Epidemiol. Infect. (1996), **117**, 349–360

Isotype-specific antibody responses to foot-and-mouth disease virus in sera and secretions of 'carrier' and 'non-carrier' cattle

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(*Accepted 12 May 1996*)

SUMMARY

Isotype-specific antibody responses to foot-and-mouth disease virus (FMDV) were measured in the sera and upper respiratory tract secretions of vaccinated and susceptible cattle challenged with FMDV by direct contact or by intranasal inoculation. A comparison was made between cattle that eliminated FMDV and those that developed and maintained a persistent infection. Serological and mucosal antibody responses were detected in all animals after challenge. IgA and IgM were detected before the development of IgG_1 and IgG_2 responses. IgM was not detected in vaccinated cattle. Challenge with FMDV elicited a prolonged biphasic secretory antibody response in FMDV 'carrier' animals only. The response was detected as FMDV-specific IgA in both mucosal secretions and serum samples, which gained statistical significance ($P < 0{\cdot}05$) by 5 weeks after challenge. This observation could represent the basis of a test to differentiate vaccinated and/or recovered convalescent cattle from FMDV 'carriers'.

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious viral disease of wild and domesticated even-toed ungulates. In FMD-free countries the financial implications of an outbreak of the disease include not only the direct costs of slaughter-compensation and lost productivity, but also the indirect costs related to the loss of export trade with FMD-free partners. The causal agent of the disease is a small, non-enveloped RNA virus found within the *Picornaviridae* (reviewed in [1]). The seven serotypes of foot-and-mouth disease virus (FMDV) are presently the sole members of the aphthovirus genus. Infection with a virus of one serotype does not confer immunity to another.

Recovery from FMD and protection from reinfection are predominantly associated with the presence of circulating neutralizing antibody (reviewed in [2]). Persistent inapparent infection is a common sequel to clinical and sub-clinical infection of susceptible and vaccinated ruminants [3, 4]. The duration of persistent FMDV infection can be 3·5 years in cattle [5] and 9 months in sheep [6]. The epidemiological significance of the 'carrier state' is ambiguous, although transmission of FMDV from persistently infected buffalo to susceptible buffalo [7] and cattle [8, 9] has been shown under controlled conditions. However, there is little doubt that FMDV 'carriers' represent a reservoir of potential infection [10], especially when mixed with a non-immune cattle population. The recent phasing out of prophylactic FMD vaccination has led to a gradual decline in herd immunity to a point where the 'European herd' may now be considered as almost totally susceptible to FMD. As a result there has been a resurgence of interest in the FMDV 'carrier' animal, more particularly into the mechanism of FMDV persistence and the development of a reliable means of diagnosing the 'carrier' state.

In FMDV 'carrier' animals long-term viral replication is restricted to the oropharynx [4, 11]. The determination of persistent FMDV infection is by the intermittent isolation of virus and/or viral RNA from

oropharyngeal scrapings and mucus collected with a probang sampling cup [12, 13]. Since Hyslop [14] first described the presence of specific virus-neutralizing activity in the saliva of cattle infected with FMDV several other studies have confirmed these findings in saliva and other secretory fluids [15–18]. There has been particular interest in the local oropharyngeal immune response to FMDV infection because this region is the most common natural route of infection with FMDV in ruminants [19] and is the site of primary virus replication [20, 21].

Previous studies of isotype-specific antibody responses to FMDV, as measured in serum [22] and secretions [15, 23], have been reported. Some have described the secretion of IgA in FMDV convalescent cattle [18, 24, 25], but none has specifically examined the influence of individual antibody isotype responses upon the outcome of FMDV infection in terms of virus elimination or the development and maintenance of persistent infection. This study analyses both serum and secretory antibody responses to FMDV challenge in terms of persistence or elimination of the virus using well-defined isotype-specific reagents in a sensitive ELISA [26, 27].

METHODS

Experimental design and animals

Three trials on yearling Friesian-cross cattle were conducted in the isolation facility at the Institute for Animal Health, Pirbright over a 2-year period. For the purposes of analysis the three experimental groups have been pooled and individual animals categorized as vaccinated ($n = 9$) or non-vaccinated ($n = 13$) prior to challenge. The vaccinated group received a single dose of either a trivalent aqueous inactivated FMD vaccine containing a European type O_1 strain or a similar polyvalent vaccine containing both a European and a Middle Eastern type O_1 strain. Challenge with FMDV was either by direct contact with an animal showing clinical signs of FMD following inoculation with a Middle Eastern type O_1 strain, or by the intranasal instillation of a 1·0 ml inoculum containing 10^6 $TCID_{50}$ of a European type O_1 strain of FMDV [28].

Sampling procedures

Oropharyngeal fluid. A probang sampling cup was used to collect cells, mucus and saliva from the oropharnyx and cranial oesophagus [3], collectively referred to as 'probang fluid'. Each sample was divided into two equal portions, one for antibody assay and one for virus isolation. The latter was immediately diluted (1:1) in Eagle's medium containing 20 mM HEPES, 200 IU/ml penicillin, 200 IU/ml streptomycin, 200 IU/ml neomycin, 200 IU/ml polymixin B and 2·5 IU/ml Fungizone (Squibb, Hounslow, England). Following temporary storage on dry ice, long-term storage was at −70 °C. The undiluted samples for antibody assay were clarified in a Beckman GPR bench centrifuge (3500 rpm, 10 min) and the supernate stored at −20 °C. Probang fluid was collected approximately weekly for the duration of the study. Samples contaminated with blood were discarded.

Blood. Blood was collected from a superficial vein and allowed to clot for 30 min at 37 °C. After 3–4 h at 4 °C the serum was separated by centrifugation prior to storage at −20 °C. Samples were collected approximately weekly for the duration of the study.

Virus isolation and typing

Each probang fluid sample was used to inoculate monolayers of primary bovine thyroid cells (BTY) essentially as described by Snowdon [29]. BTY culture tubes were examined daily for cytopathic effect (cpe). After 72 h the supernate from negative samples were 'blind passaged' onto further BTY tubes. The tissue culture medium from all cpe positive tubes was harvested, clarified and assayed for the presence of type O FMDV in an indirect sandwich ELISA [30].

Antibody assay

The FMDV type O_1-specific antibody titre in serum and secretion samples was determined in the following isotype-specific assays. For brevity only the results of the serum and probang samples are reported.

Isotype-specific indirect double antibody sandwich ELISA (IDAS)

An anti-viral sandwich ELISA was used to measure FMDV-specific IgA, IgG_1, IgG_2 and IgM in samples using monoclonal antibodies specific for these isotypes (ID-DLO, Lelystad, Netherlands). These reagents have been shown in our laboratory and by others [26] not to cross-react in ELISA. The assay was based

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upon that of Mulcahy and colleagues [31]. Ninety-six-well flat-bottomed Nunc Maxisorp ELISA plates (Nunc, Roskilde, Denmark) were coated overnight at room temperature with a solution of rabbit anti-FMDV type-specific hyperimmune antiserum (1:4000) in 0·1 M carbonate/bicarbonate buffer, pH 9·6 (100 μl/well). Coated plates were subsequently incubated for 1 h at 37 °C (100 μl/well) with pre-titrated inactivated FMDV antigen in excess. Duplicate threefold dilution series of each sample were made. Following incubation for 1 h at 37 °C monoclonal antibodies specific for bovine isotypes were added at 2 μg/ml, followed by the addition of HRPO-conjugated rabbit anti-mouse immunoglobulins (Dako, Glostrup, Denmark) (1:2000) and incubation for 1 h at 37 °C. All reagents were diluted in 0·04 M PBS containing 3% (v/v) soya milk and 0·05% (v/v) Tween 20 (Sigma). Plates were washed three times with PBS containing 0·05% (v/v) Tween 20 between each step. After a final wash, 100 μl/well of substrate consisting of 0·04% (w/v) *O*-phenylenediamine dihydrochloride and 0·005% (w/v) H_2O_2 in citric acid-phosphate buffer, pH 5·0, was added. The reaction was terminated by the addition of 1·25 M H_2SO_4 after 10 min. Optical densities (OD) were read at 492 nm on a Titertek Multiskan reader (Flow Laboratories, Irvine, Scotland). Controls included convalescent FMDV type O_1 bovine serum and secretory fluid positive controls, and normal bovine serum and saliva negative controls for IgM/IgG and IgA assays, respectively. One hundred μl volumes were used throughout. In these assays it was found that the point on the titration curve corresponding to A_{492} of 1·0 invariably fell on the linear part of the curve. Antibody titres were therefore expressed as the reciprocal of the last dilution calculated by interpolation to give an absorbance of 1·0 OD unit above background. However, IgM titres in probang samples were expressed as the absorbance in OD units at a dilution of 1:2.

Isotype-specific antibody capture ELISA (ACA)

It has been suggested that intra-isotypic competition may occur in this type of serum assay, which can distort the titre of minor isotypes. Reagents were used in excess to reduce this effect, which in secretory fluids would have been minimal due to the low antibody content of these samples compared to serum [32]. However, following initial evaluation of the anti-viral sandwich ELISA for the detection of FMDV-specific serum IgM and IgA, the assay was found to lack sensitivity, possibly through competition with the more prevalent IgG isotypes. These findings are similar to those of others working with isotype-specific assays for antibody to bovine rotavirus [26], bovine respiratory syncitial virus [33], bovine herpesvirus 1 [27] and Aujeszky's disease virus [34].

Therefore a second isotype-specific ELISA for the detection of FMDV-specific serum IgA and IgM was developed as an antibody capture assay (ACA) to measure FMDV type O_1-specific IgM and IgA titres in serum. Ninety-six-well flat-bottomed Nunc Maxisorp ELISA plates (Nunc, Roskilde, Denmark) were coated overnight at room temperature with a solution of monoclonal anti-bovine IgA or IgM-specific antibody at 1 μg/ml in 0·1 M carbonate/bicarbonate buffer, pH 9·6 (100 μl/well). Coated plates were incubated consecutively at 37 °C with duplicate threefold dilution series of each sample for 2 h, pre-titrated inactivated FMDV antigen in excess (100 μl/well) for 1 h, rabbit anti-FMDV type-specific hyperimmune antiserum (1:4000) for 1 h and finally HRPO-conjugated goat anti-rabbit immunoglobulins (Dako, Glostrup, Denmark) (1:2000). The remainder of the procedure and control samples were as above for the IDAS. Antibody titres were expressed as the 492 nm absorbance in OD units at a serum dilution of 1:30.

Statistical analysis

Neither isotype-specific assay was quantitative, therefore the only valid statistical analyses were between animal groups for individual antibody isotypes and not between different isotypes. Statistical analyses were made using the Student's *t* test.

RESULTS

Clinical outcome of infection

All of the nine animals in the vaccinated group were protected against clinical disease following FMDV challenge. FMDV was isolated from all nine of the vaccinated cattle on at least one occasion. All 13 of the animals in the non-vaccinated group showed signs of clinical FMD, including pyrexia (rectal temperature > 39·5 °C), hypersalivation, anorexia, lameness, and erosions around the oral cavity and the coronary bands of the feet.

Development of persistent FMDV infection

Table 1 shows the interpretation of the results of virus isolation from probang samples collected following

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Table 1. *Outcome of challenge of experimental cattle with FMDV type O_1. On the basis of the consistent isolation of FMDV from probang samples after 28 days after challenge, animals were classified as either 'carriers' or 'eliminators'*

Status before challenge	Group size	'Carriers'	'Eliminators'	'Carriers' (%)
Non-vaccinated/susceptible	13	10	3	77
Vaccinated/protected	9	7	2	78

challenge with FMDV from both groups of cattle. On the basis of virus recovery three of the non-vaccinated and two of the vaccinated cattle eliminated the virus early in the course of infection and were classified as 'eliminators'. Probang samples collected from ten non-vaccinated and seven vaccinated cattle were consistently positive for FMDV for longer than 28 days after challenge and were classified as 'carriers'. In the non-vaccinated and vaccinated groups of cattle 77% and 78% respectively became FMDV 'carriers'.

Isotype-specific antibody responses

Serum IgG_1

All vaccinated cattle had detectable serum IgG_1 on the day of challenge (Fig. 1), and responded to FMDV challenge similarly. The IgG_1 response peaked at 14 days in both vaccinated and non-vaccinated groups, with the peak mean titre being higher in the non-vaccinated animals than the vaccinates. Titres declined steadily in both groups during the study period. There were no significant differences in the mean IgG_1 response profiles between the FMDV 'carriers' and 'eliminators' in either group with the exception of a significantly higher mean titre in the non-vaccinated 'eliminator' group 37 days after challenge compared to the non-vaccinated 'carriers'.

Probang sample IgG_1

IgG_1 was not detected in the probang samples from any animal on the day of challenge (Fig. 1). In all vaccinated animals an early IgG_1 response was detectable at 7 days after challenge, whereas in the non-vaccinated animals a response was only found in some and at lower titres. Peak mean IgG_1 titres occurred at 14 days after challenge in both groups and were higher in the non-vaccinated animals. In the non-vaccinated group there was a rapid decline in mean titres from the peak at 14 days after challenge. During the period 49–98 days after challenge many samples from the non-vaccinated group contained no detectable IgG_1. Both 'carrier' and 'eliminator' mean antibody titres had a similar profile, and there was no significant difference despite higher mean titres from 14 days after challenge in the 'eliminators'. In the vaccinated group there was evidence of a low titre late IgG_1 response from 28 days after challenge in the 'carrier' animals only. Mean IgG_1 titre increased from day 20 to day 49 after challenge in the probang samples from these animals. The statistical significance of the apparent difference in responses between the vaccinated 'carriers' and 'eliminators' could not be determined because of the small number of 'eliminators' studied.

Serum IgG_2

All vaccinated cattle had serum IgG_2 titres on the day of challenge (Fig. 2), and showed a response to FMDV challenge by 7 days which peaked at 28 days after challenge. Peak titres in vaccinates and non-vaccinates were delayed in comparison with serum IgG_1 responses. The IgG_2 response peaked at 35 days after challenge in the non-vaccinated group, and the peak mean titres were lower in this group than in the vaccinates. There was evidence of a decline in mean titre 100–180 days after challenge in the non-vaccinated animals that eliminated FMDV, which was not seen in the 'carrier' animals. There were no significant differences in the mean IgG_2 response profiles between the FMDV 'carriers' and 'eliminators' in either group during the study period.

Probang sample IgG_2

IgG_2 was not detected in the probang samples from any of the animals in the study on the day of challenge (Fig. 2). In both groups of animals IgG_2 profiles were similar to the corresponding IgG_1 profiles. In many animals IgG_2 titres were undetectable and highly variable between individuals in the same groups,

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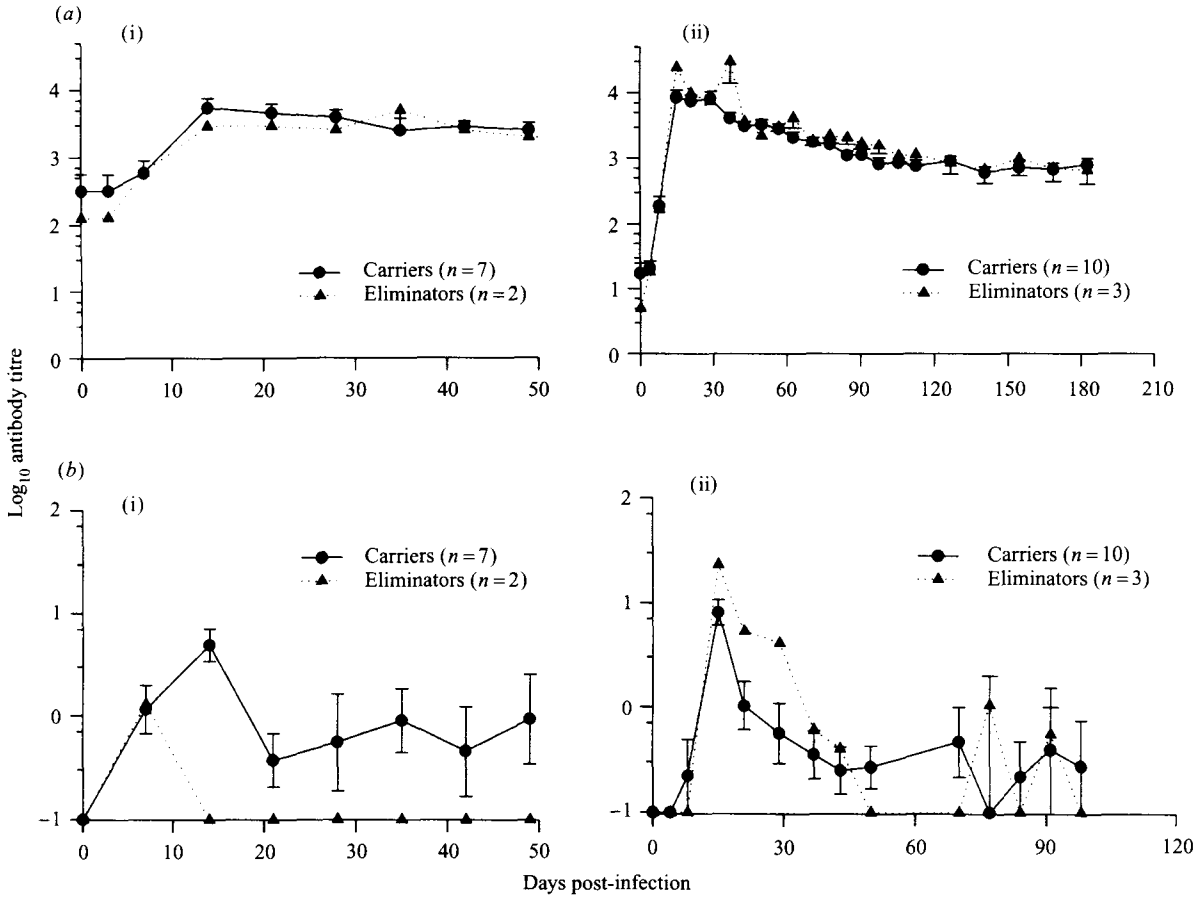

Fig. 1. The mean IgG_1 responses against FMDV in (*a*) sera and (*b*) probang samples from (i) vaccinated and (ii) non-vaccinated cattle following challenge with FMDV type O_1. Data points represent the geometric mean ± S.E.

despite a higher peak titre in the non-vaccinated 'eliminator' group. The statistical significance of the apparent difference in responses between the 'carriers' and 'eliminators' could not be determined because of the small number of 'eliminators' studied.

Serum IgM

In the vaccinated group IgM was detectable at low titres in the most recently vaccinated cattle on the day of challenge (Fig. 3). Responses were detected in some individuals at 7 days after challenge. The peak mean titre of the 'carrier' animals was at 14 days after challenge, but these declined to base-line measurements by 28 days. Wide variation was noted in the responses of individuals. Animals with higher serum IgG titres on the day of challenge developed the lowest IgM responses to challenge. Maximal responses were seen in all animals at eight and 14 days after challenge, which declined to base-line titres by 56 days. There were no significant differences between the FMDV 'carriers' and 'eliminators' in either group.

Probang sample IgM

IgM was not detectable in the vaccinated group either before or after challenge with FMDV (Fig. 3). In the non-vaccinated group all animals showed a response to challenge, which peaked at 8 days after challenge and rapidly declined to background titres by 28 days after challenge. Response profiles for the FMDV 'carrier' and 'eliminator' groups were coincident.

Serum IgA

In the vaccinated group IgA was not detectable on the day of challenge (Fig. 4). Some animals developed low titres at 4 days after challenge which peaked at 7–14 days. A second, later response beginning at 28 days, was detected in the 'carrier' group. This continued up to the end of the study at low titres, although these varied between individuals. Despite the apparent difference between mean titres of the FMDV 'carriers' and 'eliminators' there was no statistical difference between them. All members of the non-vaccinated group showed a transient response

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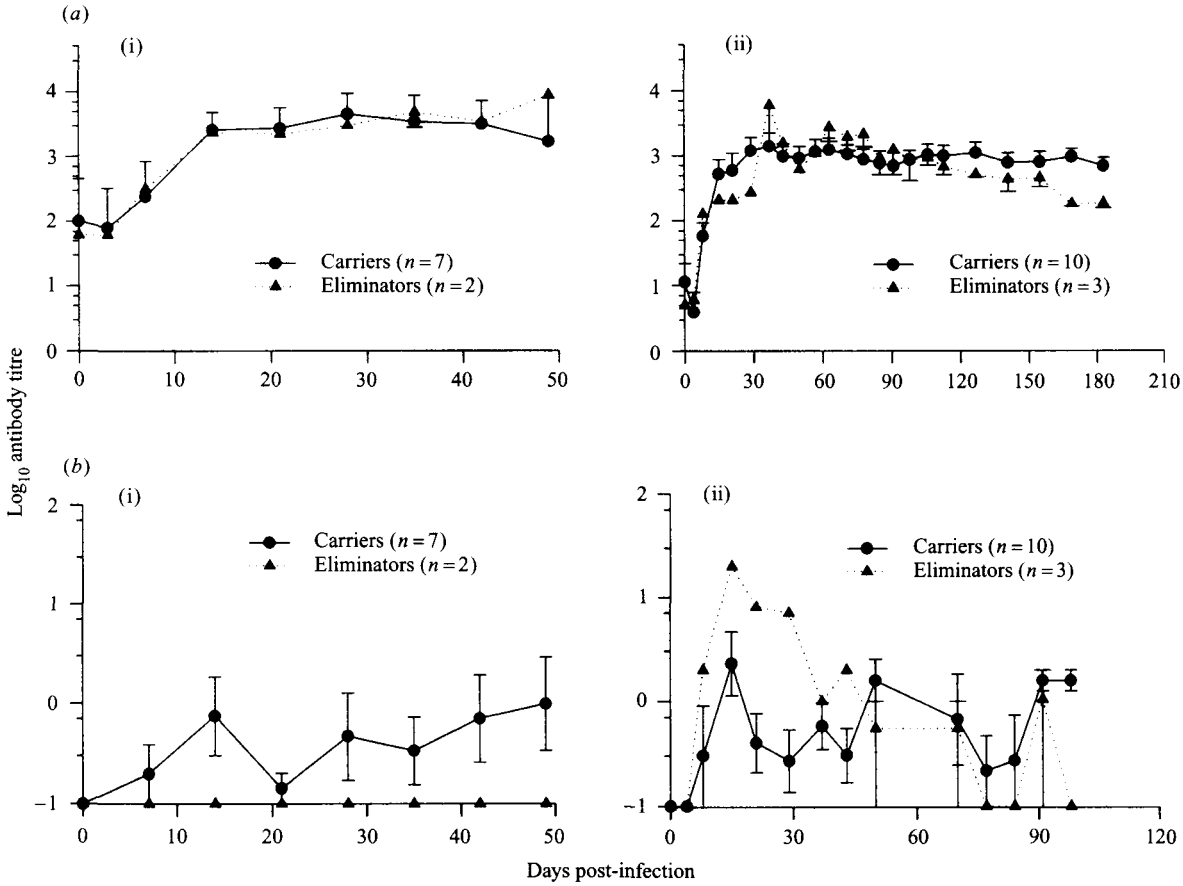

Fig. 2. The mean IgG_2 responses against FMDV in (*a*) sera and (*b*) probang samples from (i) vaccinated and (ii) non-vaccinated cattle following challenge with FMDV type O_1. Data points represent the geometric mean ± S.E.

which peaked 7 days after challenge and declined by 21 days. The mean peak titres of this early response were similar to those in the vaccinated group. The difference between the early responses in the FMDV 'carrier' and 'eliminator' animals was not statistically significant because of individual variation. A late response was seen in the non-vaccinated 'carrier' animals which significantly differed ($P < 0{\cdot}05$) from the 'eliminator' group from 37 days after challenge.

Probang sample IgA

Low titre responses were detected in the vaccinated group seven days after challenge in some individual animals (Fig. 4). All members of the group showed an early low titre response that peaked at 14 days after challenge. A second, late response, was detected at 28 days in all animals, which persisted in the 'carrier' animals at a mean titre tenfold higher than the early response. Significant differences could not be shown between the FMDV 'carrier' and 'eliminator' groups because of the small number of 'eliminators'. Similar responses were seen in the non-vaccinated cattle both in terms of the kinetics and titre of the responses.

Other secretory fluids

Similar results to the probang fluid results were obtained for the tear fluid and saliva (results not shown). In both, the titres of FMDV-specific total antibody and IgA were approximately ten-fold higher than those found in probang samples.

DISCUSSION

The mechanism by which persistent FMDV infection is established or maintained is not clear. Protective immunity to FMD is associated with neutralizing antibody, however our study concurs with the findings of others that vaccination and prevention of clinical disease offer no protection against the development of the 'carrier' state in cattle [35, 36]. The biological function of antibody is regulated by specificity, isotype and affinity. Mulcahy and colleagues [31] proposed

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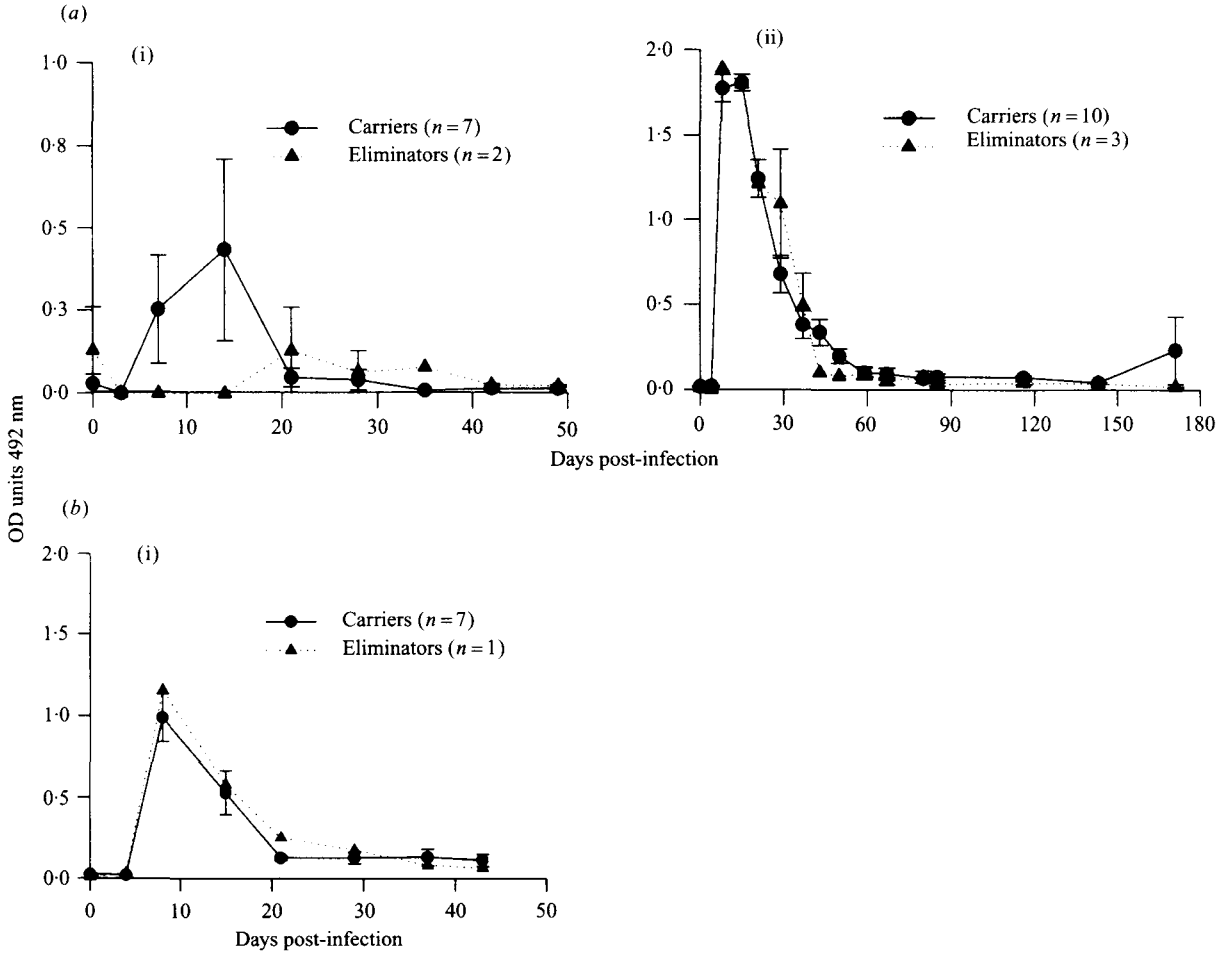

Fig. 3. The mean IgM responses against FMDV in (*a*) sera and (*b*) probang samples from (i) vaccinated and (ii) non-vaccinated cattle following challenge with FMDV type O_1. Data points represent the geometric mean ± S.E.

that differences in isotype profiles of the systemic humoral response to conventional and peptide FMD vaccines could explain, in part, functional differences between the sera. We examined, therefore, the role of one of these parameters, namely antibody isotype, in the development and maintenance of FMDV persistence. In vaccinated cattle there was no relationship between the serum antibody isotype profile before challenge and the development of persistence, and FMDV-specific secreted antibody was not detectable prior to challenge. The earlier appearance of IgA in probang samples from FMDV challenged vaccinated and non-vaccinated cattle in our study may be due to greater virus replication in animals vaccinated up to 6 months prior to challenge, compared to only 3 weeks prior to challenge of the cattle used by Francis and colleagues [23]. The similarity of the mean titres of specific IgA in probang samples of vaccinated and non-vaccinated cattle supports the theory that IgA is produced locally in the oropharynx and therefore is less susceptible to the limiting effect of serum antibody upon the systemic responses in vaccinates. However, vaccination is known to reduce the early secretion of FMDV following challenge [18, 37, 38], and interferes with the transmission of FMDV from sub-clinically infected vaccinated cattle to susceptible contacts [39]. Therefore, the more consistent early IgA response seen in the non-vaccinated cattle may reflect limited virus replication in this group.

Serum IgA in ruminants is principally dimeric [40] and is thought to originate in exocrine glands, such as the salivary glands, and mucosal tissues [41]. The temporal relationship of the early serum and probang IgA profiles in this study suggest a common origin of the antibody, most probably the pharyngeal mucosa and associated lymphoid tissue. The early detection of specific IgG_1 and IgG_2 in probang samples at a similar ratio to that found in serum, and the similarity in the kinetics of the two responses, is also indicative of a common origin, in this case systemic sites [23].

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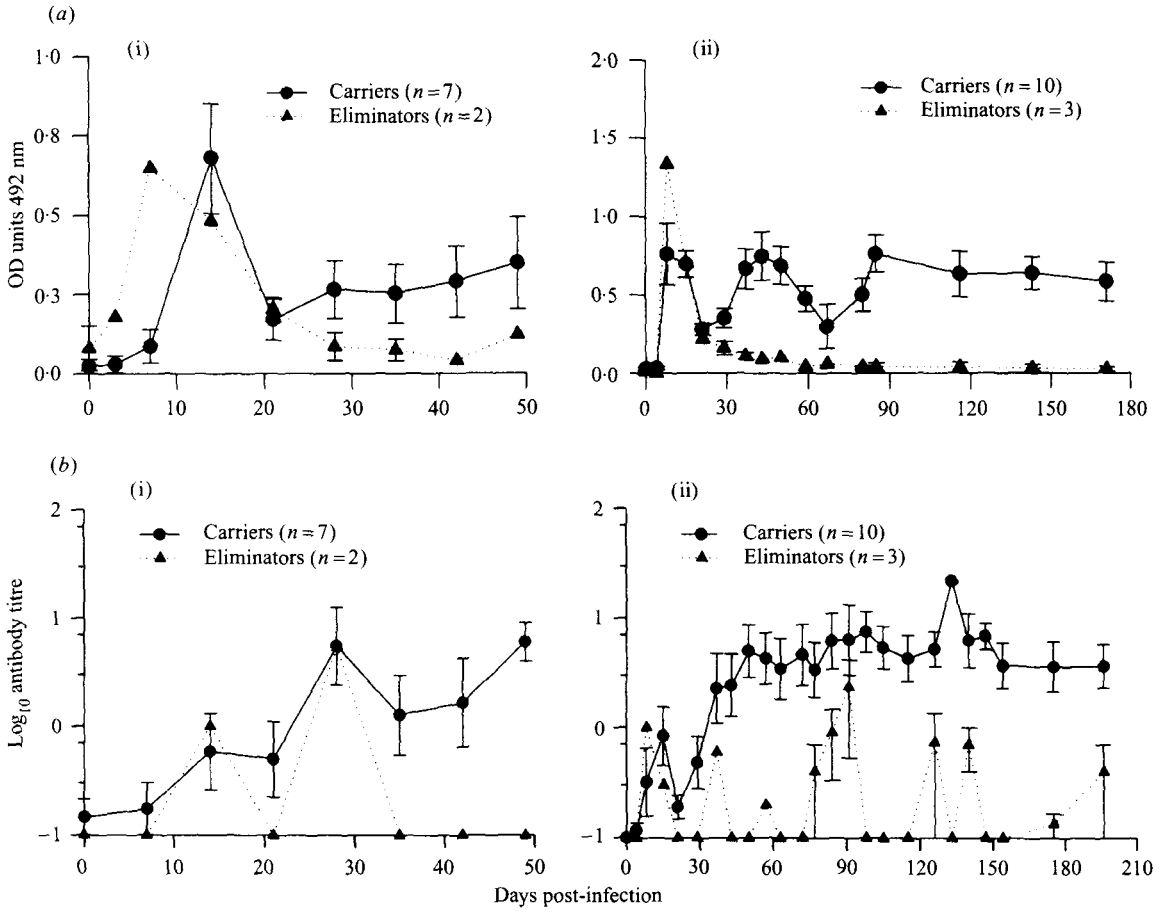

Fig. 4. The mean IgA responses against FMDV in (*a*) sera and (*b*) probang samples from (i) vaccinated and (ii) non-vaccinated cattle following challenge with FMDV type O_1. Data points represent the geometric mean ± S.E.

The results of our study show that prolonged serum IgM responses to FMDV do not occur in either vaccinated or non-vaccinated 'carrier' animals, with titres returning to baseline values 21 and 58 days after challenge, respectively. The detection of a persistent serum IgM response is the basis for the diagnosis of several chronic active viral infections in humans [42–44]. The absence of a persistent IgM response to FMDV in 'carrier' cattle suggests inadequate stimulation of the systemic immune system to maintain the response. This could be due to the low level of viral replication in 'carrier' cattle or the sequestration of the viral antigen to sites where IgM is not produced at sufficient titre to be detectable in either serum or secretions. Indeed, some 'carrier' cattle have been found previously to have serum antibody titres to FMDV which were below the titre considered positive for international trade purposes [45], or were sero-negative [46]. These observations suggest that the systemic humoral immune system is not necessarily stimulated in FMDV 'carrier' animals.

The long-term serum antibody responses of 'carrier' and 'eliminator' cattle differed in the development of a prolonged biphasic IgA response in the FMDV 'carrier' animals in both vaccinated and non-vaccinated groups. The biphasic serum IgA response was paralleled by a similar IgA response in probang samples. The similarity in the mean titres of long-term probang IgA responses in 'carrier' cattle in both groups suggests that once persistence is established viral replication is similar in both vaccinated and non-vaccinated cattle. Francis and colleagues [23] suggested that the second peak of a biphasic neutralizing antibody response in probang samples from FMD convalescent cattle was produced locally in the pharynx. Our findings indicate that this response is limited to convalescent 'carrier' animals. Mucosal antibody responses are generally of short duration following immunization. Therefore, the continued production of IgA in 'carrier' cattle presumably depends upon continued FMDV replication. In individual 'carrier' animals it was not possible to

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relate a decline in IgA to the apparent elimination of FMDV. This may in part be due to the continued presence of FMDV at undetectable levels.

There is an apparent paradox in the recovery of both FMDV type-specific IgA and infectious virus in probang samples from cattle with persistent FMDV infection. There are several possible explanations for this phenomenon, the most obvious of which is that the IgA is non-neutralizing. IgA has been shown to neutralize influenza virus *in vitro* [47] and passively protect mice against intranasal challenge [48], and prevent mucosal infection of cattle with bovine herpesvirus 1 [49] and coronavirus [50]. Furthermore, an anti-FMDV monoclonal antibody of IgA isotype has recently been shown to neutralize an Asia 1 strain of FMDV *in vitro* [51]. We have previously shown that pooled convalescent saliva and probang samples neutralized homologous FMDV infection of IB-RS-2 and BTY cells following depletion of IgG on a protein G column (data not shown). Several other studies have inferred that the neutralizing activity in mucosal secretions from FMD convalescent cattle is associated with IgA [15, 24, 25] and protection against reinfection with homologous FMDV has been shown to correlate with the presence of neutralizing IgA in probang samples [18]. Therefore, at least in *in vitro* assays, IgA has been shown to neutralize FMDV.

Our results show that all challenged cattle developed an early IgA response, and that cattle able to eliminate FMDV early in the course of infection failed to develop the second phase of the biphasic IgA response seen in 'carrier' cattle. This suggests, therefore, that the continued presence of FMDV is necessary to maintain the mucosal IgA response, and not vice-versa. Treatment of 'carrier' probang samples with organic solvent increases the titre of FMDV recovered once a local immune response had developed in the animal [52]. This was attributed to the disruption of FMDV immune complexes formed in the probang samples. However, the possibility that the organic solvent may have disrupted cells to release intracellular virus cannot be ignored. The detection of FMDV in probang samples from 'carrier' animals may, therefore, depend upon the presence of FMDV infected cells in the oropharyngeal fluid assayed *in vitro*, or the presence of infectious immune complexes. In another inapparent mucosal viral infection of cattle, the concurrent shedding of free virus and immune complexes during chronic infection with bovine enteric coronavirus has been described [53]. In fact, the receptor-mediated transcytosis of IgA immune complexes across mucosal epithelial cells has been proposed as a 'non-inflammatory' local defence function of IgA [54] thus preventing potentially more damaging immune interactions at mucosal surfaces [55]. Once voided into the lumen of the oropharynx immune complexes could be swallowed, or taken up by M cells overlying tonsillar lymphoid tissue to restimulate the local immune response, as has been reported for poliovirus, another picornavirus [56].

The failure to induce an effective or appropriate immune response is one means by which viruses avoid elimination. In this context it is noteworthy that a moderated tissue tropism of Epstein Barr virus has been attributed to IgA receptor-mediated uptake of infectious immune complexes [57]. It is possible to speculate upon the role of such a mechanism in FMDV persistence. Residual infectivity in FMDV immune complexes opsonised for Fc receptor-mediated uptake by porcine macrophages has been described [58]. This represents an alternative mode of cellular entry for FMDV not mediated by the cellular FMDV receptor route [59]. It can be envisaged that intracellular escape of virus from relatively low avidity immune complexes with IgA could occur, possibly in previously uninfected cell types.

Transmission of FMDV from 'carrier' to susceptible animals has been difficult to demonstrate under controlled conditions, despite circumstantial evidence for its occurrence in the field [5, cited in 60]. The presence of neutralizing antibody in the secretions bathing the prime site of virus persistence in the oropharynx may contribute to the low efficiency of transmission from FMDV 'carriers'. Our results suggest that factors or events which affect the mucosal production and/or secretion of IgA could also affect the infectivity of 'carrier' animals. The physiological changes associated with impending parturition and dexamethasone-simulated 'stress', that cause a redistribution of mucosal plasma cells [61] and secreted IgA [62] respectively, are examples of such factors.

Archetti and colleagues [25] have recently suggested that the detection of neutralizing antibody or FMDV-specific IgA in saliva and/or probang samples could be used to screen for cattle herds exposed to FMDV following ring-vaccination around an FMD outbreak. The findings reported here support the inclusion of testing for FMDV-specific IgA in serum in addition to mucosal fluid samples. Indeed, Madic and colleagues [63] have suggested that serum IgA was the most sensitive indicator of bovine herpesvirus 1 reinfection or reactivation. We found greater individual variation

in the IgA responses in serum than in probang samples in the 'carrier' cattle. Therefore, serological assays for IgA may be more useful for herd screening for 'carriers', whereas analysis of IgA in secretions may be required to identify individual 'carriers'. Assays of this kind have the additional potential for the differentiation of vaccinated from convalescent sero-positive cattle for international trade purposes or epidemiological studies.

ACKNOWLEDGEMENTS

The authors would like to thank the staff of the World Reference Laboratory for FMD, especially Linda Turner, Janet Oxtoby and Keith Marchant, for their technical assistance. In addition thanks are due to the animal attendants at IAH, Pirbright for their care and attention to the welfare of the animals over prolonged periods. This work was supported financially by the Ministry of Agriculture, Fisheries and Food.

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