

**SHEDDING OF BHV1 AFTER EXPERIMENTAL
CONJUNCTIVAL INOCULATION AND AFTER THE
REACTIVATION OF LATENT INFECTION IN RABBITS**

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Experimental inoculation of rabbits with two subtypes of BHV1 virus was evaluated. Different patterns of virus shedding were observed after inoculation. Cooper strain (BHV1.1 subtype) was excreted longer than K-22 strain (BHV1.2 subtype). Shedding of both subtypes after reactivation of latent infection started at the same time. In one rabbit virus was present in conjunctival swabs for 6 consecutive days. No immune response has been found during that time. The study also confirmed the usefulness of rabbit as an experimental model for the studies on the pathogenesis of BHV1 infection.

Key words: BHV1, experimental inoculation, shedding, latent infection.

Bovine herpesvirus type 1 (BHV1), also known as IBR/IPV, is the etiological agent of: infectious bovine rhinotracheitis (IBR), infectious bovine pustular vulvovaginitis (IPV), infectious balanoposthitis (IBP), conjunctivitis, encephalomyelitis, abortions and reproductive disorders (3, 4, 16). Such a wide variety of clinical syndroms of BHV1 infections leads to serious economic losses in cattle breeding all over the world. BHV1 belongs to *Alphaherpesvirinae* subfamily within *Herpesviridae* family. Its genome consists of linear, double-stranded DNA of about 136 000 base pairs, and its structure is typical for herpesviruses of group D (14). There are two main subtypes of BHV1: subtype BHV1.1, defined also as "IBR like" with predilection for respiratory system and subtype BHV1.2, defined as "IPV like" with predilection for reproductive system. Both subtypes of BHV1 have the ability to induce the latent infection, localised predominantly in neuron ganglia (1, 2, 9, 10, 11). Latent infection lasts through the life of the infected animal. The latent infection is being reactivated from time to time by stress factors and the viral shedding is the final outcome of this reactivation. BHV1 excreted into the environment infects susceptible animals.

The objective of the study was to induce BHV1 infection after experimental inoculation of rabbits with both subtypes of BHV1 and also to compare the viral shedding during the acute phase and after the reactivation of the latent infection.

Material and Methods

Experimental inoculation. In the study 16 rabbits of body weight 2-3 kg each were used. They were allocated to two groups of 8 animals in each. Within each of these groups 6 rabbits were experimentally inoculated with the virus, while the remaining two animals served as controls. First group of rabbits was inoculated with Cooper strain (subtype BHV1.1), titre $10^{6.5}$ TCID₅₀ (tissue culture infectious dose 50%), while K-22 strain of subtype BHV1.2, with the titre of $10^{5.7}$ TCID₅₀ was used to inoculate the second group of rabbits. Inoculum of appropriate virus, 0.2 ml of volume was administered intraconjunctivally to both conjunctival sacs. Control animals were given intraconjunctivally the same volume of saline. To reactivate the latent infection Dexaven (Jelfa, 8 mg/2 ml) was administered i. m. to all rabbits at the dose of 4 mg/kg of body weight for five consecutive days.

Samples for testing. Conjunctival swabs were collected before inoculation and for 30 days after, once daily during the acute phase of infection. Swabs were also collected for 10 consecutive days after administration of Dexaven to observe the beginning of the virus shedding after reactivation of the latent infection. Collected swabs were dipped in 1.5 ml of MEM supplemented with antibiotics (100 i.u. of penicillin, 100 µg of streptomycin, 5 µg of amphoterycin B), left for several hours in a refrigerator, then squeezed out and the medium was kept at -70°C until isolation test.

Isolation test. MDBK cell line in 24-well plates with flat bottom (Costar) was used for virus isolation. Prior to inoculation the samples were thawed and centrifuged for 10 min at $1000 \times g$ at 4°C . The supernatant was used for inoculation of MDBK monolayer with 150 µl/well. The plates were incubated for 1 h at 37°C , then inoculum was removed and the wells were filled with medium and incubated at 37°C . The plates were observed under an inverted microscope for 5-7 d.

Seroneutralisation test. The antibodies titre in rabbit sera was evaluated with seroneutralisation test using increasing dilutions of the tested sera. One hundred TCID₅₀ of IPV 468 was used in the test as a reference strain.

Results

Shedding of the Cooper strain after intraconjunctival inoculation of rabbits continued until the 13th d post inoculation (p.i.). Virus in swabs from conjunctival sacs of rabbit No. 1 was detected from the 1st up to the 11th d and on day 13th p.i. (Table 1).

Similar pattern of shedding was observed in rabbit No. 5. However, virus shedding period was shorter (from the 1st until the 9th d p.i.). In other rabbits the virus was detected for several days with short 1-2 d periods without shedding. In all swabs collected after the 13th d p.i. the Cooper strain was not detected. Shedding of K-22 virus occurred mostly within the first days p.i. (Table 1). On the first day p.i. virus was present in swabs from 4 rabbits. K-22 strain was detected in all rabbits on the 2nd and the 5th d p.i. while on the 4th d p.i. it was found in swabs from 5 rabbits. On the 6th and the 10th d p.i. positive swabs were found in rabbits No. 2 and 3 and in rabbits No. 1 and 6, respectively. K-22 strain was not detected in conjunctival swabs from 7th to 9th d p.i. and after the day 10th. In control rabbits from both groups virus shedding was not detected.

Four months after intraconjunctival inoculation all rabbits were injected with dexametazone (Dexaven) to reactivate the latent infection. In rabbits inoculated with Cooper strain the shedding started on the 3rd d after administration of Dexaven (rabbit

No. 6, Table 2). Virus in conjunctival swabs from rabbit No. 6 was detected for 7 consecutive days. In rabbit No. 4 virus shedding started on the 6th d after Dexaven administration and lasted continuously until the end of the experiment. In rabbits No. 5 and No. 1 shedding was detected only on the 6th and the 8th d, respectively. Also in rabbit No. 3 the shedding was detected only on the 6th and the 8th d after Dexaven administration (post Dexaven administration - p.D.a.). In rabbit No. 2 the shedding was detected on the 4th, 5th, 7th, 8th, and 10th d.

In case of K-22 strain the first shedding of the virus was also detected on the 3rd d p.D.a. (rabbit No. 1, Table 2). On the 4th d p.D.a. the virus was detected in swabs from five rabbits. Virus shedding continued for 6 consecutive days in rabbits No. 1, No. 3, and No. 5. In rabbits No. 2 and No. 6 virus was detected in conjunctival swabs on the 4th and the 7th d and on the 4th, 6th, and 8th d p.D.a., respectively. In rabbit No. 4 as well as in control rabbits virus shedding was not detected.

Table 1
Shedding of BHV1 after intraconjunctival inoculation

BHV1	rabbit	Days after inoculation																	
		-1	0*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15... 30	
Cooper strain	1																		
	2																		
	3																		
	4																		
	5																		
	6																		
	C																		
K-22 strain	1																		
	2																		
	3																		
	4																		
	5																		
	6																		
	C																		

0* – rabbit inoculation day, C – control.

Table 2
Shedding of BHV1 after reactivation of latent infection

BHV1	rabbit	Days after "Dexaven" administration												
		-1	0*	1	2	3	4	5	6	7	8	9	10	
Cooper strain	1													
	2													
	3													
	4													
	5													
	6													
	C													
K-22 strain	1													
	2													
	3													
	4													
	5													
	6													
	C													

0* – the beginning of Dexaven administration.

Discussion

Seal *et al.* (15) showed that the homology of nucleotide sequence between BHV1.1 and BHV1.2 subtypes is around 95%. Despite such a high level of homology, the strains of both subtypes differ in biological properties. Such differences have been also observed in this study. Shedding of BHV1.1 continued for much longer period than BHV1.2 after intraconjunctival inoculation of rabbits. The Cooper strain was detected continuously in conjunctival swabs from the 1st until the 11th d p.i. while K-22 was found only from the 1st until the 6th d p.i. Rock *et al.* (12) isolated Cooper strain continuously from the 9th until the 15th d p.i. Virus in swabs from individual rabbits was detected until the 24th d p.i. On the other hand, Mweene *et al.* (7) detected the virus (Los Angeles strain of BHV1) in inoculated calves until the 10th d p.i.

Shedding of the both strains after dexametazone reactivation of latent infection started in this study at the same time. Just on the 3rd d p.D.a. both Cooper and K-22 strains were detected in conjunctival swabs from the inoculated rabbits. The pattern of virus shedding in rabbits of both groups (Cooper group and K-22 group) had the similar course and the shedding continued almost until the end of the observation period. Latent infection was successfully reactivated in all the rabbits inoculated with Cooper strain and in 5 out of 6 rabbits inoculated with K-22. It is worth mentioning that in the rabbit No.3 inoculated with K-22, the virus was present in swabs for 6 consecutive days. No specific antibodies could be detected in this animal before Dexaven was administered (Table 3).

Table 3
Seroneutralisation titres in rabbits inoculated with K-22 strain before the reactivation of the latent infection with Dexaven

Rabbit no.	Result	SN titre
1	positive	2
2	positive	2
3	negative	
4	negative	
5	positive	2
6	positive	2
C	negative	

Such a possibility exists also in cattle, where BHV1 infected animals can have such low levels of specific antibodies that they can not be detected in routine serological testing. These animals after a spontaneous or induced reactivation of latent infection start the virus shedding and become the main source of infection for susceptible herd mates. Rock *et al.* (13) reactivated latent infection after dexametazone administration in all 22 rabbits inoculated with Cooper strain. They detected the presence of virus in conjunctival swabs for a period of 48-72 h p.D.a. Pastoret *et al.* (8), using the same method, reactivated latent infection in calves previously vaccinated with a thermosensitive strain of BHV1. The vaccine virus was excreted from the 5th d after reactivation of the latent infection. Kaashoek *et al.* (5, 6), on the other hand, used

dexametazone to reactivate latent infection in calves immunized with a live modified vaccine and deleted gE BHV1 vaccine (gE⁻). The vaccine virus was detected only in nasal swabs in a half of the calves vaccinated with a live, modified vaccine also on the 5th d after dexametazone administration. In case of calves vaccinated with gE⁻ vaccine, the virus was not detected in swabs.

The results presented in this paper also confirm the usefulness of a rabbit as an experimental model for studies on pathogenesis of BHV1 infection.

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References

1. Ackermann M., Peterhans E., Wyler R.: DNA of bovine herpes virus type 1 in the trigeminal ganglia of latently infected calves. *Am. J. Vet. Res.*, 1982, **43**, 36-40.
2. Ackermann M., Wyler R.: The DNA of an IPV strain of bovid herpesvirus 1 in sacral ganglia during latency after intravaginal infection. *Vet. Microbiol.*, 1984, **9**, 53-63.
3. Gibbs E.P.J., Rweyemamu M.M.: Bovine herpesviruses. Part I. Bovine herpesvirus 1. *Vet. Bull.*, 1977, **47**, 317-343.
4. Kahrs R.F.: Infectious bovine rhinotracheitis: a review and update. *J. Am. Vet. Ass.*, 1977, **171**, 1055-1064.
5. Kaashoek M.J., Moerman A., Madic J., Rijsewijk F.A., Quak J., Gielkens A.L., van Oirschot J.T.: A conventionally attenuated glycoprotein E-negative strain of bovine herpesvirus type 1 is an efficacious and safe vaccine. *Vaccine*, 1994, **12**, 439-444.
6. Kaashoek M.J., Rijsewijk F.A., Ruuls R.C., Keil G.M., Thiry E., Pastoret P.P., Van Oirschot J.T.: Virulence, immunogenicity and reactivation of bovine herpesvirus 1 mutants with a deletion in the gC, gG, gI, gE, or in both the gI and gE gene. *Vaccine*, 1998, **16**, 802-809.
7. Mweene A.S., Okazaki K., Kida H.: Detection of viral genome in non-neural tissues of cattle experimentally infected with bovine herpesvirus 1. *Jpn. J. Vet. Res.*, 1996, **44**, 165-174.
8. Pastoret P.P., Babiuk L.A., Misra V., Griebel P.: Reactivation of temperature-sensitive and non-temperature-sensitive infectious bovine rhinotracheitis vaccine virus with dexamethasone. *Infect. Immun.*, 1980, **29**, 483-488.
9. Pastoret P.P., Thiry E.: Diagnosis and prophylaxis of infectious bovine rhinotracheitis: the role of virus latency. *Comp. Immun. Microbiol. Infect. Dis.*, 1985, **8**, 35-42.
10. Pastoret P.P., Thiry E., Brochier B., Derboven G.: Bovine herpesvirus 1 infection of cattle: pathogenesis, latency, consequences of latency. *Ann. Rech. Vet.*, 1982, **13**, 221-235.
11. Rock D.L.: The molecular basis of latent infections by alphaherpesviruses. *Seminars in Virology*, 1993, **4**, 157-165.
12. Rock D.L., Beam S.L., Mayfield J.E.: Mapping bovine herpesvirus type 1 latency-related RNA in trigeminal ganglia of latently infected rabbits. *J. Virol.*, 1987, **61**, 3827-3831.

13. Rock D., Lokensgard J., Lewis T., Kutish G.: Characterization of dexamethasone-induced reactivation of latent bovine herpesvirus 1. *J. Virol.*, 1992, **66**, 2484-2490.
14. Roizman B.: The family *Herpesviridae*: an update. *Arch. Virol.*, 1992, **123**, 425-449.
15. Seal B.S., Jeor S.C., Taylor R.E.: Restriction endonuclease analysis of bovine herpesvirus 1 DNA and nucleic acid homology between isolates. *J. Gen. Virol.*, 1985, **66**, 2787-2792.
16. Straub O.C.: BHV1 infections: relevance and spread in Europe. *Comp. Immun. Microbiol. Infect. Dis.*, 1991, **14**, 175-186.