Appendix 39

Emergency FMD Vaccine: Effect of antigen payload on protection, sub-clinical infection and persistence following direct contact challenge of cattle

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Abstract:

Introduction: Previous work, in sheep vaccinated with emergency foot-and-mouth disease vaccine, indicated the benefits of antigen payload in inhibiting local virus replication and consequently persistence following homologous indirect aerosol challenge. The work presented investigates this possibility further using cattle and a more severe heterologous direct contact challenge. The quantitative dynamics of virus replication and excretion in both vaccinated and non-vaccinated cattle following challenge is examined. Materials and Methods: Two experiments were carried out each involving 20 vaccinated and 5 non-vaccinated cattle. An O_1 Manisa vaccine (18 PD_{50}) was used for the first experiment. The same vaccine was used for the second experiment except the antigen payload was increased 10-fold per bovine dose. Twenty-one days post vaccination the cattle received a 5 day direct contact challenge from 5 further cattle infected 24 hours earlier with O UKG 34/2001. Blood and probang samples for antibody and virus analyses were regularly taken following vaccination and challenge alongside frequent examination for clinical signs. Results: All vaccinated cattle regardless of antigen payload were protected against clinical disease. Localised sub-clinical infection at the oropharynx was detected in animals from both experiments but quantitative RT-PCR showed that the level of virus replication shortly after direct contact challenge was significantly reduced in vaccinated animals. Cattle immunised with the 10-fold antigen payload cleared the virus more readily and consequently at 28 days post challenge fewer animals were persistently infected compared to the single strength vaccine. Neutralising antibody titres were shown to be significantly higher for the 10-fold antigen payload ($P<0.05$). Discussion: Following an extremely severe challenge, the results show that use of emergency vaccine can prevent or decrease local virus replication and thereby dramatically reduce the amount of virus released into the environment, particularly during the early post-exposure period. Additionally, increasing the antigen payload of the vaccine may be a further means of reducing such sub-clinical infection, leading to less persistently infected and subsequent carrier animals.

Introduction:

Acceptance of FMD vaccines into the UK Defra strategic reserve is presently determined by a cattle potency test as described in the European Pharmacopoeia Monograph (1993) and OIE Manual of Standards (2000), from which a PD_{50} value for a vaccine is calculated based on protection from clinical disease i.e prevention of generalisation of FMDV to the feet. For a vaccine to be acceptable as an 'emergency vaccine' it has to achieve a PD_{50} of ≥6. It is well documented that despite being protected from disease some vaccinated ruminants can maintain replicating virus in the upper respiratory tract and become persistently infected carriers with consequential impact on control policies relating to vaccination.However, the potency test takes no account of how effective the vaccine is at reducing sub-clinical infection at the oropharynx.

A number of previous research studies have provided evidence that FMD vaccination, particularly emergency FMD vaccines, formulated to higher potency than conventional vaccines, can have some inhibitory influence on local virus replication and excretion in the oropharynx, thereby limiting transmission of disease to other susceptible animals (Barnett and Carabin, 2002, Donalson and Kitching, 1989). Although most of these studies assume the antigen payload used is higher than that incorporated in conventional vaccine with the same strain , they did not attempt to evaluate the effect of antigen payload directly, being primarily designed to investigate rapidity of protection. A more recent study in sheep, however, examined the ability of three similarly formulated vaccines, which differed only in antigen payload, to decrease or inhibit local virus replication (Barnett et al, 2004). This study indicated that higher payload vaccines were capable of inhibiting local virus replication and consequently persistence following homologous indirect aerosol challenge. The work presented investigates this possibility further using cattle and a more severe heterologous direct contact challenge. The quantitative dynamics of virus replication and excretion in both vaccinated and non-vaccinated cattle following challenge is examined.

Materials and Methods:

Animals

Two experiments, each using thirty Holstein/Friesian cattle (steers) aged 4-8 months were used for this study. All work was performed in a disease secure isolation unit at the Institute for Animal Health's Pirbright Laboratory. In both experiments cattle were housed in a group of twenty-five and a further five cattle were used as a source of virus for the direct contact challenge.

Vaccine and vaccination

In the first experiment (EXPT. 1) an O_1 Manisa vaccine was prepared from antigen concentrate stored over liquid nitrogen, which is being held at a commercial facility as part of a new UK strategic reserve. In accordance with the European Pharmacopoeia Monograph, this commercially produced oil adjuvanted vaccine had been shown to have a PD_{50} value of 18. Twenty-one days prior to challenge, twenty cattle were vaccinated intramuscularly in the side of the neck with a full bovine dose (2ml volume). A further five animals remained unvaccinated as controls. The same vaccine was used for the second experiment (EXPT. 2) except the antigen payload was increased 10-fold per bovine dose.

Infection of donor cattle and challenge of vaccinates and controls

Five cattle in each experiment were used as a source of virus for direct contact challenge. These cattle were inoculated intradermalingually (into the tongue) with 10^5 $TCID_{50}$ /0.2ml live FMDV O UKG 34/2001 24 hours prior to the start of the direct contact challenge period, during which time they were housed separately. The direct contact challenge was carried out by allowing all cattle to mingle freely with each other for 5 days in a common holding area of $125m^2$, after which the five needle challenged cattle and the control unvaccinated cattle were removed and housed separately from the vaccinates. All animals were examined regularly for clinical signs of FMD until 28 days post challenge and various samples were taken. Rectal temperatures were recorded daily until 10 days post challenge.

Sample collection

Clotted blood for serology and heparinised blood for virus isolation (whole blood) were collected at regular intervals prevaccination, post vaccination, at challenge, and up to 28 days post challenge. Additionally, oesophageal-pharyngeal fluid (probang) samples were collected at similar intervals for virus isolation by cell culture and for detection of viral RNA by reverse transcriptase polymerase chain reaction (RT-PCR). All samples were stored at -70^{o} C except sera, which were stored at -20^{o} C, until required for testing. The material for RT-PCR (200µl) was added to an 300µl of lysis/binding buffer (Roche, UK) before freezing.

Virus isolation and detection of FMD viral RNA by RT-PCR

Heparinised blood samples and probang samples were examined for the presence of virus by inoculation of monolayers of primary calf thyroid (BTY) cells (Snowdon, 1966). Four BTY tubes were each inoculated with 250 µl sample and incubated at 37^{o} C on roller drums. At 24, 48 and 72 hours post inoculation cell monolayers were examined for cytopathic effect (cpe). ELISA was used to confirm the presence of FMDV in cultures showing cpe (Ferris and Dawson, 1988). BTY cell culture supernatants from samples showing no sign of cpe after 72 hours were pooled and repassaged once.

The probang samples were also tested by quantitative real-time RT-PCR using similar automated programmes for total nucleic acid extraction and RT and PCR set up, to those described by Cox et al. (2004, at press).

Serology

Serum samples were examined for both anti-FMDV neutralising antibodies (OIE , 2000) and for the presence of antibodies to the FMDV non-structural proteins (NSP) 3ABC (Ceditest FMDV-NS (Cedi-Diagnostics)).

Results:

Development of clinical FMD and viraemia

Within 3 days of needle challenge, all of the donor cattle, in both experiments, had developed typical FMD foot and tongue lesions. Although the amount of virus excreted from these animals was not measured, previous studies in cattle injected by the same route with the same challenge strain have shown that up to $10^{4.3}$ $TCID_{50}$ per animal per 24 hours may be excreted by aerosol from 1-3 days post infection (Alexandersen et al., 2002). The donor cattle were therefore a potent source of virus for the challenge, probably from day 1.

All of the vaccinated cattle in both experiments, regardless of antigen payload, were protected against clinical disease and none developed a detectable viraemia in samples collected at regular intervals up to 28 days after contact exposure with the needle challenged cattle. The five cattle in the

unvaccinated groups, however, all developed clinical signs of FMD including nasal discharge, with foot and tongue lesions appearing between 2-10 days and viraemia from 2-7 days after introduction of the needle challenged cattle.

Local virus replication, detection of FMD viral RNA and development of antibodies against non-structural FMDV polyprotein 3ABC

Following observation of BTY cells for signs of cpe and confirmation by ELISA, FMDV was recovered from probang samples collected from most vaccinated cattle and all unvaccinated cattle in both experiments, at various time points after the direct contact challenge, although detection, and therefore occurrence of local virus replication, varied greatly between individual animals (Tables 1 and 2). Likewise, FMDV RNA was variably detected by quantitative RT-PCR in the probang samples taken from individual animals of both groups and copy numbers per ml are also shown in Tables 1 and 2. Samples found positive on virus isolation were not necessarily positive by RT-PCR and vice versa. Combining the results of the two virological tests, virus or viral genome was confirmed in all but three animals from the vaccinated group in both experiments although an additional four vaccinated animals in EXPT. 1 and five in EXPT. 2 only had oropharyngeal virus or genome detected whilst the donor animals were still present. This might have been of environmental origin rather than virus replication. The total number of animals with some form of viral recovery at 28 days after challenge exposure, i.e. persistently infected, was 9 vaccinates and 1 unvaccinated control in EXPT.1 and 2 vaccinates and 2 unvaccinated controls in EXPT. 2. Samples from one vaccinated animal (UY83) and one unvaccinated animal (UY 97 - dead) were unavailable for testing at 28 days post challenge exposure.

NSP serology results at 28 days post challenge are also shown in tables 1 and 2. Thirteen of the 20 vaccinated animals in EXPT. 1 and 15 of the 20 in EXPT. 2 showed no evidence of non-structural antibody development, particularly those appearing to clear the virus. The unvaccinated cattle in both experiments all showed an NSP antibody response by 28 days post challenge exposure.

The mean quantity of FMD viral RNA $[\log_{10}(\text{copies ml}^{-1})]$ in probang samples from both vaccinated and unvaccinated control cattle at different times after contact with the donor cattle, as measured by quantitative RT-PCR, are presented in Figure 1. During the period between 4 and 10 days post challenge exposure, the unvaccinated controls in both experiments, had highest average levels of detectable viral RNA in their oropharynxes, the levels being between 10^2 - 10^3 greater than that seen in the vaccinated animals at the same time points.

The unvaccinated animals demonstrated a very high initial load, peaking at 7 days, and then falling again. In contrast, some persistently infected animals, particularly in EXPT. 1 were unable to clear the virus as efficiently, and had their highest levels of detectable viral RNA at 28 days after the introduction of the needle challenged cattle.

In order to assess whether antigen payload was affecting the amount of sub-clinical infection and persistence (as measured by virus recovery either by virus isolation or RT-PCR), a comparison of how often virus was detected by either technique was made. The percentage number of animals positive at each time point is shown in Table 3. Results for the unvaccinated animals from both experiments were combined and are also included.

Virus neutralising antibody induction

Figure 2 shows mean serum neutralising antibody responses against O_1 Manisa in vaccinated and unvaccinated cattle, up to 14 days post challenge exposure, following vaccination and direct contact with infected donor cattle. Neutralising antibody responses were detected as early as 5 days post vaccination in some animals from both experiments. At 7 days post vaccination, nine animals in EXPT. 1 and twenty in EXPT. 2 had seroconverted. Neutralising antibody titres, at every time point, were significantly higher for the 10-fold antigen payload group ($P<0.05$).

Discussion:

In order to assess how well emergency vaccines will protect from direct contact challenge a commercially prepared O_1 Manisa vaccine antigen, held as part of the new UK strategic reserve, was formulated to provide an oil vaccine with a calculated PD_{50} of 18. Additionally, a similar vaccine was prepared which contained ten times more antigen. The vaccinated cattle of both groups were subjected to a severe heterologous direct contact challenge from 5 cattle infected with O UKG 34/2001, over a period of 5 days. All twenty cattle, regardless of antigen payload, were protected from clinical disease confirming that high potency emergency vaccines are capable of providing protection after a single application, even in the face of this severe direct challenge exposure. Further studies, however, utilizing shorter time periods between vaccination and challenge, are needed to identify how quickly such protection is achieved.

Protection from clinical disease did not always coincide with prevention of localised, sub-clinical infection. In the first 10 days after challenge exposure, FMDV was frequently recovered from the oropharynx of vaccinated cattle receiving both antigen payloads although to a lesser extent in animals receiving the higher antigen payload by 10 days post challenge exposure. There is a possibility that the virus recovered in the first five to seven days following start of the challenge exposure could have been directly inhaled or ingested rather than being an indication of oropharyngeal replication. During the first ten days after challenge exposure, the amount of virus produced was considerably less in the vaccinated groups due to the transient or intermittent extent of virus replication. Furthermore, even comparing the levels of viral RNA found in positive samples, the average levels were approximately 100-1000 times higher in the unvaccinated animals until 10 days after challenge exposure. These findings demonstrate the ability of the vaccines to either prevent or reduce viral replication shortly after direct contact challenge at the site of primary infection, thereby further limiting the amount of infectious material released to the environment from sub-clinically infected animals. The 10-fold antigen payload vaccine, however, did not add much in the way of additional benefit over the single antigen payload vaccine.

It is documented that conventionally vaccinated cattle may succumb to local infection and subsequently become persistently infected 'carriers'. This study shows that persistence can be observed following the administration of an emergency vaccine (X1) and severe direct contact exposure for 5 days. However, if the antigen payload of the vaccine is increased, the number of persistently infected animals is reduced. Additionally, virus was less frequently recovered on most other occasions from animals vaccinated with the higher antigen payload.

It is interesting that the levels of viral RNA found in probang samples collected from persistently infected animals receiving the lower antigen payload beyond ten days post challenge exposure was often higher than those in equivalent samples from unvaccinated animals. Although it cannot be excluded that unvaccinated animals with a similar response might have been identified if there had been a comparable number of unvaccinated to the vaccinated subjects it is possible that the local immune response to sub-clinical infection in vaccinated animals will be different from that of unvaccinated animals, resulting in a prolonged clearance of virus. However, it should be noted that a similar result was not seen in animals receiving the higher antigen payload which perhaps suggest an improved local immune response at the oropharynx following higher payload vaccination.

It is difficult to assess the relationship between RNA copy number and infectivity. Results presented here show that the correlation between isolation of live virus and detection of viral RNA from probang samples is variable. The most likely reason for this lack of correlation is the involvement of antibody, either from serum or local production at the mucosa following sub-clinical infection, which neutralises the virus effectively making it non-infectious. It is clear therefore that although detection of FMDV RNA may be useful for diagnostic purposes, as in determining whether an animal has had contact with the virus, RNA copy number alone may not be useful as an indicator for determining whether a persistently infected animal is likely to present a risk for disease transmission. Future studies will need to investigate more precisely the correlation between viral RNA recovery (copy number) and actual tissue culture infective dose at different time intervals following challenge in both vaccinated and unvaccinated animals.

The mean systemic neutralising antibody response for both groups of vaccinated cattle was examined to see whether any differences were evident as a result of antigen payload. Overall, the 10-fold higher antigen payload vaccine resulted in a quicker response, with titres at every time point being significantly higher than for the single strength vaccine. Although it is difficult to gauge the effect of such a systemic response on the local environment, the reduction seen in virus replication and viral persistence obtained with the 10-fold payload perhaps suggests that an improved immune response involving correlates of protection not investigated, as well as an improved neutralising antibody response, has been achieved.

Conclusions:

- High potency emergency vaccines are effective at preventing clinical disease and reducing local virus replication in the all important early post exposure period and therefore dramatically reduce the amount of virus released into the environment following severe direct contact challenge.
- Increasing antigen payload results in an improved immune response which has an effect on local virus replication and persistence.
- Emergency vaccines, as presently selected using potency tests based on development of clinical disease, may contain sub-optimal levels of antigen for combating sub-clinical infection.

Recommendations:

- Investigate further the effect of antigen payload on sub-clinical infection in order to optimise vaccines for emergency use.
- Investigate further the kinetics of protection in cattle under severe direct contact challenge conditions
- Rec. no. 3

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Animal Ref	Days post challenge																		
	2		4		7		10		12		14		16		21		28		
Vacc:	VI	PCR	VI	PCR	VI	PCR	VI	PCR	VI	PCR	VI	PCR	VI	PCR	VI	PCR	VI	PCR	NSAb
UV2	-	4.09*	+	3.79	+	3.84	+	0	-	0	-	0	IS	4.78	-	0	-	4.39	-
UV3	-	0	-	0	-	0	-	0	-	0	-	0	IS	0	-	0	-	0	-
UV4	-	0	+	5.46	-	0	-	0	-	0	-	0	IS	0	-	0	-	0	-
UV5	-	0	+	5.18	-	4.86	+	3.66	+	4.1	+	3.73	IS	4.69	+	3.99	-	2.85	+
UV6	-	0	-	0	+	0	-	0	-	0	-	0	IS	0	-	0	-	0	-
UV7	-	0	-	0	-	0	-	0	IS	0	-	0	IS	0	-	0	-	0	-
UV8	-	0	-	0	-	0	-	0	-	0	-	0	IS	0	-	0	-	0	-
UV9	+	4.77	+	0	+	5.05	+	4.28	+	4.68	+	5.42	IS	5.75	+	4.35	-	6.22	+
UV10	+	5.58	-	0	+	5.26	+	5.52	+	5.11	+	5.46	IS	4.15	+	4.57	-	6.09	+
UV11	+	0	+	5.77	+	6.13	+	4.05	-	4.2	+	3.5	IS	3.64	+	4.38	+	6.06	+
UV12	-	3.06	+	3.71	+	0	+	0	-	0	-	0	IS	0	-	0	-	0	-
UV13	+	5.27	+	4.01	+	4.43	+	4.29	+	5.12	+	5.31	IS	4.28	+	5.34	-	5.59	+
UV14	+	0	+	3.54	+	4.78	+	0	+	3.41	+	0	IS	0	+	4.3	-	3.65	-
UV15	-	0	-	4.95	-	0	-	0	-	0	-	0	IS	0	-	0	-	0	-
UV16	+	0	-	3.29	-	0	-	0	-	0	-	0	IS	0	-	0	-	0	-
UV17	IS	IS	+	3.38	+	5.41	+	4.2	+	4.78	+	5.14	IS	5.27	+	5.32	-	5.63	-
UV18	IS	IS	-	0	-	0	-	0	+	0	-	0	IS	0	+	0	-	0	+
UV19	+	3.86	+	4.68	+	4.97	+	3.2	-	4.94	+	4.12	IS	4.8	+	5.19	-	5.96	-
UV20	+	6.66	+	6.2	-	0	-	0	-	4.25	-	0	IS	0	-	0	-	0	+
UV21	+	3.4	+	4.76	-	0	-	0	-	0	-	0	IS	0	-	0	-	0	-
Unvacc:																			
UV22	+	5.25	IS	6.05	+	6.71	-	3.27	-	0	-	0	IS	3.09	-	0	-	0	+
UV23	+	0	+	4.96	+	8.2	+	5.64	-	4.42	-	0	IS	0	-	3.41	-	0	+
UV24	-	3.27	+	5.51	+	6.84	-	5.06	-	4.2	-	0	IS	0	+	0	-	0	+
UV25	+	5.04	+	3.44	+	8.99	+	7.22	-	5.45	+	3.35	IS	0	+	3.36	-	3.25	+
UV26	+	7.29	+	8.52	+	5.97	+	4.38	-	3.86	+	3.43	IS	3.49	+	0	-	0	+

IS: Insufficient sample * Viral RNA levels [$\log_{10}$(copies ml^{-1}) 0: No viral RNA detected

+: Virus or nonstructural antibodies detected '-: No virus or nonstructural antibodies detected

Table 1: Virus isolation (VI) and PCR results from probang samples and nonstructural antibody (NSAb) results from vaccinated and unvaccinated cattle (EXPT. 1)

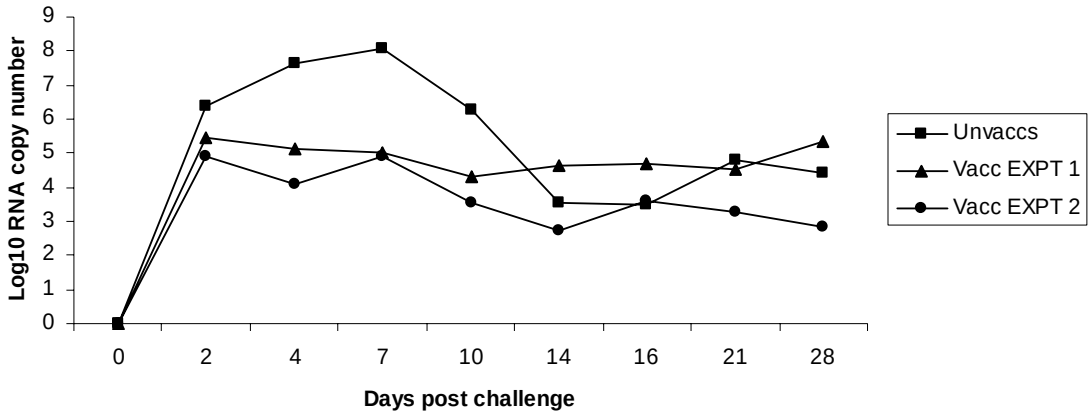
Animal Ref	Days post challenge																
	2		4		7		10		14		17		21		28		
Vacc:	VI	PCR	VI	PCR	VI	PCR	VI	PCR	VI	PCR	VI	PCR	VI	PCR	VI	PCR	NSAb
UY72	-	0	+	0	-	3.49*	IS	IS	-	0	IS	IS	-	0	-	0	-
UY73	-	0	-	0	+	0	-	0	-	0	-	0	-	0	-	0	-
UY74	-	0	+	4.07	-	0	-	0	-	0	-	0	-	0	-	0	-
UY76	+	4.32	+	4.37	+	0	+	4.57	-	0	+	3.96	+	4.44	+	4.1	+
UY77	-	0	+	0	-	IS	-	0	-	0	-	0	-	0	-	0	-
UY78	-	0	-	0	-	0	IS	IS	-	0	IS	IS	-	0	-	0	-
UY79	-	0	+	4.99	+	5.9	IS	IS	-	0	-	0	-	0	-	0	-
UY80	-	0	+	0	+	5.71	+	3.96	-	0	-	0	+	0	-	0	+
UY81	-	0	-	0	+	4.13	-	0	-	0	-	0	-	0	-	0	+
UY82	+	3.86	-	0	+	3.58	-	0	-	0	-	0	-	0	-	0	+
UY83	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	-	3.33	IS	IS	-
UY84	+	0	+	3.95	-	0	-	0	-	0	-	0	-	0	-	0	+
UY85	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
UY86	-	0	-	0	+	0	-	0	-	0	-	0	-	0	-	0	-
UY87	+	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
UY88	IS	IS	IS	IS	IS	IS	IS	IS	-	0	IS	IS	-	0	-	0	-
UY89	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	+
UY90	+	6.14	+	4.56	+	5.61	+	3.36	+	3.99	+	4.74	+	3.77	+	0	-
UY91	+	0	-	0	+	5.56	+	3.88	-	0	+	3.6	-	0	-	0	+
UY92	+	3.27	+	4.61	+	4.55	-	0	-	0	-	0	-	0	-	0	-
Unvacc:																	
UY93	IS	IS	IS	IS	-	6.28	IS	IS	IS	IS	-	0	IS	IS	-	0	+
UY94	+	5.79	+	7.6	+	5.98	-	4.16	-	0	-	0	-	0	-	0	+
UY95	+	5.09	+	6.32	+	6.5	-	4.68	+	4.36	+	4.4	+	5.74	+	5.33	+
UY96	+	0	+	4.23	+	5.97	-	5.16	-	0	+	0	+	0	+	4.46	+
UY97	+	4.58	+	3.96	+	6.23	-	3.7	-	3.43	-	0	-	0	ND	ND	+

IS: Insufficient sample * Viral RNA levels [$\log_{10}$(copies ml^{-1}) 0: No viral RNA detected

+: Virus or nonstructural antibodies detected '-: No virus or nonstructural antibodies detected

Table 2: Virus isolation (VI) and PCR results from probang samples and nonstructural antibody (NSAb) results from vaccinated and unvaccinated cattle (EXPT. 1)

Figure 1: Mean RNA copy number detected over time in RT-PCR positive samples



Treatment	Days post Challenge							
	2	4	7	10	14	16/17	21	28
0 (Expt.1 & 2)	100	100	100	100	44	40	67	33
X1 (Expt. 1)	61	70	55	50	40	40	45	45
X10 (Expt. 2)	39	50	61	27	5	18	20	11

Table 3: Percentage of animals from which virus recovered at different time points post challenge exposure

Figure 2: Mean neutralising antibody titres over time