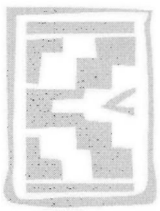

RESEARCH COMMUNICATION

Teratogenicity of a mutagenised Rift Valley fever virus (MVP 12) in sheep

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ABSTRACT

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A 5-fluorouracil mutagenised Rift Valley fever virus strain, which was shown to be attenuated and immunogenic in cattle and sheep, was evaluated for its ability to cause teratogenic effects in pregnant sheep. A group of 50 sheep at various stages of pregnancy was inoculated with the virus and the pregnancies followed to term. There were two abortions and 14 % of the lambs produced by vaccinated ewes showed teratogenic effects, the most prevalent being spinal hypoplasia, hydranencephaly, brachygnathia inferior and arthrygryposis. The foetal malformations of the central nervous and musculo-skeletal systems were mostly consistent with those observed in sheep vaccinated with the attenuated Smithburn RVF strain. The teratogenic effects of MVP12 were not seen in previous experiments by other authors as immunisation of sheep took place in the second to third trimester of pregnancy, when the foetal brain tissue has completed most of its cell division.

Keywords: Mutagenised, MVP 12, Rift valley fever virus, sheep, teratogenicity

INTRODUCTION

Rift Valley fever (RVF) is a mosquito-borne virus disease of humans and animals. Although it is endemic in Africa it has the potential to spread outside the continent and become established where the vectors occur, as recent outbreaks in the Middle East have demonstrated (CDC report 2000).

Vaccines available currently for immunising stock are formalin-killed vaccines (Harrington, Lupton, Crabbs, Peters, Reynolds & Slone 1980; Barnard & Botha 1977; Yedloutschnig, Dardiri, Mebus, Walker & Eddy 1981) and the live attenuated Smithburn strain (Smithburn 1949). Both types of vaccines have disadvantages and therefore alternative vaccines have been investigated.

A RVF virus strain isolated from a human patient was mutagenised by passaging it in the presence of 5-fluorouracil (Caplen, Peters, & Bishop 1985). At the 12th passage the mutagenised virus (MVP 12) was shown to be attenuated in hamsters (Rossi & Turrell 1988) and adult mice (Caplen *et al.* 1985). This vaccine was tested in pregnant ewes and said to be non-abortigenic in sheep (Morrill, Jennings, Caplen, Turrell, Johnson & Peters 1987; Morrill, Carpenter, Taylor, Ramsburg, Quance & Peters 1991), and effective in cattle on virulent challenge (Morrill, Mebus & Peters 1997a; Morrill, Mebus & Peters 1997b). The MVP-12 strain was shown to be safe when used as a vaccine in young lambs and protected them against challenge (Hubbard, Baskerville& Stephenson 1991).

Since the live attenuated Smithburn RVF strain has been shown to be teratogenic in a proportion of

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pregnant ewes (Coetzer & Barnard 1977; Davies 1981) the MVP 12 strain was investigated more extensively for these characteristics in this study. Ewes at various stages of pregnancy were vaccinated with MVP 12 strain and allowed to carry the lambs to term.

MATERIALS AND METHODS

Virus strain

The MVP 12 strain (ZH548-P12), prepared by the Salk Institute (Swiftwater, PA, 18370, USA; Code TSI-GSD 223) on MRC-5 cells, was supplied as a freeze-dried preparation in glass vials. The freeze-dried virus preparations were titrated in a plaque assay on Vero cells to determine the virus titre.

Animals

Susceptible seronegative Merino type ewes were housed under insect-free conditions throughout the experiment.

Experimental design

The ewes were synchronised as described by Kellerman, Prozesky, Schultz, Rabie, Van Ark, Maartens & Lubben (1991). Rams were placed with the ewes for 30 days, after which pregnant ewes were identified using sonar. The ewes were then separated into groups for vaccination at different stages of pregnancy. Each of the five groups contained ten animals and groups were vaccinated at 28, 35, 42, 49 and 56 days, respectively, using a vaccine dose of 1 x 10^6 plaque forming units. The ewes' temperatures were taken from days 2–14 and monitored for clinical signs. Thereafter they were inspected daily for signs of abortion or other complications. Stillborn foetuses and dead lambs were submitted for post-mortem examination.

RESULTS

All vaccinates seroconverted, confirming inoculation with the virus (results not shown). No signs of fever or malaise were seen in the vaccinated ewes. However, there were severe effects on the foetuses in all groups, with the exception of the group vaccinated on the 28th day of pregnancy (Table 1). Two foetuses were aborted at 12 and 13 weeks but were too decomposed for examination. Between 15–23 % of the total number of lambs born to each group was affected, and 14% of the total number of lambs produced by all the groups. The teratogenic effects seen in the lambs are summarized in Table 2.

DISCUSSION

The mutagenised MVP 12 vaccine strain induced a high percentage of malformations of the nervous and musculo-skeletal systems. Lambs of ewes vaccinated from 35–56 days of pregnancy were affected, while those vaccinated at 28 days were not. The teratogenic effects were mostly similar to those recorded in experimental work with the neurotropic Smithburn strain as described by Coetzer & Barnard (1977) who found that vaccinating nine ewes at 42–74 days of pregnancy with the Smithburn strain produced teratogenicity in 4 out of the 13 lambs born to the group. The lesions described in these lambs were hydranencephaly, microencephaly and arthrogryposis. MVP12 did not induce teratogenicity in previous experiments in sheep probably due to the fact that the sheep were 90–110 days pregnant at the time of inoculation (Morrill *et al.* 1987, 1991).

Teratogenic viruses appear to have an affinity for rapidly dividing cells of the nervous tissue, which in sheep foetuses occurs approximately in the first trimester of pregnancy (McIntosh, Baghurst, Potter

TABLE 1 A summary of the results obtained when ewes were vaccinated with MVP12 at various stages of pregnancy

Time of vaccination (days)	No. of ewes	No. of lambs	Abortions	No. of lambs with teratogenic effects	Remarks
28	9	13	–	–	–
35	10	19	1*	3	3**
42	10	17	1*	3	–
49	10	13	–	2	–
56	11	13	–	3	–

* Foetuses too decomposed for analysis
** Death due to dystocia and stillbirth unrelated to teratology

TABLE 2 Summary of teratogenic effects on lambs born to ewes vaccinated with MVP12

Group[a]	Ewe no.	Cerebellar hypoplasia	Spinal hypoplasia	Hydran–encephaly	Prognathia inferior	Brachygnathia inferior	Arthro–gryposis	Scoliosis	Lordosis	Kyphosis	Domed head
35	6042/3	–	–	–	–	–	–	+	–	–	+++
	"	–	–	–	–	–	–	+	–	–	+
	6049/8[b]	–	–	–	+	–	–	–	–	–	+
42	6090/5	–	+++	+++	–	+++	++	+++	–	+++	–
	"	–	+++	+++	–	+++	++	–	–	–	–
	"	–	+++	+++	–	+++	+++	++	–	–	–
49	4095/6	–	+++	+++	–	++	+++	+	+	+	–
	"	–	+++	+++	–	+++	+++	+	+	+	–
56	3373/0	+++	–	+	–	–	–	–	–	+	–
	3804/9	+	–	–	+	–	+	+	+	+	–
	3118/8	–	+++	+++	–	++	++	+++	–	+	–

[a] Day of pregnancy on which ewes were vaccinated
[b] Ewe had twins but only one was affected
\+ Indicates the severity of teratology

& Hetzel 1979). It has been shown with other teratogenic viruses, such as attenuated bluetongue virus, that injection at 50–58 days of pregnancy causes hydranencephaly, but when injected at 100 days causes only a mild encephalitis (Coetzer 1994).

Another interesting feature of the study was that in the case of one multiple birth, one twin was affected while the other showed no teratogenic effects. Since twinning in synchronized sheep is usually dizygous, the difference between the twins may be due to genetic susceptibilities to the virus which are known to occur in other species (Peters & Anderson 1981). Humoral and cellular immune functions develop fairly early in foetal lambs (Tizard 1992).

In conclusion, the MVP 12 vaccine strain appears to have marked neurotropic characteristics and it therefore has no advantages over the currently used live Smithburn vaccine.

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