J. Vet. Malaysia (2010) 22(1 & 2): 13 - 17

COMPARISON OF THE RESTRICTION ENZYME PROFILES OF HERPESVIRUS ISOLATES FROM CAPTIVE WILDLIFE

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SUMMARY

Herpesvirus has been commonly isolated from captive wildlife in animal sanctuaries. Other then identification of the virus, attempts to trace the origin of the virus were initiated based on DNA restriction enzyme (RE) analysis. Two isolates from gaurs (UPMV4/05, UPMV5/05) and an isolate from a Malayan sun bear (UPMV19/05) were examined for a homologous relationship among them. As expected, the DNA from UPMV4/05 and UPMV5/05 showed close RE similarity in comparison to UPMV19/05. The RE patterns from this herpesvirus isolates indicate a possible evolution from the same origin, bovine herpes virus (BHV). The present study showed the DNA of isolates UPMV4/05, UPMV5/05 and UPMV19/05 to have an average molecular weight of 112 x 10^6 Dalton.

Keywords: Captive wildlife, herpesvirus, restriction endonuclease, Malayan sun bear, gaur, DNA fingerprinting

INTRODUCTION

Most wildebeest in zoos are carriers for herpesvirus. Several other wild ruminants in Africa, including the species of oryx and addax are reservoirs of the virus (Pastoret *et al.,* 1988). In its natural hosts, animal herpesvirus produces a highly fatal infection that can affect other types of animals either pets, livestock or wildlife animals (Li *et al.,* 2000). Some important diseases caused by this virus include Infectious Bovine Rhinotracheitis (IBR) and Malignant Catarrhal Fever (MCF) in bovine, Equine Laryngotracheitis, Equine Abortion and Equine Coital Exanthema (ECE) in equine, Canine Herpesvirus in canines and Feline Rhinotracheitis in felines (Ardans, 1992). Despite the wide range of herpesvirus infectivity in various species, the infection in wildlife usually goes unnoticed. This study examined three different hespesvirus isolates (UPMV4/05, UPMV5/05 and UPMV19/05) from two different captive wildlife species (the gaur and the Malayan sun bear) from two different areas in Malaysia. In this study, both single and double digestions by restriction enzymes (RE) were used to draw comparisons between the isolates.

MATERIALS AND METHODS

Virus

Isolate UPMV19/05 was obtained from an oral swab of an adult Malayan sun bear, whereas isolates UPMV4/05 and UPMV5/05 were from plasma samples of two gaurs. All isolates were from clinical cases of captive wildlife from different regions in Malaysia which were submitted to the Virology Laboratory of Faculty of Veterinary Medicine, Universiti Putra Malaysia for viral disease diagnosis. All three isolates were propagated in Vero cells, purified using sucrose gradient and examined under TEM for confirmation.

DNA extraction and purity determination

Viral DNA was extracted and ethanol precipitated from 300 μl of purified virus suspension using standard protocol (Sambrook *et al.,* 1989). The viral DNA pellet was air dried in room temperature before dissolving in 50μl of sterile distilled water. The final step was removal of the RNA by incubating DNA with RNase at 37 °C for an hour. The concentration and purity of the DNA was determined by spectrophotometer according to the method outlined by Davis *et al.* (1989).

DNA digestion with Restriction Endonuclease Enzyme

DNA digestion was done according to manufacturer's recommendations. Single digestion of DNA was performed with either *Bam*HI, *Hind*III or *Eco*RI All samples were digested in PCR machine for 15 h at 37°C and deactivated at 65°C for 20 min. All samples were either stored at 4°C or proceeded to electrophoresis in 0.8% agarose gel slab using 27 V, 40 mA for 17 h. Molecular weight of digested viral DNA fragments was estimated from captured image by using standard ladders and Alpha Ease EC software.

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RESULTS

Molecular weight estimation

The average genome molecular weight for the herpesvirus isolates was estimated to be 112 x 10^6 Dalton or 172 Kb multiplied by 650 (from *Bam*HI RE pattern in Table 1), in which 1bp = 650 Dalton (Chanock *et al.*, 1995).

Restriction pattern with single digestion

Molecular weight determination was done from RE digestion pattern for each isolate (Table 1). Findings revealed variation in the number of RE digested fragments by each enzyme. Generally a range of 15 to 27 fragments was produced for each digestion. The DNA digestion patterns indicated close similarity between UPMV4/05 and UPMV5/05, with minor variations in a few bands in terms of size and position (Table 1, Figure 1).

DNA digestion with *Bam*HI produced 24, 22 and 27 fragments for UPMV4/05, UPMV5/05 and UPMV19/05 respectively. UPMV4/05 and UPMV19/05 did not have fragment 4 (14.87 kb) which was present in UPMV5/05. Instead, both UPMV4/05 and UPMV19/05 generated fragment 11 (8.6kb and 8.78kb respectively). On the other hand, UPMV4/05 did not generate fragment 12 which was present in UPMV5/05 and UPMV19/05 with sizes of 8.07kb and 8.01kb, respectively. UPMV4/05 had a smaller fragment 13(7.21kb). Although fragment 16 was missing in UPMV19/05, *Bam*HI digestion generated fragment 22 with 2.23kb size. For fragments below 2kb, only fragments 24 (1.30 – 1.48kb) and 33 (0.30 – 0.36 kb) were seen in all three isolates. In addition, fragments 23, 25 and 31 (1.71kb,1.27kb, 0.46kb) were noticed in UPMV4/05, whilst fragment 32 (0.4kb) was seen in UPMV5/05. UPMV19/05 had the entire range of fragments, that is, between fragments 24 to 33 (1.46 kb – 0.30 kb).

Table 1: RE digested DNA fragment size of herpesvirus isolates

RE	*Bam*HI (kb)			*Hind*III (kb)			*Eco*RI (kb)		
	V4	**V5**	**V19**	**V4**	**V5**	**V19**	**V4**	**V5**	**V19**
1	22.20	21.89	22.36	30.36	30.02	28.55	19.02	18.12	17.62
2	15.84	16.76	17.86	19.44	18.60	19.33	13.44	12.98	12.98
3	15.19	15.59	15.84	17.59	17.69	18.05	12.89	12.54	12.54
4	-	14.87	-	15.73	15.73	16.09	10.91	10.91	11.23
5	14.16	14.26	14.16	14.55	14.47	14.47	9.56	9.69	9.63
6	12.13	12.56	-	13.09	13.17	13.39	8.04	8.21	8.32
7	11.23	11.07	11.71	8.87	9.17	9.17	7.39	7.29	7.24
8	10.39	10.25	10.76	7.26	7.06	7.30	6.80	6.66	6.66
9	9.49	9.69	9.96	6.21	6.35	6.49	5.56	5.56	6.00
10	9.09	9.16	-	5.87	-	-	4.71	4.72	5.48
11	8.60	-	8.78	5.65	-	5.68	4.15	4.01	4.94
12	-	8.07	8.01	4.97	4.83	4.83	3.61	3.56	3.98
13	7.21	-	-	3.93	3.87	3.68	3.28	3.19	3.37
14	6.44	6.35	6.18	3.08	3.09	3.04	2.91	2.82	2.72
15	5.92	5.52	5.29	2.42	2.57	-	2.64	2.52	-
16	5.44	5.11	-	1.87	1.71	1.71	2.16	2.24	2.19
17	4.83	4.70	4.70	1.13	-	-	1.83	1.75	-
18	4.38	4.20	3.86	0.79	0.79	0.78	1.39	1.47	1.39
19	3.65	3.67	3.65	-	-	-	1.18	1.14	-
20	2.79	2.81	2.95	-	-	-	0.99	0.91	-
21	2.41	2.58	2.66	-	-	-	0.82	-	-
22	-	-	2.23	-	-	-	0.71	0.70	0.65
23	1.71	-	-	-	-	-	0.51	0.58	-
24	1.48	1.30	1.46	-	-	-	0.46	0.48	0.49
25	1.27	-	1.29	-	-	-	0.39	0.38	0.35
26	-	-	1.14	-	-	-	-	-	-
27	-	-	0.86	-	-	-	-	-	-
28	-	-	0.67	-	-	-	-	-	-
29	-	-	0.61	-	-	-	-	-	-
30	-	-	0.56	-	-	-	-	-	-
31	0.46	-	0.48	-	-	-	-	-	-
32	-	0.40	0.39	-	-	-	-	-	-
33	0.36	0.33	0.30	-	-	-	-	-	-
Total	176.67	181.14	158.72	167.57	149.12	152.41	125.21	122.56	118.58

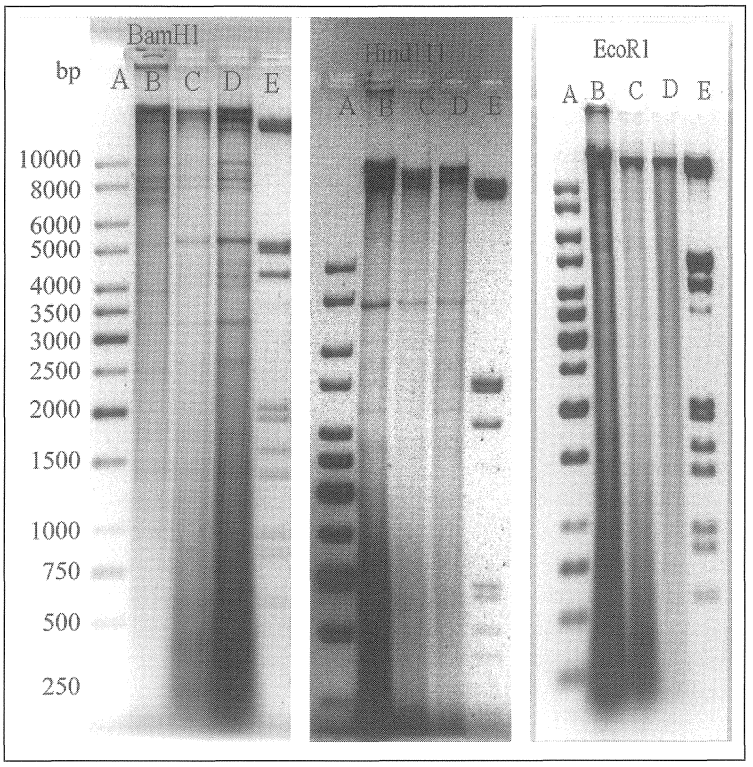

Figure 1. RE pattern of herpesvirus isolates

DNA extracted from herpesvirus isolates were digested with BamHI (Lane B:UPMV19/05, Lane C:UPMV5/05,UPMV4/05); HindIII (B:UPMV4/05, Lane C:UPMV5/05,UPMV19/05) and EcoRI (B:UPMV4/05, Lane C:UPMV5/05,UPMV19/05). DNA ladders: Lane A and Lane E.

*Hind*III digestion generated the least number of fragments with only 15 to 18 fragments. The big fragment sizes generated by *Hind*III were 20.55 kb (UPMV19/05), 30.02kb (UPMV5/05) and 30.36 kb (UPMV4/05). Digestion of UPMV4/05 DNA generated 18 fragments, whilst only 15 fragments were seen in UPMV5/05 and UPMV19/05. *Hind*III digestion pattern revealed a closer relationship between UPMV19/05 and UPMV5/05 in view of the presence of common similar size fragments and missing fragments 10 (5.87kb) and 17 (1.13 kb). In comparison to UPMV4/05, fragments 11 (5.65 kb) and 15 (2.42 – 2.57 kb) were missing in UPMV5/05 and UPMV19/05 respectively.

Digestion with *Eco*RI revealed 19 to 25 fragments. The digestion pattern of UPMV5/05 was almost similar to UPMV4/05 except for missing fragment 21 at 0.82kb. Although the first 14 fragments were intact in UPMV19/05, a total of 6 fragments were absent downwards (fragments 15, 18, 20, 21, 22 and 24).

DISCUSSION

The genome size of the isolates was estimated to be from 118-181 kb which were within the expected range for herpesvirus (Chanock *et al.,* 1995). Previously, the genome molecular weight for herpesvirus was estimated to vary from 79 – 145 x 10^6 Dalton by Heinz *et al.* (1988). Correspondingly, the range that we obtained was 77-118 x 10^6 Dalton with a maximum average of 112 x 10^6 Dalton (172 kb multiplied by 650), based on *Bam*HI RE pattern (Table 1).

Restriction endonuclease patterns obtained from *Bam*HI and *Eco*RI digestion differentiated UPMV19/05 from the other two isolates but demonstrated a close relationship withUPMV4/05 and UPMV5/05, which was expected because both were isolates from the same species origin (gaur) but different animals. Interestingly, the *Hind*III digestion pattern linked UPMV19/05 (bear isolate) with UPMV5/05. Therefore, it is not possible to conclude that the bear and gaur isolates were different. Previously an alpha herpesvirus, such as an equine herpesvirus 9 (EHV9) had managed to cross species and infect a polar bear and the case resembled a fatal herpesvirus encephalitides (Schrenzel *et al.,* 2008; Donovan *et al.,* 2009). Likewise, the *Hind*III restriction pattern of the bear isolate seemed to be closely related to the gaur isolate UPMV5/05.

According to de Wind *et al.* (1993), the *Eco*RI restriction site is absent from porcine herpesvirus such as the pseudorabies virus Therefore, there is no possibility of these isolates originating from porcine herpesvirus because their DNA were digested by *Eco*RI.

The RE pattern comparison with pseudorabies virus (Porat *et al.,* 1984), feline herpesvirus (Sigrid *et al.*,1984) and equine herpesviruses 1, 4 and 3 (Studdert *et al.*, 1985; Robertson and Whalley, 1985) showed vast differences.

It is interesting to note that *Eco*RI and *Hind*III digestion patterns of bovine herpesvirus 1 (BHV1) demonstrated a close relationship especially for the first five fragments; 19.02- 9.56kb for *Eco*RI and 30.36- 14.47kb for *Hind*III (Shih *et al.*, 1989; Pidone *et al.*, 1999). The corresponding presence of the signature fragment size from 18kb to 8kb (11th fragment) shown in the *Bam*HI RE pattern from another study (Osorio *et al.,* 1985) of BHV1 (alphaherpesvirus) further justified our findings. However, none of the isolates had the same restriction pattern with BHV 4, which is a gammaherpesvirus (Donofrio *et al.*, 2000; Fichtelova and Kovarcik, 2010).

Although evidence of bovine herpesviruse in free-ranging wildlife species has been reported, the risk of intraspecies transmission, especially between captive wildlife and domestic livestock is poorly understood. Incidence of BHV1 in buffalo in Malaysia is one example of virus infecting related species in close contact (Ibrahim *et al.,* 1983). The ability of BHV to infect a totally different species such as the sun bear may have important implications for control or eradication efforts.

CONCLUSION

In conclusion, all the 3 herpesvirus isolates from captive wildlife had a signature RE pattern to BHV1. With these RE profiles and further confirmation by gene profiling and sequencing, it should be valuable data for monitoring the prevalence of BHV in captive wildlife species.

ACKNOWLEDGEMENT

We wish to express our thanks to the Bacteriology Laboratory of Faculty of Veterinary Medicine for providing imaging facilities. We express special and sincere appreciation to Prof Dr Abdul Rani Bahaman for his efforts in proofreading this manuscript.

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