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COMPARISON OF THE SUSCEPTIBILITY OF THE RED FOX (VULPES VULPES) TO A VACCINIA-RABIES RECOMBINANT VIRUS AND TO COWPOX VIRUS

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ABSTRACT

Sylvatic rabies can be efficiently controlled by vaccination of foxes with a vaccinia-rabies recombinant virus. However, the risk of recombination between the engineered vaccine virus and other orthopoxviruses endemic in wildlife, such as cowpox virus, still needs to be investigated. In this study,f oxes inoculated orally and intradermally with cowpox virus were found to be not very susceptible to cowpox virus, which was isolated from only the oropharynx and tonsils, at low titre andfor onlyfive days after inoculation. Thus the risk of recombination between these viruses in foxes is very low.

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Rabies remains a disease of major importance in Europe where the main vector is the red fox. Research has shown that control of sylvatic rabies is possible by vaccinating foxes with a vaccinia-rabies recombinant vaccine which expresses the immunogenic glycoprotein of rabies virus[1] Previous experiments have demonstrated the efficacy and innocuity of this vaccine in target and non-target species[2-5]. The use of this vaccine is therefore preferable to the use of conventional attenuated strains of rabies virus which are pathogenic to some non-target species[6-10]. Several large-scale fox vaccination campaigns with this vaccine have led to almost complete elimination with this vaccine have led to almost complete elimination of rabies in Belgium[11,12]. However, before the recombinant vaccine can be routinely used in wildlife, it is important to know how its efficacy and safety might be affected by possible interaction with other orthopoxviruses, particularly cowpox virus, that are endemic in European wildlife. Cowpox virus is believed to circulate in populations of wild rodents from which transmission to other species, particularly domestic cats and man, may occur [14-18]. The possibility of genetic recombination in the field between cowpox virus and the engineered vaccinia virus should not be ignored. For genetic vaccinia virus should not be ignored of the sites of virus replication after intradermal and oral inoculation.

Materials and methods

ANIMALS

Twenty foxes aged between 4 and 5 months were captured in Belgium and kept in quarantine for one month before use in this study.

VIRUSES

Low-passage (sixth passage in Vero cells) cowpox virus strain L9720 and a recombinant vaccinia (Copenhagen strain) virus which expresses the rabies (ERA strain) glycoprotein (VVTGgRAB-26D3 187XP)[1] were used in this study. Both viruses were propagated on Vero cells with Dulbecco's Modified Eagle Medium (DMEM) supplemented by 5% fetal calf serum.

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EXPERIMENTAL PROTOCOL

The susceptibility of foxes to cowpox virus was studied after intradermal or oral inoculation with different amounts of virus. Two foxes (foxes 1 and 2) were inoculated intradermally on the flank with 100 µl containing 0.25, 5, 10^2, 2 x 10^3, 4 x 10^4 and 2 x 10^5 plaque-forming units (p.f.u.) of cowpox virus and two (foxes 3 and 4) were inoculated intradermally with 7 x 10^2, 7 x 10^3, 7 x 10^4, 7 x 10^5 and 7 x 10^6 p.f.u. of cowpox virus. Each fox was also inoculated with 100 µl of DMEM as control. A further two foxes (foxes 5 and 6) were inoculated with 100 µl containing 3 x 10^2, 3 x 10^3, 3 x 10^4. 3 x 10^5 and 3 x 10^6 p.f.u. of vaccinia-rabies recombinant virus and DM EM. Two weeks after inoculation, foxes were killed by intracardiac injection of T61 (Hoechst Veterinar) after sedation with Hypnorm (0.1 ml kg^{-1} (Janssen Pharmaceutica). Ten foxes (foxes 7 to 16) were inoculated orally with 8 x 10^6 p.f.u. cowpox virus and two others (foxes 17 and 18) with 3 x 10^7 p.f.u. of the vaccinia-rabies recombinant virus (1 ml administered directly into the oral cavity). Two foxes (foxes 19 and 20) were inoculated orally with DMEM. Foxes were killed at various times after inoculation (Tables 1 and 2). Rectal temperatures were measured every two days. Blood samples and oropharyngeal and nasal swabs in 1 ml of medium were taken every two days and on the day of death. Buffy coats and plasma were separated by centrifugation at 1300*g* for 30 min and stored at -80°C for virus isolation and serology.

SEROLOGY

Antibody to Orthopoxvirus was determined by immunofiuorescence (IF) and haemagglutination inhib- ition (HAl)[21]. IF antibody titres were determined using cowpox virus-infected Vero cells in 96-well plates and an anti-dog IgG FITC conjugate (Sigma). Titres were expressed as the reciprocal of the greatest dilution of serum which gave fluorescence. Rabies virus-neutralizing antibodies were determined by a fluorescence inhibition technique (RFFIT). Antibody titres were expressed in IU (international units) per ml as determined by comparison with a standard serum (titre = 4.4 1U ml^{-1}) [22]. The arbitrarily defined level of 0.5 IU ml^{-1} in humans is considered indicative of successful rabies immunization.

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VIRUS ISOLATION AND TITRATION

After death, the following tissues were collected for virus isolation from each orally inoculated animal : tonsils, buccal mucosa, maxillary and parotid salivary glands, submaxillary, retropharyngeal, axillary, bronchial and mesenteric lymph nodes, thym us, spleen, liver, kidney, small and large intestine, Jung and brain. Tissues were washed in sterile phosphate-buffered saline containing antibiotics and stored at -80°C. Samples were weighed, ground, freeze-thawed three times and clarified by centrifugation for 15 min at 1300g. Swabs, buffy coats, plasma and clarified tissue samples were inoculated in Vero cells in 24-well plates. Three days after inoculation, cells were examined for cytopathic effect and negative samples were passaged once more. Virus isolated from tissues at first passage was titrated on Vero cells. Titres were expressed in p.f.u. ml^{-1} for swabs and p.f.u. g^{-1} for tissues.

HISTOLOGICAL EXAMINATION

Skin samples from normal or depilated areas were fixed in 10% formol-saline or in 4% paraformaldehyde, and plastic-embedded (JB-4; Polysciences Ltd) before being processed and stained with haematoxylin and eosin. Imm unoperoxidase staining was undertaken using a rabbit hyperimmune anti-cowpox virus serum as previously described. [23]

Results

CLINICAL SIGNS

No obvious clinical signs were observed in any foxes apart from small depilated areas observed at the intradermal inoculation sites of ?105 p.f.u. cowpox or recombinant virus and a small induration in one fox (fox 2) at 102 p.f.u. from 4 to 8 days postinoculation (dpi). No pyrexia was detected in any fox.

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SEROLOGY

No antibody to orthopoxviruses was detected in any fox by either IF or HAI assays at the beginning of the experiment. Foxes inoculated intradermally with cowpox virus developed detectable IF and HAI antibody by 6-15 dpi (Table I). A strong correlation was found between HAI and IF assays in all foxes. Of two foxes inoculated intradermally with vaccinia-rabies recombinant virus, one seroconverted at 7 dpi but no antibody was detected in the other. One fox inoculated orally with cowpox virus (fox 16) seroconverted at 9 dpi but no antibody was detected in any other fox inoculated orally with either virus (Table I). Five foxes had detectable antibody against rabies prior to the beginning of the experiment (Table I). These titres did not significantly vary during the experiment except in fox 5 which developed an increase in ra bies antibody titre 13 days after intradermal inoculation with recombinant virus (data not shown).

VIRUS ISOLATION

No viraemia was detected in any fox and no virus was isolated from swabs or tissues from foxes inoculated intradermally with either virus. From foxes inoculated orally, no virus was isolated from nasal swabs but cowpox virus was isolated at up to 10^3 p.f.u. ml^{-1} from oropharyngeal swabs until 5 dpi and vaccinia-rabies recombinant virus was isolated from oropharyngeal swabs at 1 dpi (Table 2). Cowpox virus was isolated from tonsils at up to 104 p.f.u. g^{-1} for 2-3 dpi and after passage until 5 dpi. The recombinant virus was reisolated for only one day from tonsils (Table 2). No virus was detected in any other tissues tested.

HISTOLOGICAL EXAMINATION

Examination of the mild skin lesions which developed at some sites of intradermal inoculation revealed none of the changes usually associated with cowpox virus infection [2,3]; no characteristic A-type inclusions were seen and no Orthopoxvirus antigen was detected by immunoperoxidase staining (data not shown).

Discussion

Genetic recombination between orthopoxviruses and the production of recombinant viruses with novel biological properties after dual infection of cell and tissue cultures is a well known phenomenon [24-27]. Similar recombination has also occurred in whole animals, for example among capripoxviruses [28] and leporipoxviruses [29]. The purpose of the experiments described here was to

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assess the risk of genetic recombination between cowpox and vaccinia-rabies recombinant viruses in foxes in the field. In order to evaluate the risk of such recombination, it was essential to determine first whether foxes were susceptible to cowpox virus. Intradermal titration of cowpox virus in the flanks of four foxes demonstrated that foxes are not very susceptible to infection by this route. Indeed, unlike cats, in which as little as 5 p.f.u. can cause primary lesions at the inoculation site, viraemia and secondary skin lesions[2,3], only mild skin lesions, with no evidence of virus replication, were provoked when ≥ 2 x 10^5 p.f.u., or 10^2 p.f.u. in one case, of cowpox virus were inoculated into foxes. These lesions may be similar to those reported after abortive Avipoxvirus infection of mammals [30-32]. No lesions at all were observed in foxes inoculated orally, although cowpox virus was isolated from sils and oropharyngeal swabs until 5 dpi, indicating that transient local infection probably did occur. However, no evidence of systemic infection was found in any fox. None of the foxes had detectable antibody against cowpox virus at the beginning of the experiment but all of those inoculated intradermally and one fox inoculated orally seroconverted at 6-15 dpi. Five foxes had detectable rabies antibody at the beginning of the experiment. The age and health of the foxes suggest that this antibody was unlikely to have been due to either rabies virus infection or maternally derived antibody. However, the foxes may have eaten baits containing the recombinant vaccine brought back to the earth by their mothers during a recent vaccination campaign. If some foxes had been previously exposed to the recombinant vaccine, then pre-existing immunity might have reduced the susceptibility of some of these foxes to cowpox virus infection and might particularly have reduced the amounts of virus reisolated from foxes 8 and 9. However, only three of 14 foxes inoculated with cowpox had pre-existing rabies antibody so this argument does not affect the overall conclusion that foxes are relatively unsusceptible to cowpox.

The prevalence of antibody to cowpox virus in wild foxes is low: none of 72 foxes from South Belgium had detectable Orthopoxvirus antibody (unpublished data). The low prevalence of Orthopoxvirus infection in foxes and the restricted replication of both cowpox and vaccinia-rabies recombinant viruses 19 suggests that the risk of recombination between these two viruses in foxes is very low. This work therefore adds support to arguments in favour of the use of the vaccinia-rabies recombinant vaccine in large-scale vaccination campaigns to eliminate rabies in Europe.

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Table 1. *Serology for cowpox (IF) and rabies (RFFIT) in foxes inoculated orally or intradermally with cowpox or recombinant rabies-vaccinia virus*

			Cowpox antibody IF titre[b] (days after inoculation)										
Fox	**Virus / route[a]**	**Rabies RFFIT titre (IU ml^{-1}) at day 0**	**0**	**1**	**2**	**3**	**5**	**6**	**7**	**9**	**11**	**13**	**15**
1	cpx/i.d.	6.3	<5		<5		<5	10		40	20	20	20·
2		<0.5	<5		<5		<5	<5		<5	<5	<5	10·
3		<0.5	<5			<5	<5		<5	<5	<5	10	20·
4		<0.5	<5			<5	<5		<5	20	80	160	80'
5	rec/i.d.	4.0	<5			<5	<5		80		160	160	160'
6		<0.5	<5			<5	<5		<5	<5	<5	<5	<5.
7	cpx/oral	<0.5	<5	<5'									
8		4.9	<5	<5'									
9		4.3	<5		<5.								
10		<0.5	<5		<5'								
11		<0.5	<5			<5'							
12		<0.5	<5			<5*							
13		<0.5	<5			<5	<5*						
14		<0.5	<5			<5	<5		<5*				
15		<0.5	<5			<5	<5		<5	<5*			
16		<0.5	<5			<5	<5		<5	10	20	40*	
17	rec/oral	1.3	<5	<5*									
18		<0.5	<5		<5*								
19	media control	<0.5	<5			<5	<5		<5	<5	<5	<5*	
20		<0.5	<5			<5	<5		<5	<5	<5	<5*	

Légende de la table : [a]cpx, cowpox virus; rec, rabies-vaccinia recombinant virus; i.d., intradermal - [b]Reciprocal of last dilution which gave fluorescence - *Indicates the day on which foxes were killed

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Table 2. *Reisolation of cowbox and recombinant vaccinia viruses from oropharyngeal swabs and tonsils after oral inoculation*

Fox	Virus		2	3	5	7	9	11	13	15	Isolation from tonsils (p.f.u. g^{-1})
7	Cowpox	–*									25
8		–*									–
9			200*								4000
10			–*								35
11				300*							1800
12				1000*							(+)
13				5	–*						(+)
14				30	–	–*					–
15						–	–*				–
16					+	–	–	–	–*		–
17	Recombinant	55*									250
18			–*								–
19	Media control		–	–	–	–	–	–	–	–*	–
20			–	–	–	–	–	–	–	–*	–

Légende de la figure. : indicates the day on which foxes were killed : - Indicates that no virus was isolated after two passages / +Indicates that virus was isolated but the titre was not determined (very low) (+) Indicates that virus was isolated after passage

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References

Kieny, M.P., Lathe, R., Drillien, R., Spehner, D., Skory, S.. Schmitt, D. et al. Expression of rabies virus glycoprotein from a recombinant vaccinia virus. Nature 1984, 312, 163-166

Pastoret, P.P., Brochier, B., Blancou, J., Artois, M., Aubert, M.. Kieny, M.P. et al. Development and deliberate release of a vaccinia-rabies recombinant virus for the oral vaccination of foxes against rabies. In: Recombinant Poxviruses (Eds Binns, M.M. and Smith, G.L.) CRC Press, Boca Raton, FL, USA, 1992, pp. 163-206

Blancou, J., Kieny, M.P., Lathe, R., Lecocq, J.P., Pastoret, P.P. Soulebot, J.P. and Desmettre, P. Oral vaccination of the fox against rabies using a live recombinant vaccinia virus. Nature 1986, 322. 373-375

Brochier, B., Languet, B., Blancou, J., Kieny, M.P., Lecocq, J.P. Costy, F. et al. Use of a recombinant vaccinia-rabies virus for oral vaccination of fox cubs (Vulpes vulpes, L.) against rabies. Vet. Microbial. 1988, 18, 103-108

Brochier, B., Blancou, J., Thomas, I., Languet, B., Artois, M., Kieny, M.P. et al. Use of recombinant vaccinia-rabies glycoprotein virus for oral vaccination of wildlife against rabies: lnnocuity to several non-target bait consuming species. J. Wild/. Dis. 1989, 25. 540-547

Winkler, W.G., Shaddock, J.H. and Williams, LW. Oral rabies vaccine: evaluation of its infectivity in three species of rodents. Am. J. Epidemiol. 1976, 104, 294-298

Wandeler, A., Bauder, W., Prochaska, S. and Steck, F. Small mammal studies in a SAD baiting area. Comp. lmmunol. Microbial. Infect. Dis. 1982, 5, 173-176

Leblois, H. and Flamand, A. Studies on pathogenicity in mice of rabies virus strains used for oral vaccination of foxes in Europe. In: Vaccination to control rabies in foxes (La vaccination antirabique du renard) (Eds Pastore!, P.-P., Brochier, B., Thomas, I. and Blancou, J.) Commission of the European Communities, EUR 11439 ·EN-FR, 1988, pp. 101-104

Bingham, J., Foggin, G.H., Gerber, H., Hil, F.W.G., Kappeler, A., King, AA et al. The pathogenicity of SAD rabies vaccine in chacma baboons (*Papio ursinus*) given by the oral route. Vet. Rec. 1992, 131, 55-56

Artois, M., Guittre, C., Thomas, I., Leblois, H., Brochier, B. and Barra, J. Potential pathogenicity for rodents of vaccines intended for oral vaccination against rabies: a comparison. Vaccine 1992, 10, 524-528

Brochier, B., Kieny, M.P., Costy, F., Coppens, P., Bauduin, B., Lecocq, J.P. et al. Large-scale eradication of rabies using recombinant vaccinia-rabies vaccine. Nature 1991, 354, 520

Brochier, B., Coppens, P., Costy, F., Peharpre, D., Marchal, A., Hallet, L et al. Programme d'eradication de la rage en Belgique par la vaccination du renard : bilan 1992. Ann. Med. Vet. 1993, 137, 285--291

Saxby, D. Is cowpox misnamed - a review of 10 human case. BMJ 1977, i, 1379-1381

Marennikova, S.S., Ladnyj, l.D., Ogorodnikova, Z.1., Shelukhina, E.M. and Maltseva, N.N. Identification and study of a poxvirus isolated from wild rodents in Turkmenia. Arch. Viral. 1978, 56, 7-14

Baxby, D., Gaskell, R.M., Gaskell, C.J. and Bennett, M. Ecology of orthopoxviruses and use of recombinant vaccinia vaccines. Lancet 1986, II, 850-851

Egberink, H.F., Willemse, A. and Horzinek, M.C. Isolation and identification of a poxvirus from a domestic cat and a human contact case. J. Vet. Med. B. 1986, 33, 237-240

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Bennett, M., Gaskell, C.J. Saxby, D., Gaskell, R.M., Kelly, D.F. and Naidoo, J. Feline cowpox virus infection. J. Small Anim. Pract. 1990, 31, 167-173

Maenhout, T., Declercq, J., De Keuster, T. and Hoorens, J.K. Orie gevallen van koepokkeninfektie bij de kat in Belgie. Vlaams Diergeneeskd Tijdschr. 1991, 60, 64-67

Thomas, I., Brochier, B., Languet, B., Blancou, J., Peharpre, D., Kieny, M.P. et al. Primary multiplication site of the vaccinia-rabies glycoprotein recombinant virus administered to foxes by the oral route. J. Gen. Virol. 1990, 71, 37-42

Gaskell, R.M., Gaskell, C.J., Evans, R.J. Dennis, P.E., Bennett, A.M., Udall, N.D. et al. Natural and experimental poxvirus infection in the domestic cat. Vet. Rec. 1983, 112, 164-170

Bennett, M., Baxby, D., Gaskell. R.M., Gaskell, C.J. and Kelly, D.F. The laboratory diagnosis of Orthopoxvirus infection in the domestic cat. J. Small Anim. Pract. 1985, 26, 653--661

Smith, J.S., Jager, PA and Baer, G.M. A rapid time culture test for determining rabies neutralizing antibodies. In: Laboratory Tech- niques in Rabies, 3rd ed. (Eds Kaplan, M.M. and Koprowski, H.) World Health Organization, Geneva, 1973, pp. 354-357

Bennett, M., Gaskell, R.M., Gaskell, C.J., Saxby, D. and Kelly, D.F. Studies on poxvirus infection in cats. Arch. Virol. 1989, 104, 19-33

Fenner, F. and Comben, B.M. Genetic studies with mammalian poxviruses. I. Demonstration of recombination between two strains of vaccinia virus. Virology 1958, 5, 530-548

Ball, LA. High-frequency homologous recombination in vaccinia virus DNA. J. Virol. 1987, 61, 1788--1795

Chernos, V.I., Antonova, T.P. and Senkevich, T.G. Recombinants between vaccinia and ectromelia viruses bearing the specific pathogenicity markers of both parents. J. Gen. Viral. 1985, 66, 621--626

Chernos, V.1., Senkevich, T.G., Chelyapov, N.V., Antonova, T.P., Vovk, T.S. and Mitina, l.V. Biologic properties and genome structure of the recombinants between ectromelia and rabbitpox viruses. Acta Virol. 1987, 31, 193-202

Gershon, P.D., Kitching, R.P., Hammond, J.M. and Black, D.N. Poxvirus genetic recombination during natural virus transmission. J. Gen. Virol. 1989, 70, 485-489

Strayer, D.S., Cabirac, G., Sell, S. and Leibowitz, J.L Malignant rabbit fibroma virus: Observations on the culture and histo- pathologic characteristics of a new virus-induced rabbit tumor. J. Natl Cancer Inst. 1983, 71, 91-104

Taylor, J., Weinberg, R., Languet, B., Desmettre, P. and Paoletti, E. Recombinant fowlpox virus inducing protective immunity in non-avian species. Vaccine 1988, 6, 497-503

Taylor, J., Trimarchi, C., Weinberg, R., Languet, B., Guillemin, F., Desmettre, P. and Paoletti, E. Efficacy studies on a canarypox--rabies recombinant virus. Vaccine 1991, 9, 100-193

Baxby, D. and Paoletti, E. Potential use of non-replicating vectors as recombinant vaccines. Vaccine 1992, 10, 8-9