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Transmission of ovine herpesvirus 2 among adult sheep

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Abstract

Previous studies from this laboratory have defined the pattern of acquisition of ovine herpesvirus 2 (OHV-2) in lambs under natural flock conditions. This study examined the question of whether OHV-2 could be transmitted between adult sheep. Two potential routes of transmission were examined: (1) direct inoculation of either viable leukocytes or whole blood from OHV-2 positive sheep, and (2) horizontal transmission through natural contact with OHV-2 positive sheep. Two groups of OHV-2 negative adult sheep were inoculated with material from infected sheep, one with 5×10^8 viable peripheral blood leukocytes (PBL), and the other with 100 ml of whole peripheral blood. No PCR signals were detected in any of the three sheep inoculated with the PBL during the 20 weeks following inoculation. In the group of five sheep inoculated with whole blood, two became PCR-positive at 7 and 8 weeks post-inoculation, respectively, and the remaining three sheep maintained their negative status until termination of the experiment at 20 weeks post-inoculation. In two experiments conducted in different flocks, a total of 20 adult sheep were used to examine horizontal transmission by contact; all animals became PCR-positive within 12 months of mixing the uninfected and infected animals. The results of these experiments support two conclusions. First, the susceptibility to OHV-2 is not limited to young lambs; adult sheep remain fully susceptible. Second, the fact that whole blood, but not PBL, from infected sheep was able to transmit the infection to only two of five inoculated sheep suggests that the infection in peripheral blood cells may be largely non-productive. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Ovine herpesvirus 2; Herpesvirus; Sheep-viruses; Transmission

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1. Introduction

Sheep are natural carriers of a gammaherpesvirus designated as ovine herpesvirus 2 (OHV-2) (Roizman et al., 1981), the causative agent of sheep-associated malignant catarrhal fever (SA-MCF) in cattle, deer and certain other ruminants (Heuschele, 1988; Plowright, 1990; Reid, 1992). The sheep agent, though widespread and well-adapted in domestic sheep (Rossiter, 1981; Metzler, 1991; Li et al., 1995) and apparently in a number of other ruminant species, persists in its natural hosts in a state that to date has defied *in vitro* cultivation attempts (Daniels et al., 1988; Plowright, 1990), greatly hindering research progress. Recent technological developments, namely monoclonal antibody and PCR, have provided avenues around this significant obstacle. A competitive-inhibition ELISA based on a MCF-specific epitope (15A) was developed that is broadly conserved among all the known strains of MCF virus, including those of both ovine and alcelaphine origin (Li et al., 1994). Another major recent advance is the cloning of a fragment of the SA-MCF virus genome (Bridgen and Reid, 1991). PCR assays for the sheep agent were subsequently developed (Baxter et al., 1993), validated, and applied in field investigation (Li et al., 1995, 1998; Muller-Doblies et al., 1998). With these tools, the detailed events occurring between this virus and its host have begun to be examined.

The transmission pattern of OHV-2 in domestic sheep varies significantly from that of alcelaphine herpesvirus 1 (AHV-1), the wildebeest-associated MCF virus (Li et al., 1995). Unlike wildebeest calves, most of which are infected with AHV-1 well before 2 months of age and serve as an important source of virus for transmission (Mushi et al., 1980, 1981), most lambs are not infected with OHV-2 until after 3 months of age. Recent data have shown that production of individual sheep that are free of MCF virus is possible by separation of lambs from OHV-2 infected sheep at the proper time (Li et al., 1999). This provides an alternative to species separation for the prevention of SA-MCF in clinically susceptible species. However, maintaining the virus-free status is a challenge, because of the many undefined factors involved in transmission, such as age-related host susceptibility, mechanisms of virus transfer, and possible transmission distances. Like other gammaherpesviruses, OHV-2 infects lymphocytes (Baxter et al., 1997). Whether the infection in lymphocytes is permissive has not been determined. Isolation of the virus from lymphocytes of sheep has been unsuccessful (Daniels et al., 1988; Plowright, 1990). OHV-2 DNA was detected by PCR in sheep nasal secretions (Li et al., 1998). Results of a quantitative study showed that some sheep had significantly greater quantities of viral DNA in their nasal secretions than in their blood (Hua et al., 1999), suggesting that nasopharyngeal secretions are an important vehicle for OHV-2 transmission among sheep. This study examined whether OHV-2 could be transmitted between adult sheep: (1) by inoculation of viable leukocytes or whole blood and (2) horizontally, via natural contact.

2. Materials and methods

2.1. *OHV-2 negative sheep and inoculation experiments*

All OHV-2 negative sheep used for the study were raised in isolation facilities after separation from the OHV-2 positive flock at 2–3 months of age as described previously

(Li et al., 1999). The OHV-2 negative status of these sheep was examined monthly by both PCR and competitive inhibition ELISA (Li et al., 1994). OHV-2 positive sheep were defined by the presence on multiple examinations of an OHV-2 specific amplicon in gels following PCR amplification of DNA from peripheral blood leukocytes (PBL) (Li et al., 1995). Two inoculation experiments were conducted. In Inoculation Experiment I, conducted at the University of Wyoming, Laramie, six 1.5-year-old OHV-2 negative adult sheep were divided into two groups. One group of three negative sheep was inoculated intravenously with 5×10^8 viable leukocytes from OHV-2 positive sheep and maintained in the isolation facility. The presence of OHV-2 viral DNA in these isolated leukocytes was confirmed by PCR. The control group consisting of the three remaining negative sheep was maintained in a separate facility. Leukocytes for inoculation were obtained from the pooled blood of three OHV-2 positive sheep. Erythrocytes were lysed with 0.87% ammonium chloride-Tris-HCl buffer and the leukocytes washed with PBS. The viability of the cells was evaluated by trypan blue exclusion. Blood samples were taken weekly from both groups of animals over the subsequent 20 weeks.

Inoculation Experiment II was carried out at Washington State University, Pullman. A total of eight OHV-2 negative sheep, ranging from 1 to 2 years of age, were divided into two groups: five sheep in the experimental group and three in the control group. The groups were maintained in separate BL-2 isolation facilities. Individuals in the experimental group were inoculated intravenously with 100 ml of whole blood from an OHV-2 positive sheep. The blood was obtained and refrigerated for about 24 h until inoculation. In three of these five experimental sheep, the blood for inoculation originated from their own twin siblings. The three control members received 100 ml whole blood from unrelated OHV-2 negative sheep. The OHV-2 positive or negative status in these 'donor' sheep was confirmed by PCR amplification of a sample of the inoculum. Blood samples were taken weekly from each individual until termination of the experiment at 20 weeks post-inoculation. Any inoculated sheep that were found to be OHV-2 positive by PCR were immediately removed from the group to minimize the possibility of horizontal contact transmission.

2.2. Horizontal transmission via natural contact

Two experiments were conducted at different locations to evaluate the possibility of transmission between adult sheep by natural contact. In Contact Experiment I, conducted at the University of Wyoming, 12 OHV-2 negative, 2-year-old sheep were housed with 11 OHV-2 positive adult sheep, which ranged between 3 and 6 years old. Blood samples were taken monthly from each sheep in the negative group throughout the 12 months of the experiment. Nasal swab samples were obtained from OHV-2 positive sheep on two occasions. The procedure for the collection of nasal secretions was described previously (Li et al., 1998).

Contact Experiment II was conducted at the USDA Sheep Experiment Station, Dubois, Idaho. Eight 1-year-old OHV-2 negative sheep, raised in isolation at Washington State University, were shipped to Dubois and placed into a flock of over 50 OHV-2 positive sheep, which ranged from 1 to 6 years old, and maintained under normal husbandry

conditions. Four OHV-2 negative sheep, flock-mates of the animals shipped to Dubois, were kept in the isolation facility at Washington State University as controls. Blood samples were collected at monthly intervals from all of the test sheep. Nasal swab samples were obtained bimonthly from 10 randomly selected OHV-2 positive sheep in the flock during the first 6 months of the experiment.

2.3. Transmission without direct contact

In order to monitor the feasibility of maintaining OHV-2 negative sheep in an open-air facility, 10 OHV-2 negative sheep (10 months of age) were kept in a fenced paddock at the USDA-ARS animal facilities in Pullman, Washington. A flock of 50 OHV-2 positive sheep was maintained in a pasture that was geographically separated from the negative sheep. The pasture for the positive sheep was located 0.5 miles southeast of the negative sheep at a slightly higher elevation (ca. 50 ft.). No water drained from one facility to the other. Care was taken to avoid cross-contamination by animal care-workers and other personnel. Monthly blood samples were taken and examined by PCR to monitor for OHV-2 infection over a 1-year period of observation.

2.4. DNA preparation and PCR assay

All the blood samples and nasal swabs collected from sheep at the University of Wyoming or the US Sheep Experiment Station at Dubois, ID, were refrigerated and shipped to Pullman by overnight express delivery. The procedures for DNA preparation, described previously (Maniatis et al., 1982), utilized protease K digestion, phenol-chloroform extraction, and ethanol precipitation. Purified DNA was quantitated by spectrophotometry at 260 nm and stored at -20°C until assayed. The procedures for both conventional PCR (Baxter et al., 1993; Li et al., 1995) and quantitative PCR (Hua et al., 1999) were previously described.

3. Results

3.1. Transmission by inoculation

In Inoculation Experiment I, the PBL of none of the three sheep that were inoculated with 5×10^8 viable leukocytes (over 95% viability) became PCR positive during the 20-week period of observation (Table 1). The three non-inoculated control sheep also remained PCR-negative until termination of the experiment. In Inoculation Experiment II, two out of the five sheep inoculated with 100 ml of whole blood from OHV-2 positive sheep became PCR-positive at 7 and 8 weeks post-inoculation, respectively. Upon PCR conversion, the sheep promptly were removed from the group and housed separately to preclude horizontal contact transmission. Both of these sheep remained PCR-positive throughout the life of the experiment. The inoculum for one of the two sheep that became infected in this experiment originated from its congenital twin sibling. The remaining three sheep, including two that received blood from their infected twin, and all of the

Table 1
Appearance of OHV-2 DNA in PBL of adult sheep inoculated with purified PBL or whole blood from OHV-2 infected sheep

Inoculation experiment	Sheep	Inoculum	Weeks post inoculation									
			0	5	6	7	8	9	10	15	20	
I ^a	1	PBL (+)	— ^d	—	—	—	—	—	—	—	—	—
	2	PBL (+)	—	—	—	—	—	—	—	—	—	—
	3	PBL (+)	—	—	—	—	—	—	—	—	—	—
	4	None	—	—	—	—	—	—	—	—	—	—
	5	None	—	—	—	—	—	—	—	—	—	—
	6	None	—	—	—	—	—	—	—	—	—	—
II ^b	1	Blood (+)	—	—	—	—	—	—	—	—	—	—
	2 ^c	Blood (+)	—	—	—	—	—	—	—	—	—	—
	3 ^c	Blood (+)	—	—	—	—	—	—	—	—	—	—
	4	Blood (+)	—	—	—	+ ^d	+	+	+	+	+	+
	5 ^c	Blood (+)	—	—	—	—	+	+	+	+	+	+
	6	Blood (—)	—	—	—	—	—	—	—	—	—	—
	7	Blood (—)	—	—	—	—	—	—	—	—	—	—
	8	Blood (—)	—	—	—	—	—	—	—	—	—	—

^a In Inoculation Experiment I, sheep #1–3 were inoculated with 5×10^8 viable PBL from OHV-2 positive adult sheep; control animals (#4–6) were not inoculated.

^b In Inoculation Experiment II, inoculum for animals #1–5 consisted of 100 ml of whole blood from OHV-2 infected adult sheep; control animals (#6–8) were inoculated with 100 ml blood from OHV-2 negative adult sheep.

^c The inoculum blood was obtained from the recipient's twin sibling, which was naturally infected with OHV-2.

^d Results of PCR assay on PBL DNA.

three sheep in the control group maintained their PCR-negative status until the termination of the experiment at 20 weeks post inoculation.

3.2. Transmission by contact

In Contact Experiment I, 12 OHV-2 uninfected adult sheep were penned with 11 infected adult sheep for a period of 12 months. Two of the negative sheep became PCR-positive 4 months post-mixing, and an additional five negative sheep became PCR-positive by 7 months. All of the original negative sheep had become PCR-positive by 12 months (Fig. 1). Levels of OHV-2 DNA in the nasal secretions of the 11 positive 'donor' sheep were examined on two separate occasions as a general assessment of the level of viral shedding to the environment. In the 11 infected 'donor' sheep in this experiment, the signals were generally weak or absent. In only eight samples out of the 22 examined were detectable signals obtained, and these measured less than 100 copies per 2 µg total DNA by quantitative PCR (data not shown).

In Contact Experiment II, wherein eight uninfected sheep were pastured with a flock of 50 OHV-2 positive sheep, the first negative sheep had become PCR-positive by 1 month after mixing. The remainder had become PCR-positive when examined at the following

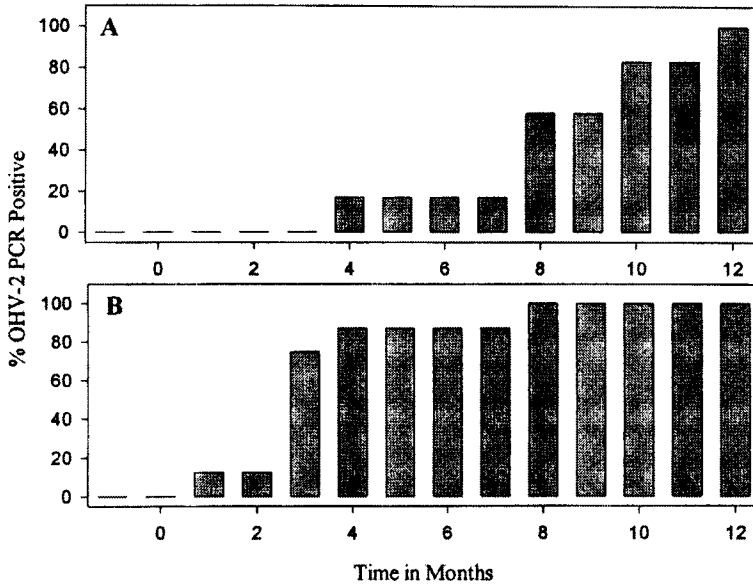


Fig. 1. Horizontal transmission by natural contact between adult domestic sheep. In Contact Experiment I (A), 12 OHV-2 negative sheep were housed in an open pen with 11 OHV-2 positive sheep for 12 months. In Contact experiment II (B), 8 OHV-2 negative sheep were pastured with a flock of more than 50 OHV-2 positive sheep for 12 months. The numbers along the y-axis are the percentage of the originally OHV-2 negative sheep that had become PCR-positive at that time.

months: 6 (75%) at 3, 7 (87%) at 4, and 8 (100%) at 8 months post-mixing (Fig. 1). The four OHV-2 negative control sheep that had been kept in isolation remained negative during the study period. To roughly assess the levels of OHV-2 shedding from the 'donor' sheep in this flock, a total of 30 nasal swabs from 10 randomly selected sheep from the group (that were all PCR-positive by blood) were collected during the first 6 months post-mixing. Fifteen samples were strongly PCR-positive (more than 1000 copies per 2 μ g total DNA) and the remaining 15 samples were weakly positive (less than 100 copies/2 μ g DNA) or negative (data not shown).

In the experiment monitoring for transmission between infected and uninfected adult sheep over a separation distance of approximately 0.5 miles, none of the 10 OHV-2 negative sheep became PCR-positive during the 12-month period of observation.

4. Discussion

The pattern of acquisition of OHV-2 by lambs under natural conditions was examined previously (Li et al., 1998). Most lambs are not infected until sometime after 3 months of age, but virtually all are infected by the time they are 1-year-old. The susceptibility to OHV-2 infection had not been previously examined because of two reasons: (1) the diagnostic methodology to do so has only just recently become available and (2)

uninfected animals, both lambs and adults, have not been available for experimental studies, due to the ubiquity of OHV-2 infection in sheep. Based on recently developed information concerning the pattern of OHV-2 transmission in lambs, it is now feasible to produce OHV-2-free adult sheep by separation of lambs from the positive flock at the appropriate time (Li et al., 1998). This information subsequently formed the basis of a management program for production of virus-free sheep (Li et al., 1999). The question of the transmissibility of OHV-2 between adult sheep is critical to the feasibility of preserving the virus-free status of an OHV-2-free flock. The results of this series of experiments showed that (1) adult sheep maintain full susceptibility to OHV-2, (2) horizontal contact is the predominant OHV-2 transmission mode among sheep and (3) transmission by inoculation of PBL or whole blood is much less efficient than contact transmission.

The experiments examining contact transmission were replicated on two different premises, with 100% transmission ultimately achieved in both the experiments. The rate, however, of transmission from infected to uninfected sheep varied between the two experiments. This rate difference may be due to the following factors: (1) a greater number of infected sheep in the positive group at Dubois, Idaho, than in the group at Wyoming, (2) a higher level or intensity of viral shedding by the individual infected sheep in the flock at Dubois which was as revealed by measurement by quantitative PCR of OHV-2 DNA in the nasal secretions (data not shown).

The findings reported herein are relevant to successful implementation of a program for production and maintenance of OHV-2-free sheep (Li et al., 1999). The maintenance of a high level of susceptibility of sheep into adulthood and the relative efficiency of horizontal transmission between adults necessitates absolute exclusion of infected sheep from the vicinity of uninfected individuals. In this study, a separation distance of 0.5 miles was adequate to prevent sheep-to-sheep transmission under the environmental conditions that existed. What the actual minimum safe distance is, and how changes in environmental conditions or other unknown factors affecting transmission may influence that distance, is not yet known. Outbreaks of MCF in very susceptible species such as bison are often observed wherein the closest sheep are over a mile away, which leads to speculation about the possible participation of indirect modes of transmission by vectors such as insects or birds, or by moving air or water (Crawford et al., unpubl. data). Unexplained patterns of transmission have been reported from Africa (Barnard and Van de Pypekamp, 1988). These routes are yet to be defined. At present, the safest policy at present is to keep the negative flock as far from infected sheep as possible.

Although the number of animals in this study was limited, the overall efficiency of transmission by blood or PBL was much lower than by natural contact, suggesting that the infection in PBL may be largely non-productive. The predominant host cell for OHV-2 in sheep is the lymphocyte, but the subclass has not been firmly established. B-cells have been suggested as the target cells in sheep (Baxter et al., 1997), but data from this laboratory (unpublished) do not support this observation. Whatever their subclass, the transferred non-productively infected lymphocytes presumably would be eliminated eventually by the recipient's immune system, due to histoincompatibility. In this study, PBL samples failed to transmit the virus at all, but 40% of the animals infused with 100 ml of blood became infected. The reason for the difference is not clear. It could

simply reflect a relative loss of viability of leukocytes subjected to the purification process relative to those in whole blood. Alternatively, the occasional successful transfer of infection could reflect a low level of permissivity of the host lymphocytes, as is seen with *in vivo* B-lymphocyte infection by Epstein-Barr virus, the prototype gammaherpesvirus (Schwarzmann et al., 1998). The low infection rate also could be due to a small amount of cell-free virus in the plasma, arising from other, as yet unidentified cell sources.

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