

# The persistence of hepatitis B antigen in the bloodmeal of the potential medicinal leech, *Asiaticobdella buntonensis*

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**Abstract** The persistence of the hepatitis B virus surface antigen (HBsAg) was used as an index of the survival time of this virus within the gastro-intestinal tract of the potential southern African medicinal leech, *Asiaticobdella buntonensis*. HBsAg was tested for in blood/faecal material at five intervals over 15 weeks. Samples from both the midgut and the rectum remained positive for the entire test period, although with decreasing strength. The results are compared with reports on other arthropods which indicate increasing antigen persistence with increasing body size. The findings implicate medicinal leeches as mechanical vectors of HBV and possibly of other medically important viruses, and argue against using leeches of suspect or unknown origin in the alleviation of venous congestion in failing microsurgical procedures.

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Since the medicinal leech came back into use to alleviate venous congestion in failing microsurgical procedures, there have been several reports of leech-related nosocomial infections,<sup>1,3</sup> with *Aeromonas hydrophila* the causative agent. Bacteriological investigation of the gut flora of leeches has been performed on *Hirudo medicinalis* and other bacteria, notably water-borne varieties, have been isolated,<sup>4,5</sup> but not as yet implicated in leech-related infections. However, the presence of diverse gut flora in medicinal leeches and their occasional role in leech-associated infection reveal little about the risk of cross-infection between patients with infective agents associated with the previous bloodmeal. For this reason, and because of limited data on the fate of viruses in the bloodmeal of leeches,<sup>5,6</sup> the persistence of hepatitis B surface antigen (HBsAg) in the bloodmeal of the leech, *Asiaticobdella buntonensis*, was investigated.

Hepatitis B virus (HBV) was chosen because its hardiness makes it a likely candidate for transmission by leeches, and one against which the persistence of less robust viruses can be inferred. In addition, HBV's persistence and potential for transmission by various arthropods have been studied by several authors, making for interesting comparisons with regard to the persistence of this virus in sanguivorous leeches.

## Material and methods

Leeches that weighed between 0,50 g and 1,0 g, housed under laboratory conditions (temperature 26 + 1°C, 12:12 hours light/dark) without a bloodmeal for more than 4 months were fed HBV-infected blood through a membrane feeder until satiated. Blood was obtained from the Natal Blood Transfusion Services and its HBsAg-positivity confirmed by radio-immunoassay (RIA).<sup>7</sup> Positivity was expressed using the positive ratio (specimen count/cut-off value). The leeches were divided into batches of five, and housed separately in plastic ice-cream containers covered with fine gauze netting.

A pilot study revealed that HBsAg remained strongly positive for more than 28 days. In the present study, batches were killed by freezing at intervals of 3, 6, 9, 12 and 15 weeks (21, 42, 63, 84 and 105 days) respectively so as to document the duration of the viral marker more completely. The gut was exposed by a mid-dorsal incision and swabs of blood/faecal material were taken from the midgut and the rectum. The swabs were eluted in 1% bovine serum albumin (BSA) (pH 7,0) and tested for HBsAg.

## Results and discussion

The survival time of the HBsAg in the bloodmeal of *A. buntonensis* is shown in Table I. Because the gut contents of most of the leeches (48/50) remained positive for HBsAg as determined by RIA for the duration of the trial, the results were broken down according to the categories used by the Department of Virology at King Edward VIII Hospital, Durban. These are negative (-), weak positive (+), strong positive (++) and very strong positive (+++).

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**TABLE I.**  
**The fate of HBsAg in the bloodmeal of the leech *A. buntonensis***

Batch	Days elapsed post feeding	Gut region investigated	RIA result				Overall positive (N = 5)
			-	+	++	+++	
E	21	M				5	5
		R		4	1		5
D	42	M		1	2	2	5
		R	1	4			4
C	63	M		1	3	1	5
		R	1	4			4
B	84	M		1	1	3	5
		R	1	3	1		4
A	105	M	1	1		3	4
		R	1	4			4

- = negative; + = weak positive; ++ = strong positive; +++ = very strong positive; M = midgut; R = rectum.

The occurrence of +++ results for swabs taken from the midgut region in the absence of the same from the rectal fluids, probably do not reflect differences in the digestive processes or gut morphology. The blood and its breakdown residuals reach the rectum long after ingestion, and rectal swabs taken soon after feeding are comprised largely of the remnants of the previous uninfected bloodmeal.

The presence of the HBsAg marker is all that need remain intact for a sample to test positive by RIA. Thus their occurrence in blood that is not freshly obtained from a patient, and therefore not subject to continual removal of antigens by the immune system, may well contain intact antigenic fragments that reveal little about the presence, viability or replicative activity of the pathogen. Caution must therefore be exercised in interpreting positive RIA readings from invertebrate bloodmeals as indicative of the presence of viable viruses. This is particularly so where there is no evidence of viral replication, and antigenic persistence is correlated with the digestion and excretion of the bloodmeal, as is the case with *A. buntonensis*. In the case of a voluminous bloodmeal that remains in the leech for extended periods with little putrefaction or active digestion, the persistence of antigen markers for protracted periods gives only an indication of the gastric conditions to which HBV or any other virus would be exposed. The results indicate a benign environment in which many medically important viruses could survive.

Although leeches have been discounted as biological carriers of viruses, they have been found to harbour viruses in their gastro-intestinal tracts for up to 120 days and there is evidence that leeches can act as mechanical vectors in the transmission of infection from one animal to another.<sup>6</sup> Studies on mosquitoes,<sup>8-12</sup> tannans<sup>13</sup> and bedbugs<sup>14,15</sup> as vectors of HBV agree that this virus has the potential to be mechanically transmitted. HBsAg persists in mosquitoes from a few hours<sup>18</sup> to 28 days,<sup>28</sup> for longer than 5 weeks in the bedbug, *Cimex lectularis*,<sup>14-17</sup> and for over 779 days in the tannan tick, *Ornithodoros moubata*.<sup>20</sup> These findings indicate that the duration of survival of the virus in the gastro-intestinal tract of invertebrates increases with the size of the carrier. The protracted viral persistence in *O. moubata* may be influenced by its gut morphology, which takes the form of a small intestine with a blind ending. Excretion is performed by the malpighian tubules while osmoregulation and bloodmeal concentration are controlled by the coxal organ.<sup>20</sup> This unusual method of expelling wastes may account for the long survival of HBsAg in the tick's gut.

The survival time of HBsAg in the bloodmeal of *A. buntonensis* is particularly interesting in the light of bloodmeal size (2 - 5-fold increase in weight after feed-

ing) and the digestive processes of these obligate sanguivores. *H. medicinalis* is dependent on commensal bacteria for all of its general proteases, haemolysins, lipases and amylases and 64% of peptidases.<sup>21</sup> Bacteria also provide lecithinase and haemolysin. Both the morphological and bacteriological similarity of these two leeches suggest that the above applies equally to *A. buntonensis*. The passive nature and the duration of leech digestion, with intact erythrocytes still present after several months,<sup>22</sup> predisposes the leech to protracted harbouring of this virus in its bloodmeal. This, and the evidence of HBV transmission by certain arthropods, argue very strongly against using leeches of unknown or suspect origin in surgery and support the widely accepted principle of avoiding any situation that could effect leech-mediated cross-infection in the clinical context.

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