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High prevalence of active and occult hepatitis B virus infections in healthcare workers from two provinces of South Africa

Tsakani H. Sondlane [a], Lesego Mawela [b], Lufuno L. Razwiedani [c], Selokela G. Selabe [a], Ramokone L. Lebelo [a], J. Nare Rakgole [a], M. Jeffrey Mphahlele [d], Carine Dochez [e], Antoon De Schryver [e], Rosemary J. Burnett [a,*]

[a] Department of Virology, Sefako Makgatho Health Sciences University, Pretoria, South Africa
[b] Department of Public Health, Sefako Makgatho Health Sciences University, Pretoria, South Africa
[c] Department of Community and Occupational Medicine, Sefako Makgatho Health Sciences University, Pretoria, South Africa
[d] South African Medical Research Council and Sefako Makgatho Health Sciences University, Department of Virology, Pretoria, South Africa
[e] Department of Epidemiology and Social Medicine, University of Antwerp, Belgium

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ABSTRACT

Background: Hepatitis B (HB) is a vaccine-preventable liver disease caused by infection with the blood-borne hepatitis B virus (HBV). South African healthcare workers (HCWs) may be at high risk of occupational exposure to HBV infection, since previous studies have found suboptimal levels of protection against HBV in HCWs.

Methods: A descriptive prevalence study based on self-administered questionnaires with data on demographics and HB vaccination status, and stored serum samples collected from 2009 to 2012, from 333 HCWs working or studying in Gauteng and Mpumalanga province hospitals or nursing colleges, was conducted. Samples were tested for HB surface antigen (HBsAg), antibodies to HBsAg (anti-HBs), antibodies to HB core antigen (anti-HBc), and HBV deoxyribonucleic acid (DNA).

Results: The majority of HCWs from whom the serum samples were drawn were black (91.4% [298/326]), female (82.6% [275/333]) and had received at least one dose of HB vaccine (70.9% [236/333]). The average age was 38.8 years (range: 19–62). Of the HCWs, 23.2% (73/314) were susceptible (negative for all markers); 9.6% (30/314) were infected (HBsAg and/or DNA positive); 29.0% (91/314) were exposed (positive for either HBsAg, anti-HBc, or DNA); 18.8% (59/314) were immune due to natural infection (anti-HBs and anti-HBc positive only); while 47.8% (150/314) were immune due to vaccination (anti-HBs positive only). Furthermore, HBV DNA was detected in 8.6% (27/314) and occult HBV infection (OBI) (HBV DNA positive but HBsAg negative) was found in 6.7% (21/314) of samples.

Discussion and conclusion: This study, which is the first to report OBI in South African HCWs, found high rates of active HBV infection and sub-optimal protection against HBV in HCWs. There is a need to strengthen vaccination programmes through a policy that ensures protection for all HCWs and their patients.

1. Introduction

Hepatitis B (HB) is a vaccine-preventable liver disease caused by hepatitis B virus (HBV) infection. It is a major global public health concern, with approximately 240 million people being chronic carriers (i.e. HB surface antigen [HBsAg] positive for >6 months), of which up to 700,000 die each year from cirrhosis or hepatocellular carcinoma [1]. HBV is highly endemic (⩾8% of the population is HBsAg positive) in sub-Saharan Africa [2].

Before the introduction of universal infant vaccination against HB in South Africa in 1995, HBsAg carriage in the black population was estimated at ~10%, while >70% had been exposed (i.e. positive for any HBV marker) to HBV [3]. HBV is a blood-borne virus, thus healthcare workers (HCWs) who work with patients' blood and body fluids are at high risk of occupational exposure. South African HCWs are at particularly high risk, since human immunodeficiency virus (HIV)/HBV co-infection is common in South African patients, with HIV co-infection being a well-established risk factor for increased HBV replication and transmission [4].

* Corresponding author at: South African Vaccination and Immunisation Centre, Department of Virology, Sefako Makgatho Health Sciences University, PO Box 173, Medunsa, 0204 Pretoria, South Africa. Tel.: +27 12 521 3880; fax: +27 12 521 5794.
E-mail address: rose.burnett@smu.ac.za (R.J. Burnett).

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Immunisation of HCWs against HBV is crucial for infection control, thus the South African National Department of Health (SANDoH) recommends all HCWs be vaccinated against HBV before being exposed to patients. However, limited studies investigating antibodies against HBsAg (anti-HBs) among South African HCWs have found sub-optimal levels of protection against HBV, with only 30.6–52.4% having protective levels of anti-HBs (i.e. anti-HBs ⩾ 10mIU/ml) [5,6]. This study aimed to determine the prevalence of HBV markers of susceptibility, protection, exposure and infection in HCWs working in two provinces of South Africa, stratified by vaccination status.

2. Methods

This descriptive prevalence study used (a) demographic and HB vaccination status data and (b) blood specimens collected from various sub-studies of a larger cross-sectional study on HB prevention and control in South African HCWs, which surveyed various HCW populations of Gauteng and Mpumalanga provinces, and offered free HBV testing. Only HCWs at high risk of being exposed to patients' blood or body fluids were surveyed in these sub-studies, while HCWs who had limited patient contact were excluded. The first population (SP-A) comprised of 113 HCWs (nurses, nursing college students, and medical doctors) from various Gauteng Province hospitals and nursing colleges, who participated in three sub-studies on HB vaccination in HCWs ($N = 725$) conducted in 2009–2010 as described elsewhere [7], and accepted an offer of free HBV testing. The second population (SP-B) comprised of 125 HCWs (student/qualified nurses, doctors and auxiliary staff handling patient specimens/cleaning contaminated equipment) from a Gauteng referral hospital in Tshwane that was not included in the 2009–2010 survey, who participated in a study on occupational exposures and HB vaccination ($N = 390$) in 2011 [8], and accepted an offer of free HBV testing. The third population (SP-C) comprised of 95 HCWs (student/qualified nurses, medical doctors, lay counsellors performing HIV testing and auxiliary staff) from a Mpumalanga Province district hospital, who participated in a study on HBV infections in HCWs ($N = 95$) in 2012, for which all HCWs at risk of exposure to HBV (6 doctors, 168 nurses, 98 student nurses, 5 lay counsellors performing HIV testing, 4 laboratory technicians and 57 cleaners) had been invited to participate (unpublished data). No sampling was conducted for this current prevalence study, with all 1210 HCWs who participated in the three studies being offered free HBV testing. Of all HCWs, 333 accepted HBV free testing. Thus 333 blood samples and their related questionnaire data were included in this study.

Blood samples were transported to the laboratory at ~4 °C and centrifuged at 1300 × gravity for 15 min. Serum fractions not tested immediately were stored at −20 °C. Samples were tested for HBsAg, anti-HBs, and antibodies to HB core antigen (anti-HBc) using Elecsys® 2010 electrochemiluminescence immunoassays (Roche Diagnostics, Penzburg, Germany) following the manufacturer's instructions. Serology testing for SP-A, SP-B and SP-C was conducted in 2009–2010, 2011 and 2012 respectively. Viral deoxyribonucleic acid (DNA) was extracted using the High Pure Viral Nucleic Acid kit (Roche Diagnostics, Penzburg, Germany) with one modification of incubating the extracts at 80 °C for 5 min during elution, as previously described [9]. The positive control included in each extraction step was used at a concentration of 9 copies/ml, as polymerase chain reaction (PCR) sensitivity testing [9] had identified that this was the minimum HBV DNA detection limit. HBV DNA was amplified using real time PCR (qPCR) (LightCycler Software Version 4.1, Roche Diagnostics, Penzburg, Germany), as previously described [10] with some modifications. These

Table 1
Summary of serology and HBV DNA results stratified by vaccination status ($n = 314$[a]).

HBV markers	Vaccinated with at least 1 dose, *n* (%)	Not vaccinated/can't remember, *n* (%)	Total, *n* (%)
Susceptible[b]	39 (17.6)	34 (37.0)	73 (23.2)
HBsAg−, anti-HBs−, anti-HBc−, and DNA−	36 (16.2)	32 (34.8)	68 (21.7)
HBsAg−, low anti-HBs+, anti-HBc−, and DNA−	3 (1.4)	2 (2.3)	5 (1.6)
Exposed[c]	60 (27.0)	31 (33.7)	91 (29.0)
Total anti-HBc+	46 (20.7)	27 (29.3)	73 (23.2)
Only HBsAg+ and DNA+	2 (0.9)	0 (0.0)	2 (0.6)
Only HBsAg+	0 (0.0)	1 (1.1)	1 (0.3)
Only DNA+	4 (1.8)	0 (0.0)	4 (12.7)
Only HBsAg+ and anti-HBs+	1 (0.5)	0 (0.0)	1 (0.3)
Only anti-HBs+ and DNA+	7 (3.2)	3 (3.3)	10 (3.2)
Infected[d]	19 (8.6)	11 (12.0)	30 (9.6)
Only HBsAg+	0 (0.0)	1 (1.1)	1 (0.3)
Only DNA+	4 (1.8)	0 (0.0)	4 (12.7)
Only HBsAg+ and anti- HBc+	0 (0.0)	1 (1.1)	1 (0.3)
Only HBsAg+ and DNA+	2 (0.9)	0 (0.0)	2 (0.6)
Only HBsAg+, anti-HBc+, and DNA+	3 (1.4)	1 (1.1)	4 (12.7)
Only anti-HBs+, anti-HBc+, and DNA+	2 (0.9)	5 (5.4)	7 (2.2)
Only anti-HBs+ and DNA+	7 (3.2)	3 (3.3)	10 (3.2)
Only HBsAg+ and anti-HBs+	1 (0.5)	0 (0.0)	1 (0.3)
Occult HBV infection[e]	13 (5.9)	8 (8.7)	21 (6.7)
All anti-HBs ⩾ 10mIU/ml	172 (77.5)	54 (58.7)	226 (72.0)
Total anti-HBs+, anti-HBc−	130 (58.6)	30 (32.6)	160 (51.0)
Total anti-HBs+, anti-HBc+	42 (18.9)	24 (26.1)	66 (21.0)
Protected (anti-HBs ⩾ 10mIU/ml and DNA−)	163 (73.4)	46 (50.0)	209 (66.6)
Only anti-HBs+	123 (55.4)	27 (29.3)	150 (47.8)
Only anti-HBs+ and anti-HBc+	40 (18.0)	19 (20.7)	59 (18.8)
Total	222 (70.7)	92 (29.3)	314 (100)

[a] Of the total population of 333, only 314 specimens had sufficient sera to test for all markers.
[b] Negative for HBsAg, anti-HBs, anti-HBc and HBV DNA.
[c] HBsAg positive and/or anti-HBc positive and/or HBV DNA positive (Note: the results for the exposed who remain infected are repeated under infected).
[d] HBsAg positive and/or HBV DNA positive.
[e] HBV DNA positive and HBsAg negative.

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Table 2
Serology and HBV DNA results stratified by vaccination status ($n = 112$[a]), SP-A (Gauteng Province HCWs tested for all markers).

HBV markers	Vaccinated with at least 1 dose, *n* (%)	Not vaccinated/can't remember, *n* (%)	Total, *n* (%)
Susceptible[b]	16 (20.8)	14 (40.0)	30 (26.8)
Exposed[c]	18 (23.4)	14 (40.0)	32 (28.6)
Infected[d]	9 (11.7)	7 (20.0)	16 (14.3)
Occult HBV infection[e]	7 (9.1)	5 (14.3)	12 (10.7)
All anti-HBs ⩾ 10mIU/ml	56 (72.7)	18 (51.4)	74 (66.1)
Protected (anti-HBs ⩾ 10mIU/ml and DNA−)	52 (67.5)	13 (37.1)	65 (58.0)
Total	77 (68.8)	35 (32.3)	112 (100)

[a] Of the total population of 113, only 112 specimens had sufficient sera to test for all markers.
[b] Negative for HBsAg, anti-HBs, anti-HBc and HBV DNA.
[c] HBsAg positive and/or anti-HBc positive and/or HBV DNA positive (Note: the results for the exposed who remain infected are repeated under infected).
[d] HBsAg positive and/or HBV DNA positive.
[e] HBV DNA positive and HBsAg negative.

Table 3
Serology and HBV DNA results stratified by vaccination status ($n = 124$[a]), SP-B (HCWs from a Gauteng hospital not included in SP-A, tested for all markers).

HBV markers	Vaccinated with at least 1 dose, *n* (%)	Not vaccinated/can't remember, *n* (%)	Total, *n* (%)
Susceptible[b]	20 (23.3)	18 (47.4)	38 (30.6)
Exposed[c]	11 (12.8)	8 (21.1)	19 (15.3)
Infected[d]	1 (1.2)	2 (5.3)	3 (2.4)
Occult HBV infection[e]	0 (0.0)	2 (5.3)	2 (1.6)
All anti-HBs ⩾ 10mIU/ml	64 (74.4)	20 (52.6)	84 (67.7)
Protected (anti-HBs ⩾ 10mIU/ml and DNA−)	64 (74.4)	18 (47.4)	82 (66.1)
Total	86 (69.4)	38 (30.6)	124 (100)

[a] Of the total population of 125, only 124 specimens had sufficient sera to test for all markers.
[b] Negative for HBsAg, anti-HBs, anti-HBc and HBV DNA.
[c] HBsAg positive and/or anti-HBc positive and/or HBV DNA positive (Note: the results for the exposed who remain infected are repeated under infected).
[d] HBsAg positive and/or HBV DNA positive.
[e] HBV DNA positive and HBsAg negative.

include amplifying 10 µl of template in 20 µl reaction mixtures, and adding to the PCR cycling conditions an extension step at 72 °C for 15 s, and a cooling step at 40 °C for 2.5 min. The modified method was validated using positive controls with known viral loads and negative controls. HBV DNA testing for SP-A, SP-B and SP-C was performed in 2011, 2012, and 2014 respectively. Data were captured using Microsoft Excel 2007 (Microsoft Office, USA), and descriptive statistical analyses (i.e. frequency of categorical data [HBV status, HB vaccination status, sex and race], and measures of central tendency and dispersion for continuous data [age]) were conducted using Epi info version 3.5.3 (Centers for Disease Control and Prevention, Atlanta, USA). The specific HBV status categories analysed were "susceptible" (i.e. negative for all markers); "infected" (i.e. HBsAg and/or DNA positive); "occult HBV infection" (OBI) (i.e. HBV DNA positive but HBsAg negative); "exposed" (i.e. positive for any of the following markers: HBsAg, anti-HBc, or HBV DNA); "immune due to natural infection" (i.e. anti-HBs and anti-HBc positive only); and "immune due to vaccination" (i.e. anti-HBs positive only).

The study was approved by the institutional research ethics committee; permission was granted from all the relevant authorities; informed consent was obtained from all participants; and HBV test results were kept confidential. All participants were informed of their test results. Those who were not serologically protected were advised to be vaccinated and re-tested one month after completing the HB vaccination course (i.e. three doses), and those who tested HBsAg or HBV DNA positive were counselled and advised accordingly.

3. Results

All stored serum samples were unhaemolysed and had been correctly stored at −20 °C without repeated freeze–thaws. However, 2 samples were insufficient for anti-HBs, 1 sample was insufficient for anti-HBc, and 17 samples were insufficient for HBV DNA extraction. This resulted in 332 samples being tested for HBsAg and anti-HBc; 314 being tested for anti-HBs and HBV DNA; 330 being tested for all serological markers; and 314 being tested for all serological markers and HBV DNA.

Data on age were available for 302 specimens, with ages ranging from 19 to 62 years (mean: 38.8 [standard deviation (SD): 10.8]; median: 38). The majority (82.6% [275/333]) of HCWs were female. Data on race were available for 326 specimens, with 91.4% (298/326) being black; 6.7% (22/326) being white; 1.2% (4/326) being of mixed descent; and 0.6% (2/326) being Asian. Self-reported vaccination coverage with at least one dose was 70.9% (236/333), with 25.5% (85/333) being fully vaccinated (vaccination coverage with at least one dose was 69.0% [78/113] in SP-A, 69.6% [87/125] in SP-B and 74.7% [71/95] in SP-C, with respectively 28.3% [32/113], 12.0% [15/125], and 40.0% [38/95] being fully vaccinated).

The results of 314 specimens tested for all HBV markers, analysed according to HBV marker patterns (susceptible, exposed, infected [with a separate category for OBI] and protected) and stratified for vaccination status, are shown in Table 1. In addition, of the susceptible ($n = 73$) (either negative for all HBV markers tested, or low positive [<10mIU/ml] for anti-HBs only) HCWs, 53.4% (39/73) were vaccinated with at least one dose, with 15.1% (11/73) being fully vaccinated; 24.7% (18/73) were not vaccinated; and 21.9% (16/73) couldn't remember ever being vaccinated. The overall HBsAg prevalence was 2.9% (9/314), with HBsAg being detected in 3.6% (4/112), 0.8% (1/124) and 5.1% (4/78) in SP-A, SP-B, and SP-C respectively. The overall HBV DNA prevalence was 8.6% (27/314), with 12.5% (14/112), 2.4% (3/124) and 12.8% (10/78) being detected in SP-A, SP-B, and SP-C, respectively. Tables 2–4 summarise the results of sub-population specimens tested for

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Table 4
Serology and HBV DNA results stratified by vaccination status ($n = 78$[a]), SP-C (Mpumalanga HCWs tested for all markers).

HBV markers	Vaccinated with at least 1 dose, *n* (%)	Not vaccinated/can't remember, *n* (%)	Total, *n* (%)
Susceptible[b]	3 (5.1)	2 (10.5)	5 (6.4)
Exposed[c]	31 (52.5)	9 (47.4)	40 (51.3)
Infected[d]	9 (15.3)	2 (10.5)	11 (14.1)
Occult HBV infection[e]	6 (10.2)	1 (5.3)	7 (9.0)
All anti-HBs ⩾ 10mIU/ml	52 (88.1)	16 (84.2)	68 (87.2)
Protected (anti-HBs ⩾ 10mIU/ml and DNA−)	48 (81.6)	15 (78.9)	63 (80.8)
Total	59 (75.6)	19 (24.4)	78 (100)

[a] Of the total population of 95, only 78 specimens had sufficient sera to test for all markers.
[b] Negative for HBsAg, anti-HBs, anti-HBc and HBV DNA.
[c] HBsAg positive and/or anti-HBc positive and/or HBV DNA positive (Note: the results for the exposed who remain infected are repeated under infected).
[d] HBsAg positive and/or HBV DNA positive.
[e] HBV DNA positive and HBsAg negative.

all markers. The results for all specimens tested for the different markers (i.e. including the specimens with insufficient serum to test for all markers), did not differ appreciably from the prevalence of the markers reported in these tables, thus these results are not reported here.

4. Discussion and conclusion

It is encouraging to see that just over 70% of HCWs in this study had received at least one dose of HB vaccine, ranging from 69.0% in SP-A to 74.7% in SP-C. This result is supported by the much larger study from which SP-A was drawn, which found that 67.9% of 723 HCWs had received at least one dose of vaccine [7]. However, it stands in contrast to the findings from a study conducted at a Gauteng hospital not included in this study (the former Johannesburg General Hospital), where only 51.2% of 170 HCWs remembered being vaccinated [6]. On the other hand, it is disappointing that only a quarter of the HCWs in this study were fully vaccinated, having received at least three doses of HB vaccine, ranging from 12.0% in SP-B to 40.0% in SP-C. The 28.3% coverage in SP-A differs substantially from the 19.9% reported for the larger population from which SP-A was drawn [7]. This may be explained by selection bias, since only those HCWs who wished to know their HBV status volunteered to be tested. This may have resulted in an over-representation of HCWs who were concerned about HBV infection and were fully vaccinated, and an under-representation of those who were not concerned and were not fully vaccinated.

The finding in this study that 23.2% of HCWs were susceptible to acquiring HBV from their patients is concerning. Among these HCWs, 53.4% had received at least one dose of HB vaccine, with 15.1% being fully vaccinated. While some of the fully vaccinated HCWs may be non-responders (i.e. individuals who test anti-HBs negative four weeks after the final dose of HB vaccine), it is possible that most had mounted an adequate immune response, but the anti-HBs titre has now waned over time. If so, these fully vaccinated HCWs are not at risk of acquiring HBV infection since protection is presumably life-long and they will mount an anamnestic response upon exposure to HBV, and no booster dose is necessary [11,12]. While no other South African study has reported on susceptibility rates in HCWs, it is clear that the HCWs surveyed in the former Johannesburg General Hospital study, must have had a higher rate of susceptibility than that found in this study. This is because that study reported a lower rate of protection (52.4% versus 66.6% in the current study), with only 1.8% of HCWs being infected, and of the unprotected HCWs 28.2% had previously been exposed to natural infection [6]. However, HBV DNA testing was not conducted in that study, thus OBIs, which would have resulted in a lower rate of susceptibility, were missed.

When considering the results based on serological markers alone, 72.0% (ranging from 66.1% in SP-A to 87.2% in SP-C) of HCWs had protective levels of anti-HBs, with 21.0% being positive for anti-HBc, the marker of natural infection and 51.0% being positive for "anti-HBs alone". This latter group would normally be assumed to be protected due to HB vaccination, but 3.2% of these were positive for HBV DNA, and 2.2% of those supposedly protected through natural infection were also HBV DNA positive, thus only 66.6% (ranging from 58.0% in SP-A to 80.8% in SP-C) of HCWs were protected. Previous seroprotection data from Gauteng HCWs have been based solely on serology, with 30.6% to 52.4% being protected [5,6]. Thus a much higher seroprotection prevalence was detected in this study, which can be explained by the much higher vaccination coverage than that found in the previous studies.

Previous studies on the general population of South Africa have found higher HBV infection and exposure rates in rural areas than in urban areas [11], findings that are supported by this study. While overall 29.0% of HCWs were exposed to HBV, this ranged from 15.3% in SP-B (from the highly urbanised Gauteng Province), to 51.3% in SP-C (from the largely rural Mpumalanga Province). In contrast to SP-B, 28.6% of SP-A (also from Gauteng Province) were found to be exposed. This finding is supported by the 28.2% exposure rate found in HCWs from the former Johannesburg General Hospital [6]. It is also in agreement with an earlier review that included studies conducted in Soweto, which is part of the greater Johannesburg in the heart of Gauteng, where exposures in general healthy populations born and living in Soweto ranged from 23.9% in 19 year-olds, to 35.2% in pregnant women [3]. A study included in that review, where 74% of KaNgwane (a former homeland in Mpumalanga Province) residents were found to have been exposed to HBV [3], also supports the high exposure rate found in SP-C. In contrast, there are no studies that support the much lower exposure rate in SP-B, where similar results to SP-A were expected as this hospital is also in Gauteng. This finding needs further investigation, which is currently underway.

A major concern arising from this study is the high prevalence of active HBV infection. While the overall HBsAg prevalence was found to be relatively low at 2.9%, ranging from 0.8% in SP-B to 5.1% in SP-C, OBIs raised this to 9.6%, with 8.6% of HCWs having active infections as evidenced by the presence of HBV DNA. The prevalence of active infections ranged from 2.4% in urban SP-B to 12.8% in rural SP-C, again illustrating the urban–rural difference supported by previous studies, while urban SP-A also has a high prevalence of 12.5%. A possible reason for the high prevalence found in SP-A, could be because Gauteng nursing colleges were surveyed, so there may have been an over-representation of nursing students from rural provinces in that sub-population. Data on the birthplace of HCWs would have been very useful for this study, and will be collected for future studies.

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The low HBsAg prevalence of 0.8% found in SP-B is in agreement with early data on the general population of Gauteng, where only 1.3% of women born and living in Soweto were HBsAg positive [3]. It is also not very different from the 1.8% HBsAg prevalence found in the study from the former Johannesburg Hospital [6]. Again, the higher HBsAg prevalence of 3.6% found in SP-A could be because of an over-representation of nursing students from rural provinces in that sub-population. Finally, the even higher HBsAg prevalence of 5.1% found in SP-C is supported by the 8.3% reported from KaNgwane [3], as well as studies on healthy women from rural Limpopo Province, ranging from 3.2% to 8.3% [13,14].

This is the first study to report OBI in South African HCWs, and suggests that if the prevention and control of HBV in HCWs is to be taken seriously, post-vaccination serological testing alone (usually testing for anti-HBs first, followed by HBsAg and/or anti-HBc testing in non-responders [15]) will not be adequate. The first issue is that HBV DNA was detected in some of the anti-HBs positive HCWs, which is a concern because after testing for anti-HBs and finding titres of $\geqslant$10mIU/ml, HCWs are regarded as protected and no further tests are performed. The second issue is that non-responders who test HBsAg-negative are usually simply revaccinated, and are not tested for HBV DNA [15]. Thus OBI cases are likely to go undetected, and these HCWs would be unaware of their status, placing their patients at risk of being infected.

The limitations of this study include the previously mentioned unavoidable selection bias, because stored serum samples collected from volunteers who wanted to know their HBV status, were used. Also, this study suffered from recall bias, which especially in the older HCWs who may have been vaccinated a long time ago, may have resulted in an underestimation of vaccination coverage.

In conclusion, this study found a high prevalence of active HBV infection in HCWs, based on HBV DNA testing. It also found a higher prevalence of protection against HBV than previously reported, and this was in line with the higher vaccination coverage than previously reported. The most logical approach to the control of HBV infection in healthcare settings, is to ensure that HCWs are (a) fully vaccinated and protected before being exposed to patients, and (b) not chronically infected with HBV. Thus the SANDoH's recommendation for offering HCWs HB vaccination before being exposed to patients should be strengthened by making it a national policy that is enforced at all higher education institutions that train HCWs. This policy should include post-vaccination testing four weeks after the last dose, which, as the results of this study show, should include HBV DNA testing. Furthermore, the policy should stipulate that healthcare facilities require proof of protection against HBV before employing HCWs, with appropriate employment being offered to infected HCWs, who should not perform invasive procedures on patients.

Conflict of interest

None of the authors have any conflict of interest.

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