THE ROLE OF BODY FLUIDS IN THE HORIZONTAL TRANSMISSION OF HEPATITIS B VIRUS VIA HOUSEHOLD/CLOSE CONTACT

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Disclosure: The authors have declared no conflicts of interest.
Received: 27.11.15 **Accepted:** 20.01.16
Citation: EMJ. 2016;1[1]:68-75.

ABSTRACT

Hepatitis B virus (HBV) infection commonly occurs through horizontal transmission via household/close contact. Although the body fluids of patients infected with HBV are likely to play a significant role in horizontal transmission, the precise mechanism remains unclear. In the 1970s, the infectivity of body fluids including saliva, urine, and faeces was assessed for the presence of hepatitis B surface antigen (HBsAg). Over the last decade, the HBV DNA in the body fluids of chronically infected patients was quantified using real-time polymerase chain reaction. Chimpanzee, gibbon, and chimeric mice with human livers have also been used to investigate the infectivity of body fluids. HBsAg levels, HBV DNA levels, and animal experiments have indicated that saliva and tears are able to transmit HBV. Urine and faeces do not lead to horizontal transmission. The infectivity of the remaining body fluids remains controversial. Horizontal transmission is related to both virus and host factors; thus, evaluations of HBsAg and HBV DNA levels provide insufficient data to determine the infectivity of body fluids. Universal hepatitis B vaccination has been implemented worldwide (with the exception of Northern Europe); an understanding of the role that body fluids play in horizontal transmission will contribute to the eradication of HBV.

Keywords: Hepatitis B virus (HBV), infection, transmission, hepatitis B surface antigen (HBsAg), HBV DNA, animal models.

INTRODUCTION

There are three major modes of hepatitis B virus (HBV) infection that are currently considered: 1) perinatal (mother-to-child) transmission, 2) sexual transmission, and 3) unsafe injections.[1] In high-endemic areas, perinatal transmission is the most common mode. In low-endemic areas, unprotected sexual behaviour and unsafe injections are the predominant transmission routes. In addition to these modes of transmission, horizontal transmission through household/close contact also plays a crucial role in spreading HBV in high-endemic areas.[2]

Historically, horizontal transmission through household/close contact contributed to the understanding of HBV pathogenesis. Experience obtained during epidemics following disasters and wars led to the establishment of two types of hepatitis in 1947: 'infective hepatitis' (i.e. hepatitis A) and 'serum hepatitis' (i.e. hepatitis B).[3] The transmission sources of infective hepatitis were hypothesised to be food and blood, whereas serum hepatitis was thought to spread from person to person parenterally via infected blood, blood products, or the use of contaminated syringes or needles. In 1963, Blumberg et al. discovered an 'Australia antigen' (i.e. hepatitis B surface antigen [HBsAg]) and published an article about it in 1965.[4,5] Early on in the discovery process, these authors erroneously thought that this antigen was associated with leukaemia.[5] However, clinical and experimental findings revealed that the Australia antigen was related to viral hepatitis.[6,7]

Horizontal transmission through household contact in a mental health institution suggested that the Australia antigen was linked to an infectious agent.[6] Moreover, horizontal transmission through household contact provided researchers with the opportunity to determine the pathogenicity of hepatitis B. In 1953, viral hepatitis was spread through horizontal transmission at Willowbrook State School, a residential institution that cared for children with mental disabilities in New York, USA.[8] In 1967, Krugman et al. successfully identified the clinical features of hepatitis A and hepatitis B at this institution;[8,9] however, this experiment later presented ethical issues.[10,11]

The mechanism of horizontal transmission through household/close contact remains unclear. The exposure of abraded skin, cuts, minor open wounds, or mucosal surfaces to blood or body fluids containing HBV from the afflicted may lead to HBV infection. Although hepatitis B vaccination is part of the routine childhood immunisation programme in nearly all countries, investigations of the relationship between body fluids and HBV infection remain extremely important for HBV infection control. This review focusses on horizontal transmission via body fluids, summarises the past and present data regarding body fluids, and discusses the role that body fluids play in the development of HBV infection.

HEPATITIS B SURFACE ANTIGEN IN BODY FLUIDS

Early epidemiological investigations suggested that HBV infection occurred parenterally;[3] thus, suggesting that contact with blood and blood products presented a risk of HBV infection. Krugman et al. demonstrated that HBV was transmitted through the oral administration of serum from a child infected with HBV;[8,9] however, the development of HBV infection through oral administration has not been confirmed. Moreover, non-parenteral transmission was reported in the early 1970s.[12,13] To clarify the mechanism of non-parenteral HBV transmission, researchers began to study the infectivity of body fluids from people infected with HBV. At that time, the presence of HBsAg in body fluids was considered to represent a surrogate marker of infectivity. Table 1 shows the frequency of HBsAg detection in body fluid studies conducted in the 1970s. Saliva, urine, faeces, nasal washings, tears, semen, vaginal secretions, bile, sweat, pleural fluid, amniotic fluid, and breast milk were evaluated for the presence of HBsAg.[14-23] Of these body fluids, saliva showed a high frequency of HBsAg, ranging from 34–86%.[14,15,17,19] Therefore, saliva was considered to accurately reflect antigenaemia and act as a potential infectious source.[24,25] As saliva is easily collected, it has been used to diagnose HBV infection in epidemiological studies.[26,27] The frequency of HBsAg in urine ranges from 2–33%.[15,17,18] Compared with saliva, these frequencies are low. Due to the fact that the frequency of HBsAg in urine varied across studies, this fluid was not used for diagnosis. Based on the frequency of HBsAg, urine appeared to be less infectious than saliva.[25]

As with hepatitis A, an oral-faecal route was strongly suspected in the spread of HBV. An early study detected HBsAg in the faeces of patients infected with HBV.[28] However, subsequent studies showed conflicting results regarding the detection rate of faecal HBsAg.[15,17,18] Villarejos et al.[15] and Irwin et al.[17] did not find any patients with chronic HBV HBsAg-positive for faecal HBsAg, whereas Tiku et al.[18] showed that 10% of patients with chronic hepatitis B were positive for faecal HBsAg. In the late 1980s, faeces were considered unimportant for HBV transmission from an epidemiological standpoint.[25]

Other studies evaluated nasal washings, tears, pleural fluids, and sweat. Although the frequency of HBsAg varied across studies, it was detected in all of these fluids. Contact with nasal droplets, tears, and sweat is common in daily life. As the nasal cavity is connected to the oral cavity, saliva might influence the frequency of nasal washing. Sweat and tears displayed high frequencies of HBsAg (100%[20] and 56%,[22] respectively). While the infectivity of sweat is extremely important in daily contact, sweat and tears were considered to be unimportant infectious agents in HBV infection.[25] Semen, vaginal secretions, and amniotic fluids were positive for HBsAg,[14,19,23] and these fluids might represent the sources of sexual transmission and mother-to-child transmission. HBsAg was detected in the breast milk of mothers with HBV;[23] however, breast feeding did not represent an additional risk of mother-to-child transmission.[29] HBsAg quantification was lacking in these studies. Patients with acute or chronic infections were not differentiated. Moreover, the hepatitis B envelope antigen (HBeAg) serostatus was unknown. Presumably, patients with varying viral loads of HBV in the blood were enrolled.

Table 1: HBsAg-positive rate of body fluids in patients with hepatitis B infection.

Year	Author	Status of serum	Body fluid	Detection rate of HBsAg in body fluids %		HBsAg test
1974	Heathcote et al.[14]	HBsAg-positive (Acute and chronic infection)	Saliva	75	(18/24)	Radioimmunoassay
			Semen	52	(10/19)	
1974	Villarejos et al.[15]	HBsAg-positive (Acute and chronic infection)	Faeces	0	(0/120)	Radioimmunoassay
			Urine	2	(3/130)	
			Saliva	81	(75/93)	
1975	Pizza et al.[16]	HBsAg-positive (acute infection)	Bile	80	(4/5)	Immunodiffusion and immunoelectroosmophoresis
1975	Irwin et al.[17]	Chronic infection	Faeces	0	(0/30)	Radioimmunoassay
			Urine	16	(7/43)	
			Saliva	34	(14/41)	
1976	Tiku et al.[18]	HBsAg-positive (chronic infection)	Nasal washings	26	(14/53)	Radioimmunoassay
			Urine	33	(19/56)	
			Faeces	10	(3/30)	
1976	Parker et al.[19]	HBsAg-positive (acute infection)	Saliva	86	(6/7)	Radioimmunoassay
			Vaginal secretions	78	(7/9)	
1977	Telatar et al.[20]	HBsAg-positive (hepatitis and cirrhosis)	Sweat	100	(30/30)	Radioimmunoassay
1977	De Flora et al.[21]	HBsAg-positive	Pleural fluid	100	(1/1)	Radioimmunoassay
1978	Darrell et al.[22]	HBsAg-positive	Tears	56	(10/18)	Radioimmunoassay
1978	Lee et al.[23]	HBsAg-positive	Amniotic fluid	33	(17/52)	Radioimmunoassay
			Breast milk	71	(45/63)	

HBsAg: hepatitis B serum antigen.

Table 2: HBV DNA level of body fluids in patients with chronic HBV infection.

Year	Author	No. of subjects	HBeAg/Ab status or HBV DNA level in serum	Body fluid	Detection rate of HBV DNA in body fluids %		HBV DNA level in body fluids	
2004	van der Eijk et al.[30]	27	HBeAg (positive: n=15, negative: n=12)	Saliva	85	(23/27)	2.27 ×10^4	copies/mL (median)
			HBV DNA 2.1 × 10^5 genome equivalents/mL (median)					
2005	van der Eijk et al.[31]	150	HBeAg (positive: n=65, negative: n=82, unknown=3)	Saliva	47	(69/147*)	3.2	Log_{10} copies/mL (mean)
			HBV DNA 5.8 log copies/mL (mean)	Urine	32	(47/147*)	2.6	Log_{10} copies/mL (mean)
2006	Kidd-Ljunggren et al.[32]	10	HBeAg positive: n=5, HBeAb positive: n=5 HBV DNA levels were >10^5 copies/mL in all subjects	Saliva	80	(8/10)	1×10^2 –5.3×10^5	copies/mL
				Nasopharyngeal fluid	60	(6/10)	1×10^2 –7.5×10^6	copies/mL
				Tears	57	(4/7)	3×10^2 –1.4×10^4	copies/mL

Table 2 continued.

Year	Author	No. of subjects	HBeAg/Ab status or HBV DNA level in serum	Body fluid	Detection rate of HBV DNA in body fluids %		HBV DNA level in body fluids	
2010	Heiberg et al.[33]	25	HBeAg positive, HBV DNA 41.9×10^6 IU/mL (mean)	Saliva	NA	NA	33.9×10^3	IU/mL (mean)
		18	HBeAg negative, HBV DNA 880 IU/mL (mean)		0	NA	No detection	IU/mL (mean)
2012	Komatsu et al.[34]	47	HBeAg (positive: n=39, negative: n=8) HBV DNA >9 log copies/mL (median), HBV DNA was detected in all subjects	Urine	74	(14/19)	4.3	Log_{10} copies/mL (mean)
				Saliva	87	(33/38)	5.9	Log_{10} copies/mL (mean)
				Tears	100	(11/11)	6.2	Log_{10} copies/mL (mean)
				Sweat	100	(9/9)	5.2	Log_{10} copies/mL (mean)
2015	Komatsu et al.[35]	50	HBeAg (positive: n=37, negative: n=13) HBV DNA >9 log copies/mL: n=24, 6–9 log copies/mL: n=13 patients >2.1 to <6 log copies/mL: n=13	Faeces	74	(37/50)	5.6	Log_{10} copies/mL (mean)
2015	Fei et al.[36]	94	HBeAg positive, HBV DNA 8.1 log IU/mL (median)	Semen	68	NA	3.2	Log_{10} IU/mL (median)
		57	HBeAg negative, HBV DNA 3.2 log IU/mL (median)	Semen	2	NA	0	Log_{10} IU/mL (median)

NA: no data available; HBV: hepatitis B virus; HBeAG: hepatitis B envelope antigen; HBeAb: hepatitis B envelope antibody.

Radioimmunoassays were used to detect HBsAg in these studies. If advanced technologies such as enzyme immunoassays and chemiluminescent immunoassays are used to measure HBsAg,[30] then the details of HBsAg in body fluids might become clearer. These limitations may also have led to inconsistencies in the detection rate of HBsAg in body fluids.

HEPATITIS B VIRUS DNA IN BODY FLUIDS

Since the early 2000s, the level of HBV DNA in body fluids had been quantified using real-time polymerase chain reaction in Northern Europe and Japan, where the HBV vaccine was not introduced into routine child immunisation programs. Saliva, which is likely to be infectious, as well as urine, faeces, and tears, which are not likely to be infectious based on epidemiological data,[25] were evaluated in several studies. The HBV DNA levels of the body fluids of patients with chronic HBV infection are shown in Table 2.[31-37] The levels of HBV DNA in serum and HBeAg serostatus were also evaluated in these studies, and the detection rate of HBV DNA in saliva was higher than that in urine. A study from the Netherlands reported detection rates for HBV DNA in saliva and urine of 47% and 32%, respectively.[32] Similarly, a study from Japan reported HBV DNA detection rates in saliva and urine of 87% and 74%, respectively.[35] Moreover, these studies showed that the level of HBV DNA in saliva was higher than that in urine (saliva: 3.2 log_{10} copies/mL and urine 2.6 log_{10} copies/mL in a study from the Netherlands;[32] saliva: 5.9 log_{10} copies/mL and urine 4.3 log_{10} copies/mL in a study from Japan[35]). These findings suggest that saliva is more important than urine with regard to horizontal transmission. In addition, tears, sweat, faeces, and semen showed high HBV DNA detection rates (tears: 57%[32] and 100%;[35] sweat: 100%;[35] faeces: 74%;[36] semen: 68% [HBeAg positive]).[37]

Table 3: Infectivity of serum and body fluids with various routes of in human and animal experiments.

Year	Author	Model	Donor, HBeAg serostatus	Serum or body fluids	Route of inoculation				
					Oral	SC or IM	Intravaginal	Intravenous	Corneal surface
1967	Krugman et al.[8]	Human	Unknown	Serum	Yes	Yes (IM)	-	-	-
1977	Bancroft et al.[44]	Gibbon	Positive and negative mixed	Saliva	No	Yes (SC)	-	-	-
1977	Alter et al.[45]	Chimpanzee	Positive	Saliva	-	-	-	Yes	-
				Semen	-	-	-	Yes	-
1980	Scott et al.[46]	Gibbon	Positive	Saliva	No	Yes (SC)	-	-	-
				Semen		Yes (SC)	Yes	-	-
1981	Bond et al.[47]	Chimpanzee	Positive	Dried and stored plasm	-	-	-	Yes	-
1982	Bond et al.[48]	Chimpanzee	Unknown	Serum	-	-	-	-	Yes
2012	Komatsu et al.[34]	Chimeric mouse	Positive	Tear	-	-	-	Yes	-
2015	Komatsu et al.[35]	Chimeric mouse	Positive	Faeces	No	-	-	Yes	-

Yes: HBV infection is confirmed.
No: HBV infection is not confirmed.
IM: intramuscular; SC: subcutaneous; HBeAG: Hepatitis B envelope antigen; HBV: hepatitis B virus.

The levels of HBV DNA are between 2 $\log_{10}$ copies/mL and 6 $\log_{10}$ copies/mL in tears;[33,35] 5.2 $\log_{10}$ copies/mL in sweat;[35] 5.6 $\log_{10}$ copies/mL in faeces;[36] and 3.2 $\log_{10}$ copies/mL in semen.[37] Urine, tears, sweat, and faeces contain low or intermediate levels of HBV DNA. However, urine, tears, sweat, and faeces are not epidemiologically likely to transmit HBV.[25]

How Can We Explain These Findings?

The detection rate and the level of HBV DNA in body fluids were positively correlated with HBV DNA levels in the blood. The relationship between the level of HBV DNA in the saliva versus serum was described as follows: HBV DNA in saliva=1.01+0.56×($\log_{10}$ HBV DNA in serum),[31] HBV DNA in saliva=-6.63+0.92×($\log_{10}$ HBV DNA in serum),[34] and HBV DNA in saliva or tears=-3.23+1.06×($\log_{10}$ HBV DNA in serum).[35] According to these formulas, the level of HBV DNA in saliva is 10^3 to 10^6-fold lower than that in serum. This finding is consistent with that of a previous study that reported that the levels of HBV virus particles in saliva were 10^3 to 10^4-fold less than those simultaneously present in serum.[24] The most important discovery that needs to be made is the HBV DNA cut-off level for transmission. The answer to this issue might be found in the recommendations made to the management of healthcare workers (HCWs) infected with HBV. For instance, the U.S. Center for Disease Control and Prevention considers <5,000 copies/mL (<1,000 IU/mL) of HBV DNA in blood to be a safe value for exposure-prone procedures (EPPs).[38] The Society for Healthcare Epidemiology of America and the European Consensus group guidelines recommend a cut-off value of $<10^4$ copies/mL of HBV DNA in blood for HCWs to perform EPPs in patients with HBV.[39,40] The UK Department of Health guidelines determined that $<10^3$ copies/mL of HBV DNA in blood was the cut-off value to perform EPPs.[41] Although household/close contact conditions differ across EPPs, based on these recommendations, body fluids containing $\geq 10^5$ of HBV DNA have infection potential. Table 2 shows that the HBV DNA levels in tears exceeded 10^6 copies/mL. The HBV DNA levels in saliva, nasopharyngeal fluid, sweat, and faeces were $>10^5$ copies/mL. Taken together and based on HBV

DNA levels, tears, saliva (including nasopharyngeal fluid), sweat, and faeces appear to be infectious vehicles of HBV; however, all but saliva are likely to be epidemiologically unimportant with regard to HBV transmission.[25] Notably, several studies reported that the HBV DNA level in blood was not correlated with HBV infectivity in animal models.[42,43] Pre-acute-phase serum is 100-fold more infectious than late acute-phase serum.[42] Exposure to an HCW with a viral burden of 10^4 copies/mL in blood is associated with exposure to <1 virion.[39] However, details on HBV virions in body fluids remain unknown.

THE INFECTIVITY OF SERUM AND BODY FLUIDS IN HUMAN AND ANIMAL EXPERIMENTS

Animal experiments represent the gold standard for evaluating HBV infectivity. Primates such as chimpanzees and gibbons can be infected with HBV.[44-48] In addition, chimeric mice with human livers were recently developed and used for viral hepatitis experiments.[49] The infectivity of serum and body fluids in human and animal experiments are shown in Table 3.[8,35,36,44-48] These experiments have two objectives. The first is to evaluate the infectivity of serum and body fluids, and the other is to confirm the inoculation route. In 1967, Krugman proved that an intramuscular injection with serum induced HBV infection in humans. Moreover, HBV infection occurred through the oral inoculation of serum in humans.[8] Ten years after that experiment, saliva was shown to be an infectious agent via subcutaneous and intravenous inoculation.[44-46] Semen was also demonstrated to be an infectious agent via intravenous and intravaginal inoculation.[45,46] These findings suggested that intravenous, intramuscular, and subcutaneous inoculations were appropriate routes to determine the infectivity of body fluids. Interestingly, plasma that was dried and stored for 1 week was able to infect a chimpanzee through intravenous inoculation.[47] This study suggested that HBV has sufficient potential to survive as an infectious agent in humans. Semen, through intravaginal inoculation and serum corneal surface inoculation, also led to infection.[46,48] In contrast, two studies failed to demonstrate that saliva was infectious via oral inoculation in gibbons:[44,46] Krugman demonstrated HBV infection through oral inoculation with serum;[8] the difference in viral loads between serum and saliva might explain the failed oral inoculation. Thus, oral inoculation is not a potential route to evaluate body fluid infectivity in this context. Enzymes and bacteria in the gastrointestinal tract are likely to affect antigenicity and inactive HBV.[50-52] Tears were infectious using chimeric mice;[35] however, faeces (which contain low levels of HBsAg and intermediate levels of HBV DNA) were not infectious.[36] The body fluids currently shown to be infectious using animal experiments are saliva, semen, and tears.

CONCLUSIONS

As surrogate makers of infectivity, HBsAg and HBV DNA have been detected and quantified in body fluids. In addition, animal experiments have examined the infectivity of body fluids. Based on the HBsAg detection rate, HBV DNA level and animal experiments, saliva, and tears can transmit HBV through household/close contact. Urine and faeces do not appear to cause horizontal infection. The roles of the remaining body fluids remain controversial. The development of HBV infection through horizontal transmission is associated with multiple factors. Viral loads in body fluids, viral activity (intact or damaged virions) in body fluids, stage of infection (pre-acute phase, late-acute, or immunotolerant phase), the entry site of body fluids (widely open wounds, small skin cuts, abrasions, skin penetrations via needles, mucous membrane, or oral ingestion), and the individual's immune response can influence outcomes following contact with body fluids from people infected with HBV. Therefore, whether body fluids play a significant role in the horizontal transmission of HBV is difficult to determine via the evaluations of HBsAg and HBV DNA. Chimeric mice with human livers and fresh human hepatocytes isolated from chimeric mice might be useful in determining the infectivity of body fluids.[49,53] In the era of universal hepatitis B vaccine immunisation, less interest exists in the body fluids of people infected with HBV (with the exception of Northern Europeans). However, an investigation into the precise mechanism of horizontal transmission will contribute to the eradication of HBV.

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