male and 160 female) with known status for HBsAg, HIV Ab and HCV Ab. The mean age was 29.24 (SD= 8.21). Voluntary and family donors were 328 (28.2%) and 834 (71.8%), respectively. The prevalence of HBsAg, HIVAb and HCVAb after Enzyme ImmunoAssay screening of 1162 samples were 7.8% (n=91), 1.2% (n=14) and 0.95% (n=11), respectively. The prevalence of HIVAb and HCVAb after Western-blot screening of samples were respectively 0.34 % (n=4) and 0.26% (n=3) (figure 1).

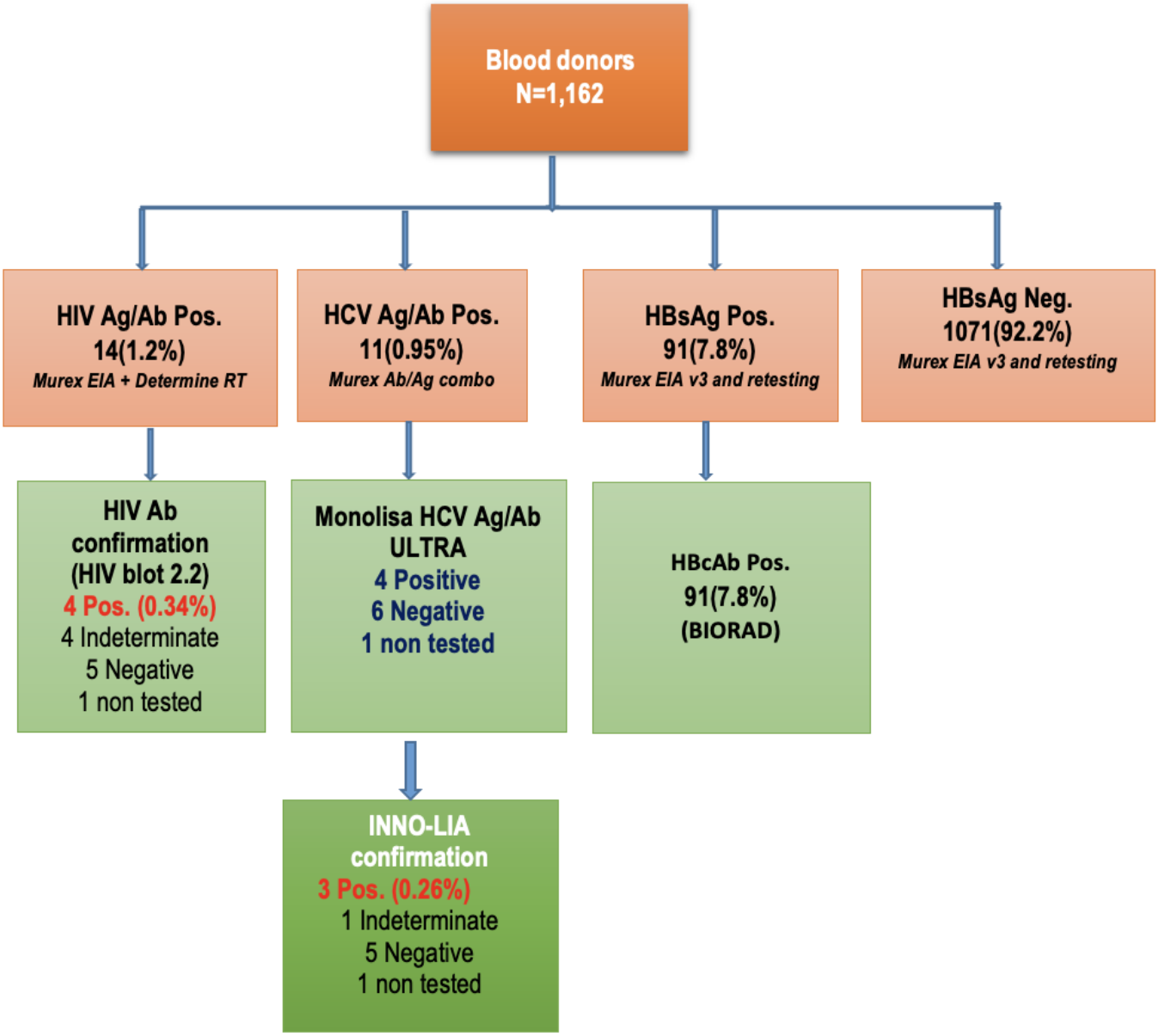

Figure 1. Flow chart of serological investigations of viral infections in blood donations collected at the University Teaching Hospital Blood Service, Yaoundé, Cameroon.

The prevalence of HIVAb and HCVAb after Western-blot screening of samples were respectively 0.26% (n=3), 0.34 % (n=4) (table 1).

Table 1. Demographic and serological characteristics of 1,162 Cameroonian blood donors at the Yaounde University Teaching Hospital in 2017

Characteristics	HBsAg[a] N=1162 n (%)		INNO-LIA HCV[b] N=1162 n (%)		HIV BLOT 2.2[c] N=1162 n (%)	
	Positive 91(7,83)	Negative 1071(92,17)	Positive 03(0,26)	Negative 1159(99,74)	Positive 4 (0,34)	Negative 1158(99,66)
Gender						
Male	81(8,08)	921(91,92)	3(0,30)	999(99,70)	4(0,40)	998(99,60)
Female	10(6,25)	150(93,75)	0(0.00)	160(100.00)	0(0,00)	160(100,00)
p-value	*NS*		*NS*		*NS*	
Type of donor						
Benevolent	25(7,62)	303(92,38)	00(0.00)	327(100.00)	0(0,00)	328(100,00)
Family	66(7,91)	768(92,09)	3(0,36)	831(99,64)	4(0,48)	830(99,52)
p-value	*NS*		*NS*		*NS*	
Age group (year)						
[18;20]	9(9,18)	89(90,82)	0(0,00)	98(100)	1(1,02)	97(98,98)
[21;30]	51(7,70)	611(92,30)	1(0,15)	661(99,85)	0(0,00)	662(100,00)
[31;40]	21(7,66)	253(92,34)	0(0,00)	274(100)	2(0,73)	272(99,27)
[41;50]	10(10,20)	88(89,80)	0(0,00)	98 (100)	1(1,02)	97(98,98)
[51;60]	0(0,00)	28(100,00)	1(3,57)	27(96,43)	0(0,00)	28(100,00)
[60;65]	0(0,00)	2(100,00)	1(50)	1(50)	0(0,00)	2(100,00)
p-value	*NS*		*NS*		*NS*	

HBsAg: hepatitis B antigen; NS: not significant; NA: not applicable.

[a]*All samples were retested by Murex HBsAg Version 3*

[b]*Only reactive samples with Murex HCV Ab/Ag combo and Monolisa HCV Ag/Ab ULTRA were confirmed with INNO-LIA HCV (Fujirebio).*

[c]*Only HIV positive samples with Murex HIV Ag/Ab Combination were confirmed using HIV BLOT 2.2 (Genelabs Diagnostic).*

Screening samples for total Anti-HBc IgG+IgM revealed that 613 (52.75%) samples were reactive. All of the 91 samples positive for HBsAg were also positive for HBcAb. In the 1071 HBsAg negative participants, the prevalence of HBcAb was 48.7% (n=522). In seronegative participants for HBsAg, HCV and HIV, the prevalence of HBcAb was 48.8% (n=511).

Discussion

Cameroon experiences critical challenges in blood safety and availability. The high prevalence of blood borne viruses

(including HIV, HBV, and HCV) remains a major concern. Although articles published in recent years show that significant change has been made in settings where WHO guidelines and quality system have been implemented, blood safety is still an important issue in Cameroon. Also, the trend of TTIs can be well monitored only it an effective quality system is put in place. That is why the prevalence of TTIs can be influenced by the screening algorithm and the tests used.

This survey determines the trends of hepatitis B, HIV and hepatitis C infection with serological confirmation in blood donors at the Blood Bank of the Yaounde University Teaching Hospital, Cameroon. In that setting, HBV screening relies only on detection of HBsAg. The seroprevalence of HBV infection in Cameroon is high. Studies show that the overall pooled seroprevalence is 11.2% (95% CI 9.7% to 12.8%) with high heterogeneity between studies (I^2=97.9%) [10].

The mean age was 29.24 (SD= 8.214), the sex ratio male/female was 6.3. The majority of participants (57.03%) were aged between 21 to 30 years old. Voluntary and family donors were 328 (28.2%) and 834 (71.8%), respectively. In 2009, Tagny *et al.* [9], reported in Cameroon the following characteristics of blood donors: 63.7% aged between 18 to 30, 28.3% female, 25.5 % benevolent. Up till now, the Yaounde University Teaching Hospital Blood Bank is still far from the strategy of the WHO Regional Committee for Africa [11].

After confirmation, the prevalence of serological markers for HBV, HIV, and HCV were respectively 7.83%, 0.34 % and 0.26%. These prevalence have dropped compared to those obtained in the same setting by Tayou Tagny *et al.* in 2009: 10.3% for HBV, 2.9% for HIV, 3.9 % for HCV [9]. This is due to the implementation of WHO recommendations in terms of screening blood donation for TTIs and also the implementation of the Standards of the African Society for Blood Transfusion. Then, the reduction of the prevalence of HBV can also lead to the reduction of OBI in blood donors.

From the 1072 HBsAg negative participants, 522 were positive for HBcAb (48.8%) and the frequency of HBcAb only positive donors was 44.4% (516/1162). In Egypt, Antar *et al.* [12] reported that 7.8% of blood samples negative for HBsAg found to be reactive to anti-HBc. In 2013, Zaid et al reported that 16.6% of HBsAg negative blood donors were positive for HBcAb in Egypt [13]. Thus, In Cameroon, blood donors seem to be highly prevalent to anti-HBc compared to blood donors in Egypt, whereas both countries are highly endemic for HBV. This may be related to the vertical transmission and horizontal transmission before age five, resulting in frequent chronic infections which lead to occult infection. This marker is associated with contact with HBV whether resolved or chronic infection results from such contact. It becomes detectable in post-acute infection some days or weeks after the peak of HBsAg and HBV DNA has been reached [14]. In high endemic areas like Cameroon, the value of the information is limited since a majority of the population of blood donation age carries anti-HBc.

This study clearly showed a high prevalence of HBV but lower prevalences of HIV and HCV among Cameroonian blood donors at the YUTH, but the trends of viral infections were lower than those observed in the same setting by Tayou Tagny *et al.* in 2009. Strategies to increase voluntary and regular donors should be intensified as such medical selection of blood donors may reduce the frequency of TTIs in blood donors. The confirmatory results of HIV and HCV underline the need to re-evaluate viral infections prevalence in Cameroonian blood donors. At the national level, a screening algorithm should be developed for each TTI. The design of an algorithm will be determined by the specific infection marker to be screened for, the expertise of the users, the infrastructure, testing conditions and quality systems of individual screening facilities.

Once an algorithm has been defined, this will guide the procurement of the specific test kits, reagents and laboratory testing systems required.

Conclusions

This study clearly showed a high prevalence of HBV but lower prevalences of HIV and HCV among Cameroonian blood donors at the YUTH, but the trends of viral infections were lower than those observed in the same setting by Tayou Tagny *et al.* in 2009. Strategies to increase voluntary and regular donors should be intensified as such medical selection of blood donors may reduce the frequency of TTIs in blood donors. The confirmatory results of HIV and HCV underline the need to re-evaluate viral infections prevalence in Cameroonian blood donors. At the national level, a screening algorithm should be developed for each TTI. The design of an algorithm will be determined by the specific infection marker to be screened for, the expertise of the users, the infrastructure, testing conditions and quality systems of individual screening facilities. Once an algorithm has been defined, this will guide the procurement of the specific test kits, reagents and laboratory testing systems required.

These findings underline the need of confirmatory strategies to avoid blood wastage and to reevaluate viral infections prevalence in African blood donors that may be overestimated.

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Authorship contributions

- **Diderot Fopa, Claude Tayou Tagny, Dora Mbanya, Syria Laperche, Daniel Candotti, Edward. L. Murphy** conception and design of the study; analysis and interpretation of data; manuscript writing and final approval of the manuscript.
- **Hany Ibrahim Kenawy and Farha El Chenawi:** design of the study, interpretation of data, manuscript review and final approval of the manuscript.
- **Claude Tayou Tagny, Syria Laperche and Edward. L. Murphy:** provision of study material
- **Diderot Fopa, Camille Doux and Daniel Candotti:** Laboratory testing
- **Diderot Fopa, Daniel Candotti and Syria Laperche:** collection, assembly, analysis and interpretation of data.

- **Diderot Fopa** is the first author.

Competing interests

The authors declare that they have no competing interests.

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