

Identification of Viral Biomarkers in Clinical Laboratory Waste (LW) and Chances of Community Pollution: A Cross Sectional Study Among Government and Private Sector Laboratories of Hooghly and Burdwan District, West Bengal, India

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ABSTRACT

Objective: Bio Medical Waste handlers may be victimized of blood borne viral diseases if clinical laboratories dispose off the biological samples without proper disinfection procedure. To assess the risks of laboratory waste related environmental contamination a cross sectional study was performed with the disposed specimens i.e. laboratory waste study (LW study) during the period of 2007 to 2012 after getting ethical clearance certificate issued by ‘Institutional Ethics Committee’.

Research Methods: LW specimens were collected from randomly selected government and private sector laboratories of ‘Hooghly’ and ‘Burdwan’ districts, West Bengal, India. Five randomly selected untreated blood samples (kept for disposal and recognized as waste) from each laboratory out of 21 selected laboratories (5 from government sector and 16 from private sector laboratories) from ‘Hooghly’ district and 20 laboratories (6 government sector and 14 private sector laboratories) from ‘Burdwan’ district (total specimen number 205) were collected with prior consent of the laboratory authorities. Standard immunological methods were adopted

for detection of three viral biomarkers of blood i.e. serum for anti HIV antibody, anti HCV antibody and HBsAg using chromatographic test kits to identify the chances of spreading of 'Hepatitis B', 'Hepatitis C' and 'HIV'.

Results: Serological findings of samples revealed that in 'Hooghly' district out of total 25 samples collected from the government sector; HBsAg, anti-HCV antibody and anti-HIV antibody were found reactive for 8%, 8% and 4% respectively. For the private sector laboratory wastes, 2.5%, 2.5% and 1.25% samples confirmed positivity for HBsAg, anti-HCV antibody and anti-HIV antibody respectively out of total 80 samples. For 'Burdwan' district, in government sector existence for HBsAg was found nil (0%), where as 3.33% positive cases were found for both of the anti-HCV antibody and 3.33% for the anti-HIV antibody out of total 30 laboratory waste samples. Out of total 70 collected samples from private laboratories, 1.43% samples with anti-HCV antibody and 1.43% sample with anti-HIV antibody were found reactive where as the positivity for HBsAg antibody was found nil (0%).

Conclusions: More positive samples were found in 'Hooghly' district indicated the greater chances of infection from the laboratory wastes than 'Burdwan' district. It may contaminate directly to the population during transportation of the laboratory wastes and surrounding community may get the viral infections through skin abrasion, injury cut etc.

Keywords: Bio Medical Waste, Laboratory waste Disposal, 'Hooghly' and 'Burdwan' districts, HBsAg, Hepatitis C, HIV.

INTRODUCTION

Probability of community pollution from the laboratory waste, especially the blood samples collected in a clinical laboratory for testing was analysed in our study. As we know all the blood samples are supposed to be potentially infectious with viruses like 'Human Immunodeficiency Virus (HIV)', 'Hepatitis-B (HBV)' and 'Hepatitis-C (HCV)' for which proper bio-safety procedure should be strictly followed during its collection, handling and disposal¹. An untreated contaminated sample of blood used in the laboratory would be of great risk to others who handle it further. For clinical laboratories biological samples may be a source of blood borne viral diseases among the Bio Medical Waste (BMW) handlers, scavengers who are used to nurture with these without sufficient knowledge or training for personal and environmental protection². Chances of community acquired viral infections for HIV, HBV and HCV from the potential hazardous laboratory waste to be considered.

According to 'Biomedical Waste (Management and Handling) Rules, 1998 of India'³ 'Biomedical Waste (BMW)' means 'Any solid, fluid or liquid waste including its containers and any intermediated product which is generated during the diagnosis, treatment or immunization of human beings or animals or in research activities pertaining thereto or in the production or testing of biological and includes the categories for same'. Majority (75-90%) of waste produced by the healthcare providers is non-risk and it is estimated that the remaining (10-25%) of health care waste regarded as hazardous which is potential for creating a variety of health problems⁴. Biomedical waste collection and their proper disposal have significant effect for both the medical and general communities. Among all health problems, there is a particular concern with 'HIV/AIDS', 'Hepatitis-B' and 'Hepatitis-C' for which there is a strong evidence of transmission through healthcare waste. The 'BMW' rule is related to all

those who generate, collect, receive, store, transport, treat, dispose or handle 'BMW' in any manner and to any institution that produces 'BMW'. The biomedical waste should be separated out at source into colour coded bags or containers and its collection as well as proper disposal should come with significant importance for both medical personnel and general community⁵.

It is estimated that annually about 0.33 million tonnes of hospital waste is generated in India and the waste generation rate ranges from 0.5-2.0 Kg/Bed/Day¹. Effective management of biomedical waste is not a legal necessity but also responsibility to community. The purpose of biomedical waste management is mainly to reduce waste generation as well as to guarantee its well-organized collection, management and safe disposal in such a way which ensures control of infection and improves safety for employees working in this system. For this a cognisant, harmonized and cooperative efforts has to be made from physicians to word boys. Based on this background this study was designed to obtain the prevalence rate of community contamination through untreated, waste blood samples collected for testing in the laboratory in two said districts. Considering the severity of blood borne infections three specific viral markers like serum for anti HIV antibody, serum for anti HCV antibody and serum for HBsAg were studied with the supplied used up, discarded blood samples.

MATERIALS AND METHODS

The cross-sectional study⁶ was followed for this experiment during the period of 2007 to 2012, which included randomly selected 21 laboratories (5 government sector laboratories and 16 private sector laboratories) from 'Hooghly' district and 20 laboratories (6 government sector laboratories + 14 private sector laboratories) from 'Burdwan' district. The

data regarding government and private registered laboratories were taken from the 'Directory of Medical Institutions–West Bengal 2004' and from the records of the office of 'Chief Medical Officer of Health' (CMOH), 'Hooghly' and 'Burdwan'. An ethical clearance certificate was issued by 'The Principal and Chairman, Institutional Ethics Committee, Medical College & Hospital', Kolkata, Vide Memo No.-5105/1(2) dated 08.12.2006 for conducting the research work. Ethical clearance certificate was also obtained from 'Institutional Ethics Committee, 'Vidyasagar University', Midnapore, Vide Memo No.-IEC/4/2/15 Dt. 19.6.15.

Study protocol was explained and written informed consent was taken in the pre-standardized format from the authorities of selected laboratories. The laboratory staff collected blood samples from the patients having different age limits and of both sexes suffering from different diseases came to the laboratories with doctor's prescription and advice for necessary laboratory investigations. According to their need laboratory staff performed the necessary laboratory tests to generate reports. The laboratory staff delivered us the used up blood samples without adding any disinfectant which were supposed to be disposed. The collected samples by the investigator were tested for various diagnostic tests except for HIV antibody, Hepatitis B surface antigen & Hepatitis C antibody as the patients were not advised for such test items. Collections of suitable specimens were done randomly. The collected samples were brought to our laboratory & processed further following the standard methods. Inclusion & exclusion criteria for the patients were not stipulated in our study protocol. No such data was maintained by us.

Five (5) blood samples from each laboratory were collected with prior consent

of the laboratory authority. The provided samples were kept for disposal and provided prior to undergo any decontamination technique or treatment for disposal of laboratory waste. After collecting the samples those were brought to our investigating laboratory. The sera were collected after centrifugation at 3000 rpm for 10 minutes. The separated sera were tested for the specified three parameters of viral bio-markers using three different immunochromatography-based test kits. The phase included the tests for three biomarkers of blood: serum for anti HIV antibody (n=205); serum for anti HCV antibody (n=205) and serum for HBsAg (n=205). Serum for anti HIV-1 and HIV-2 antibodies was detected using test kit, 'Tri-Dot' of Diagnostic Enterprise, India⁷. Serum for 'Hepatitis-B' surface antigen (HBsAg) was detected using kit of Orchid Biomedical (Virucheck)⁸. Serum for anti 'Hepatitis-C' virus (HCV) antibody was detected using test kit 'Signal HCV' supplied by Span Diagnostic Limited, India⁹. Baseline data were collected using laboratory analysis of samples, results obtained were analyzed and compared for significant difference between experimental groups using ANOVA followed by multiple comparison two-tail 't' test using standard statistical softwares (Statistica, Ver-6.0; Origin Lab, Ver-8.0; MS Excel, Ver-07). Test results may reflect the chances of community pollution if and when the samples remained untreated and not properly disposed off. Intra and inter district comparison were made with the data available and represented in tabular or graphical format to obtain any new finding.

RESULTS

Serological findings of samples from laboratory waste revealed that in 'Hooghly' district out of total 25 samples collected; HBsAg, anti-HCV antibody and anti-HIV antibody were found reactive for 8% (2), 8%

(2) and 4% (1) respectively for the government sector. For the private laboratory wastes, 2.5% (2), 2.5% (2) and 1.25% (1) samples confirmed positivity for HBsAg, anti-HCV antibody and anti-HIV antibody respectively (**Table 1, Figure 1**).

For 'Burdwan' district, in government sector existence for HBsAg was found nil (0%), where as 3.33% (1) positive cases were found for both of the anti-HCV antibody and 3.33% (1) for the anti-HIV antibody out of total 30 laboratory waste samples. Out of total 70 collected samples from private laboratories, 1.43% (1) samples with anti-HCV antibody and 1.43% (1) sample with anti-HIV antibody were found reactive. Where as the positivity for HBsAg antibody was found nil (0%) (**Table 2, Figure 2**).

Laboratory waste serology positive cases were higher in number in government sector laboratories of 'Hooghly' district than in 'Burdwan' district for all the test parameters like HBsAg, anti-HCV antibody and anti HIV antibody. Significant differences were observed in case of HBsAg and HCV antibody positivity. Only in case of comparison for HIV parameter insignificant ($p > 0.05$) difference was observed (**Table 3 and Figure 3**).

Relatively higher positive cases were found in private sector laboratories of 'Hooghly' district for each parameter of HBsAg, anti HCV antibody and anti HIV antibody. In private sector laboratories of 'Burdwan' district zero positivity was observed for HCV parameter (**Table 4 and Figure 3**).

In case of LW-HBsAg study the highest prevalence rate (3.80%) obtained in 'Hooghly' district which was followed by 'Burdwan' district (1%) and international data (0.40%)¹⁰. For anti-HCV antibody, it was found positive for 3.80% samples in 'Hooghly' district and 1% for 'Burdwan' district which was significantly ($p < 0.001$)

different from the international status (1.38%)¹⁰. Existence of anti-HIV antibody was found with significantly ($p < 0.01$) higher values in international condition (5.05%) in respect of the results obtained from 'Hooghly' district (1.90%) and 'Burdwan' district (2%). (**Table 5**)

DISCUSSION

Laboratory waste serological findings in 'Hooghly' district revealed the higher prevalence rate in government sector laboratories in all three parameters than in private sector. This scenario is also found except for HBsAg parameter in case of 'Burdwan' district LW-serology. The result in government sector may reflect the dependence of common ailing people on the government sector laboratories for their diagnosis and treatment. Majority of them may come from lower income group of the society and their life style and health awareness level might be inadequate in respect of these infectious diseases.

The results favoured that unknowingly the laboratory workers if handle highly infectious blood samples which were collected and underwent various laboratory procedures would raise the chances of environment pollution¹¹⁻¹³. For prevention of chances of migration of pollutants from laboratory to community demands the adequate knowledge of bio-safety in laboratory as well as the proper disposal technique^{14,15}. Unless proper application of inactivation procedures prior to disposal of the used up laboratory specimen there was a great chance of environmental pollution². Risks of community acquired infections were also there, where in-house waste management system was undertaken. In such institution inactivation procedure prior to disposal of the laboratory specimen should be maintained strictly.

To combat the chances of environmental pollution with these infectious diseases from the contaminated laboratory wastes especially for the handlers of the infected materials including the scavengers, street beggars immunization programme should be implemented for such viral diseases, if possible¹⁶⁻¹⁸. Cost effective application of 2-5% sodium hypochlorite solution into the blood sample containers after day to day laboratory works is suggested in this concern. Prohibition of dumping of used blood samples in the common municipal garbage discarding area without prior decontamination is strictly recommended. Stringent follow up of proper laboratory standard operation procedure (SOP) for bio-safety in laboratory and disposal of laboratory wastes is essential¹⁹. The laboratory authorities should maintain these which would be supervised by the regulatory bodies^{20,21}. Training programme at regular interval for the laboratory personnel as well as workers involved with disposal procedure may be of immense effective to control this problem^{22,23}. Laboratory waste serology in government sector showed higher positivity than private sector laboratories in 'Burdwan' district for 'HCV' and 'HIV' parameters, where as 'HBsAg' positivity was higher in private sector laboratories in the said district (Table 1 and 2). Higher positivity of the laboratory waste serology suggested more prevalence rate of infections viruses among the patients attended government sector laboratories in both the districts. It indicated that perhaps the general public with poor socio-economic standard usually attended the government hospitals for free treatment. Primarily they were habituated to avoid the private health institutes for payment of fees.

Without any discrimination of government or private sector all biological samples to be received for laboratory analysis should be treated as potentially

highly infectious in respect of the concerned viral parameters. Considering the importance of morbidity and mortality of the diseases laboratories in all sectors should follow the stringent methods of laboratory bio-safety and waste disposal. Chances of viral infections among the waste management staff and scavengers, engaged to collect the recyclable materials from wastes. Hence, procedures for inactivation or decontamination of the laboratory wastes should be strictly followed for control and prevention of infection from laboratory wastes. LW-serology revealed higher prevalence of 'Hepatitis B' and 'Hepatitis C' infected 'BMW' in our country, which indicated less awareness and knowledge about the diseases and their mode of transmission among the general population²⁴. Whereas the higher prevalence of 'HIV' infected samples were predominated in the international scenario that might be resulted from the life style of the people of developed countries²⁵. Chances of environmental pollution could be minimized following the practice of 'BMW' management procedures and rules properly²⁶. BMW like biological samples, infected materials and cultures of microorganisms should be inactivated prior to their disposal and the chances of environmental pollution could be minimized. Automation is major steps may be of immense help in this respect^{27,28}.

CONCLUSION

Chances of spreading of 'Hepatitis B', 'Hepatitis C' and 'HIV' would be initiated through the laboratory waste blood samples. More positive samples were found in 'Hooghly' district indicated the greater chances of infection from the laboratory wastes if remain untreated and not-handled properly. Those potentially infectious biological samples were suggested as responsible for environmental pollution. It

may contaminate directly other articles like, laboratory furniture, containers etc. from where during transport of the laboratory wastes to surrounding community may get the viral infections through skin abrasion, injury cut etc. Disposal of laboratory wastes can be managed under any suitable department of the state government which would be cost effective for the small laboratories in private sector. Alternatively, substitute own/in-house arrangement system for scientific disposal of laboratory waste to be encouraged where in rural areas the agency provider are less interested to collect the wastes regularly.

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Table 1: Intra-district comparison considering government and private sector laboratories in 'Hooghly' district from the view point of laboratory waste (serology) with special emphasis on specific diseases.

Laboratory waste serology for 'Hooghly' district						
	Government sector laboratories (N=5) Sample collected = 25			Private sector laboratories (16) Sample collected = 80		
	Positive	Negative	p-value	Positive	Negative	p-value
HBsAg	8% (2±0.09 ^a)	92% (23±1.28 ^b)	<0.001	2.5% (2±0.09 ^a)	97.5% (78±3.48 ^b)	<0.001
Anti -HCV Ab	8% (2±0.09 ^a)	92% (23±1.28 ^b)	<0.001	2.5% (2±0.09 ^a)	97.5% (78±3.48 ^b)	<0.001
Anti -HIV Ab	4% (1±0.04 ^a)	96% (24±1.27 ^b)	<0.001	1.25% (1±0.04 ^a)	98.8% (79 ±3.53 ^b)	<0.001

Each horizontal row represents mean ± SEM for each group. Analysis performed by Student's two tail 't' test. Values of rows with different superscripts (a, b) differ from each other significantly at the level of p<0.001.

Table 2: Intra-district comparison considering government and private sector laboratories in 'Burdwan' district from the view point of LW-serology with special emphasis on specific diseases.

LW-serology for 'Burdwan' district						
	Government sector laboratories (6) Sample collected = 30			Private sector laboratories (14) Sample collected = 70		
	Positive	Negative	p-value	Positive	Negative	p-value
HBsAg	0	100% (30 ±1.34 ^b)	<0.001	1.43% (1±0.04 ^a)	98.57% (69 ±3.08 ^b)	<0.001
Anti -HCV Ab	3.33% (1±0.04 ^a)	96.67% (29 ±1.29 ^b)	<0.001	0	100% (70 ± 3.13 ^b)	<0.001
Anti -HIV Ab	3.33% (1±0.04 ^a)	96.67% (29 ±1.29 ^b)	<0.001	1.43% (1±0.04 ^a)	98.57% (69 ± 3.08 ^a)	<0.001

Each horizontal row represents mean ± SEM for each group. Analysis performed by Student's two tail 't' test. Values of rows with different superscripts (a, b) differ from each other significantly at the level of p<0.001.

Table 3: Inter district comparison of LW–Serology in government sector laboratories ('Hooghly' district Vs 'Burdwan' district)

	Hooghly (Positive)	Burdwan (Positive)	p-value	Hooghly (Negative)	Burdwan (Negative)	p-value
HBsAg	8% (2± 0.09)	0	<0.001	92% (23±2.29 ^a)	100% (30±2.59 ^b)	<0.01
HCV-Ab	8% (2±0.09 ^a)	3.33% (1±0.04 ^b)	<0.01	92% (23±2.29 ^a)	96.67% (29 ±2.54 ^b)	<0.01
HIV-Ab	4% (1± 0.04 ^a)	3.33% (1±0.04 ^a)	>0.05	96% (24±2.36 ^a)	96.67% (29±2.54 ^a)	>0.05

For 'Hooghly' district, sample collected= 25; For 'Burdwan' district, sample collected= 30
Each horizontal row represents mean ± SEM for each group. Comparison was performed in between values of same category for same parameter. Analysis performed by Student's two tail 't' test. Values with different superscript (a, b) differ from each other significantly at the level of p<0.01 or <0.001.

Table 4: Inter district comparison of LW–Serology biomarkers in private sector laboratories of 'Hooghly' and 'Burdwan' district

	Hooghly (Positive)	Burdwan (Positive)	p-value	Hooghly (Negative)	Burdwan (Negative)	p-value
HBsAg	2.5% (2±0.09 ^a)	1.43% (1±0.04 ^b)	<0.001	97.5% (78±3.48 ^a)	98.57% (69 ±3.08 ^b)	<0.01
HCV-Ab	2.5% (2±0.09)	0	<0.001	97.5% (78±3.48 ^a)	100% (70± 3.13 ^b)	<0.01
HIV-Ab	1.25% (1±0.04 ^a)	1.43% (1±0.04 ^a)	>0.05	98.8% (79±5.03 ^a)	98.57% (69±4.98 ^a)	>0.05

For 'Hooghly' district, sample collected = 80; For 'Burdwan' district, sample collected = 70

Each horizontal row represents mean ± SEM for each group. Comparison was performed in between values of same category for same parameter. Analysis performed followed by Student's two tail 't' test. Values with different superscripts (a, b) differ from each other significantly at the level of p<0.001 or p<0.01

Table 5: Comparison of seroprevalence from the view point of LW serology with special emphasis on HBsAg, anti-HCV Ab and anti-HIV Ab among data obtained in 'Hooghly' district, 'Burdwan' district and International level.

LW serology	International status	Hooghly district	Burdwan district
	%		
HBsAg	0.40±0.1 ^a	3.80±0.22 ^b	1.00±0.17 ^c
Anti-HCV Ab	1.38±0.22 ^a	3.80±0.22 ^b	1.00±0.18 ^c
Anti-HIV Ab	5.05±0.5 ^a	1.90±0.1 ^b	2.00±0.2 ^b

Each row represents mean ± SEM for each group. ANOVA followed by multiple comparison two tail 't' test. Values of rows with different superscripts (a, b, c) differ from each other significantly at the level of $p < 0.01$.

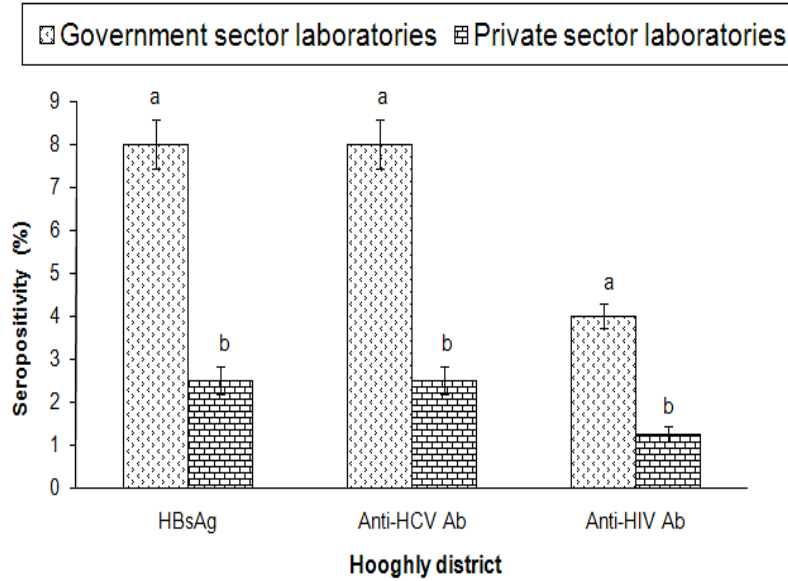


Figure 1: Laboratory waste (serology) with special emphasis on specific diseases and intra-district comparison considering government and private sector laboratories in ‘Hooghly’ district. Each bar represents mean ± SEM for each group. Analysis performed by Student’s two tail ‘t’ test. Bars having different superscripts (a, b) differ from each other significantly at the level of $p < 0.001$

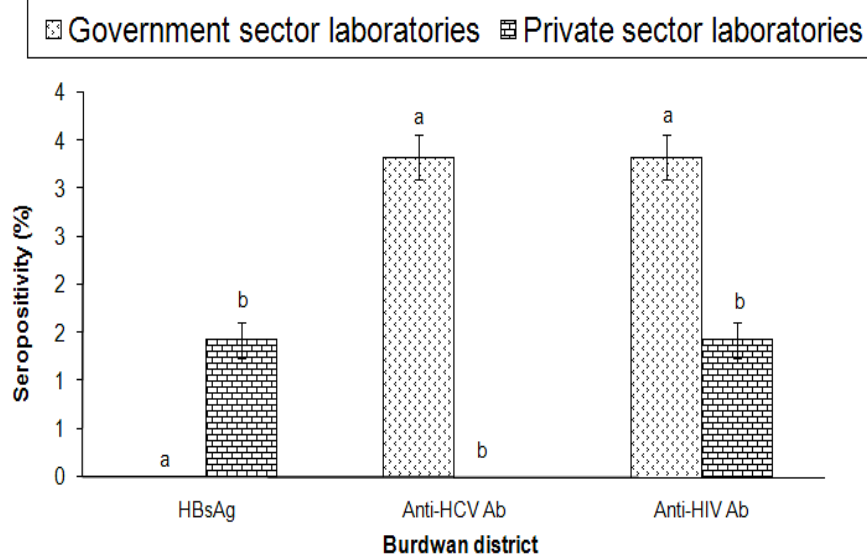


Figure 2: Laboratory waste (serology) with special emphasis on specific viral biomarkers and intra-district comparison considering government and private sector laboratories in ‘Burdwan’ district. Each bar represents mean ± SEM for each group. Analysis performed by Student’s two tail ‘t’ test. Bars having different superscripts (a, b) differ from each other significantly at the level of $p < 0.001$

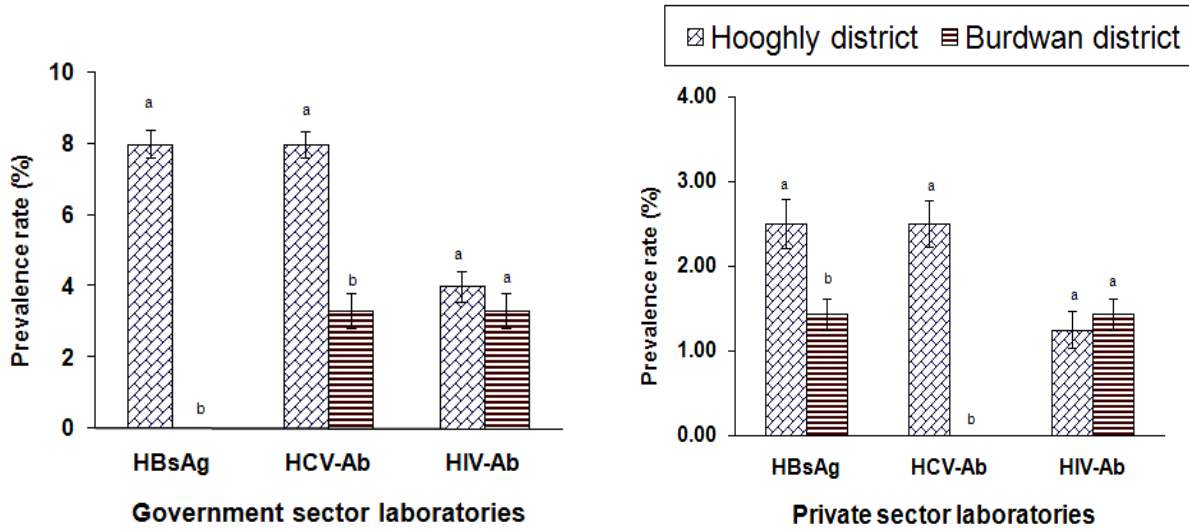


Figure 3: Inter district comparison of laboratory waste serology positivity in government and private sector laboratories of ‘Hooghly’ and ‘Burdwan’ districts from the view point of prevalence rate. Each bar represents mean ± SEM. Analysis performed by Student’s two tail ‘t’ test. Bars with different superscripts (a, b) differ from each other significantly at the level of $p < 0.001$