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RESEARCH COMMUNICATION

THE SEROLOGICAL RELATIONSHIP OF HERPESVIRUS OVIS TO OTHER HERPESVIRUSES AND ITS POSSIBLE INVOLVEMENT IN THE AETIOLOGY OF JAAGSIEKTE

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ABSTRACT

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Cross-neutralization studies showed that 3 different isolates of herpesvirus ovis from cell cultures derived from the lungs of sheep suffering from jaagsiekte were not only identical but were also related to a similar isolate made in Scotland. No relationship, however, could be established between herpesvirus ovis and common bovine or equine herpesviruses. Antibodies to herpesvirus ovis were present in roughly 70% of all animals tested and no evidence was obtained for the involvement of the virus in the aetiology of jaagsiekte. On the other hand, the absence of antibodies in sheep sera from Iceland as well as the other data obtained in this study did not exclude involvement of the virus in jaagsiekte.

Résumé

RELATION SÉROLOGIQUE DU VIRUS HERPÈS OVIN A D'AUTRES VIRUS HERPÈS ET SON IMPLICATION POSSIBLE DANS L'ÉTIOLOGIE DE L'ADÉNOMATOSE PULMONAIRE OVINE

Des études de neutralisation croisée ont montré que 3 isolats différents du virus herpès ovin, tirés de cultures cellulaires à partir de poumons de moutons atteints d'adénomatose pulmonaire (jaagsiekte), étaient non seulement identiques mais apparentés à un isolat similaire préparé en Ecosse. On n'a toutefois pu établir aucune relation entre le virus herpès ovin et les virus communs herpès (bovin ou équin). Il y avait des anticorps du virus herpès ovin dans environ 70% de tous les animaux testés et l'on n'a pas trouvé de preuves de l'implication du virus dans l'étiologie de l'adénomatose pulmonaire. D'autre part, tant l'absence d'anticorps dans des séra ovins en provenance de l'Islande que les autres données obtenues dans ce travail n'excluent pas l'implication du virus dans l'adénomatose pulmonaire ovine.

Herpesviruses have been demonstrated in the lungs of sheep suffering from jaagsiekte (ovine pulmonary adenomatosis) by Mackay (1969), Malmquist, Krauss, Moulton & Wandera (1972) and De Villiers, Els & Verwoerd (1975). In our laboratory herpesviruses were isolated after spontaneous activation from cell cultures derived from jaagsiekte lungs on 3 different occasions during the last 4 years, but similar attempts to activate a latent virus in cultures from normal lungs were unsuccessful (unpublished results). This finding suggests a possible involvement of herpesvirus ovis in the aetiology of jaagsiekte. However, all attempts by various workers to produce the tumour by inoculating experimental sheep with virus, either parenterally or into the lungs, have failed.

In an attempt to establish a possible indirect relationship between herpesvirus ovis and jaagsiekte, a serological survey was carried out to determine the distribution of antibodies to the virus in sera from both normal sheep and sheep suffering from jaagsiekte. All sera, collected from various parts of the country, were inactivated and adsorbed with sheep liver powder, and both neutralization and immunofluorescence (IF) techniques were used in the survey. The IF technique was an adaptation of that described by Pope, Walters, Scott & Gunz (1973), in which JS-3 virus infected monolayers of foetal sheep kidney (FSK) cells were used as substrate and fluorescein isothiocyanate-conjugated goat anti-sheep IgG antibody (Cappel Laboratories Inc.)* for staining. Neutralization tests were carried out as described below for the cross-neutralization studies.

The 3 herpesvirus ovis isolates obtained in this laboratory (JS-3, JS-4 and JS-5) were compared both with one another and with available herpesviruses from related host animals in cross-neutralization studies. For these experiments hyperimmune sera were prepared in rabbits, using as antigen non-purified viral suspensions grown in FSK monolayers as described previously (De Villiers *et al.*, 1975). Neutralization tests were performed in tube cultures of FSK cells following the standard constant virus concentration/serum dilution technique. Sera were inactivated and adsorbed with FSK cells before use. Titres are expressed as the reciprocals of the dilutions where 50% neutralization occurred.

From the results shown in Table 1 it can be concluded that the 3 isolates of herpesvirus ovis made in this laboratory are serologically indentical. The isolation of JS-3 from a Karakul sheep from South West Africa, and of JS-4 and JS-5 from cultures originating from a Merino submitted from the Cathcart area (Eastern Cape Province) indicate a wide geographic distribution of the virus. Furthermore, the fact that sera prepared against the South African isolates neutralized also the virus isolated in Scotland, suggests a close if not identical relationship with this virus. On the other hand, no serological relationship was found between JS-3 and 3 common bovine and equine herpesviruses (Table 2).

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TABLE 1 Antibody titres indicating the serological relationship between various herpesvirus ovis isolates

Serum	Virus			
	JS-3	JS-4	JS-5	Scottish isolate[1]
JS-3.....	640	640	320	270
JS-4.....	1 280	1 280	NT[2]	130
JS-5.....	320	NT	320	70

[1] The titrations of the Scottish isolate were kindly performed by Dr J. M. Sharp of the Moredun Institute, Edinburgh, Scotland
[2] Not tested

TABLE 2 Antibody titres indicating the serological relationship of JS-3 ovine herpesvirus to herpesviruses of other species

Serum	Virus			
	JS-3	BMV	IBR	EHV-1
JS-3.....	320	<5	<5	<5
BMV[1].....	<5	320	NT[4]	NT
IBR[2].....	<5	NT	80	NT
EHV-1[3].....	<5	NT	NT	80

[1] Bovine mammilitis virus, Allerton strain
[2] Infectious bovine rhinotracheitis virus
[3] Equine herpesvirus type 1, Kentucky strain
[4] Not tested

The wide distribution of JS-3 antibodies in normal adult sheep sera collected from all parts of South and South West Africa, as reflected by both IF and neutralization tests, suggests that herpesvirus ovis infection is common (Table 3). A similar distribution found in new-born lambs probably reflects the immune status of their mothers. When the lambs were reared in relative isolation in experimental pens, they lost all neutralizing antibodies and most of the IF activity from their serum by the age of 1 year. The high incidence of antibodies against herpesvirus ovis in normal animals does not exclude a possible role for the virus in the aetiology of jaagsiekte. In the case of Epstein-Barr virus (EBV), a human herpesvirus associated with Burkitt's lymphoma and nasopharyngeal carcinoma in certain geographic areas, the prevalence of antibodies in normal populations was found to be as high as 90% (Henle & Henle, 1966; Pope *et al.*, 1973).

No significant difference was found between the incidence of antibodies against JS-3 in normal sheep and in field cases of jaagsiekte (Table 3). In both groups 60–70% of the animals had circulating antibodies against the virus and their average titres were very similar. The latter observation is at variance with that of Henle, Henle, Clifford, Diehl, Kafuko, Kirya, Klein, Morrow, Manube, Pike, Tukei & Ziegler (1969) who recorded very high titres of IF antibody against EBV in Burkitt's lymphoma patients.

A number of sera from normal Icelandic sheep were also tested for JS-3 neutralizing antibodies. The presence of herpesvirus ovis in Iceland would have been a strong argument against its involvement in jaagsiekte, as it is the only country from which this disease has been completely eradicated. The negative results, however, (Table 3) indicate that Iceland is probably free from herpesvirus ovis and that a possible role for this virus in jaagsiekte cannot be excluded on this ground.

In new-born lambs used for transplantation studies the presence or absence of antibodies to herpesvirus ovis at the time of inoculation had no effect on the efficiency of transplantation. Furthermore, in lambs with high initial titres in which tumours developed, a decrease in the prevalence of antibodies was found similar to that observed in normal lambs. In fact, most of our experimentally-produced cases of jaagsiekte as well as some natural cases possessed no JS-3 antibodies (Table 3). Although these results seem to negate a role for herpesvirus ovis in the aetiology of jaagsiekte, they do not exclude a role for the virus in initial transformation, since it has been shown that transmission in nature can involve transplantation of tumour cells, as in the case of our experimental transmission (Coetzee, Els & Verwoerd, 1976). It is conceivable that such transformed cells may have lost the viral genome or the ability to synthesize viral antigens and thus fail to elicit antibody formation

TABLE 3 Distribution of antibodies to JS-3 in various population groups

Group	IF test		Neutralization test	
	Positive/ total	% positive	Positive[1]/ total	% positive
Normal adult sheep:				
SA.....	54/92	59	70/92	76
SWA.....	68/110	62	64/110	58
Iceland[2].....	NT	—	0/100	0
Field cases of jaagsiekte.....	11/15	73	8/15	53
Jaagsiekte negative experimental sheep:				
neo-natal.....	25/36	69	28/36	77
post-mortem[3].....	8/36	22	0/36	0
Experimental cases of jaagsiekte:				
neo-natal.....	17/27	62	20/27	74
post-mortem[3].....	6/27	22	0/27	0

[1] Sera with titres of 10 or more were regarded as positive
[2] Sera kindly provided by Dr G. Pétursson, Institute for Experimental Pathology, Keldur, Reykjavík, Iceland
[3] Lambs raised under semi-isolated experimental conditions for one year before slaughter

To summarize, the results of our serological investigations indicate that herpesvirus ovis is widely distributed in South Africa and is closely related to a similar virus isolated in Scotland. The data obtained neither support a possible role for the virus in the aetiology of jaagsiekte, nor do they exclude such a role.

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References

COETZEE, S., ELS, H. J. & VERWOERD, D. W., 1976. Transmission of jaagsiekte (ovine pulmonary adenomatosis) by means of a permanent epithelial cell line established from affected lungs. *Onderstepoort Journal of Veterinary Research*, 43, 133–142.

DE VILLIERS, ETHEL-MICHELE., ELS, H. J. & VERWOERD, D. W., 1975. Characteristics of an ovine herpesvirus associated with pulmonary adenomatosis (jaagsiekte) in sheep. *South African Journal of Medical Sciences*, 40, 165–175.

HENLE, G. & HENLE, W., 1966. Immunofluorescence in cells derived from Burkitt's lymphoma. *Journal of Bacteriology*, 91, 1248–1256.

HENLE, G., HENLE, W., CLIFFORD, P., DIEHL, V., KAFUKO, G. W., KIRYA, B. G., KLEIN, G., MORROW, R. H., MANUBE, G. M. R., PIKE, P., TUKEI, P. M. & ZIEGLER, J. C., 1969. Antibodies to Epstein-Bar virus in Burkitt's lymphoma and control groups. *Journal of the National Cancer Institute*, 43, 1147–1157.

MACKAY, J. M. K., 1969. Tissue culture studies of sheep pulmonary adenomatosis (Jaagsiekte). II. Transmission of cytopathic effects to normal cultures. *Journal of Comparative Pathology*, 79, 147–154.

MALMQUIST, W. A., KRAUSS, H. H., MOULTON, J. E. & WANDERA, J. G., 1972. Morphologic study of virus-infected lung cell cultures from sheep pulmonary adenomatosis (Jaagsiekte). *Laboratory Investigation*, 26, 528–533.

POPE, J. H., WALTERS, M. K., SCOTT, W. & GUNZ, F. W., 1973. Antibody to Epstein-Barr virus in man in Australia and New Guinea. *International Journal of Cancer*, 12, 689–698.