Detection and quantification of IgM, IgA, IgG$_1$ and IgG$_2$ antibodies against foot-and-mouth disease virus from bovine sera using an enzyme-linked immunosorbent assay

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SUMMARY

A simple solid-phase enzyme immunoassay is described for the detection of antibody classes showing activity against foot-and-mouth disease (FMD) virus in bovine sera. The assay achieves a preliminary separation of the specific class of antibody from other serum proteins through immuno-adsorption to class-specific immunoglobulin-coated wells of micro-titre plates. The specific antibody is reacted with FMD virus, which is then detected by an enzyme-labelled anti virus IgG.

INTRODUCTION

Three general methods for identifying the class of a specific antibody are in current use. Firstly, sera may be fractionated by methods such as gel filtration, ion-exchange chromatography and density-gradient centrifugation and the antibody activity of the various IgG, IgM and IgA fractions can then be measured. Secondly, specific antibodies can be eluted from antigen affinity columns and the distribution of immunoglobulin classes among the antibodies may be measured. Thirdly, techniques such as radioimmunoelectrophoresis, modified Coombs' assay and double antibody assays involving radioactive anti-species antibodies have been described which measure bound antibodies *in situ*. Of the three methods, the measurement of antibodies *in situ* is most promising, since the first general method remains qualitative and the second is complicated by the difficulty of eluting tightly bound antibodies from their immunoadsorbents.

A preliminary report describing attempts to evaluate a direct enzyme-linked immunosorbent assay (ELISA) in the study of bovine antibodies of subclasses IgG$_1$ and IgG$_2$ was made by Sloan & Butler (1978). The assay examined the uptake of the immunoglobulins directly on to polystyrene tubes and their titration using anti-subclass antibodies conjugated with enzyme. This method was not entirely satisfactory since it was difficult to standardise. This paper examines a relatively simple technique for the detection of specific anti FMD virus subclasses, using a single enzyme-conjugated antiviral antibody.

MATERIALS AND METHODS

Solid phase

Flexible polyvinyl micro ELISA plates (U-bottomed wells) (Dynatech, England). Diluents, buffers, enzyme and its substrate were as described by Abu Elzein & Crowther (1978).

Virus

FMD virus type O_1/UK/1860/1967 was grown in monolayers of BHK 21 cells and purified by sucrose density-gradient centrifugation as described by Brown & Cartwright (1963), using 1 % SDS instead of deoxycholate. Purified virus was stored at -70 °C in siliconized glass vials.

Sera

Samples of bovine sera were obtained from the Vaccine Research Department at the Animal Virus Research Institute (AVRI), Pirbright. These were collected from cattle bled up to 21 days after infection with FMD virus type O_1/UK/1860/1967. Sera were also obtained from cattle bled at various times after vaccination and challenge with O_1/UK/1860/1967 FMD virus.

Guinea-pig antiserum

Purified virus was inactivated by means of acetylethyleneimine (AEI) at a final concentration of 0·05 % and incubation at 26 °C for 30 h. One volume of inactivated virus was emulsified with one volume of Freund's complete adjuvant (Difco Laboratories, Detroit, U.S.A.). Live guinea-pigs each received 50 μg of virus intramuscularly at two sites. Animals were exsanguinated after 28 days and the sera pooled.

The IgG fraction of the serum was prepared as described previously (Abu Elzein & Crowther, 1978). Samples were stored at -20 °C in siliconized glass vials.

Commercial antisera

Rabbit antisera specific for the heavy-chain determinants of bovine IgG_1, IgG_2, IgM and IgA were obtained from Miles Laboratories (Slough, U.K.). The IgG fraction of each serum was prepared and stored as described above.

Conjugation of enzyme to IgG

Alkaline phosphatase was conjugated to the IgG fraction of the guinea-pig O_1/UK/1860/1967 FMD virus antiserum, according to the method of Avrameas (1969) except that glutaraldehyde was used at a final concentration of 0·05 % and the conjugation time was 4 h at room temperature. The working dilution of the conjugate was determined as described by Voller et al. (1976). The conjugate was stored at 4 °C after the addition of 5 % ovalbumin and 0·02 % sodium azide (final concentration).

Enzyme-linked immunosorbent assay (ELISA)

The various bovine sera were examined for specific antibodies against O_1/UK/ 1860/1967 virus using the ELISA. The specific IgGs from the rabbit antibovine antisera were diluted in carbonate buffer to a final concentration of 10 μg/ml. These were then added to the micro-ELISA plates (100 μl/well) and incubated for 3 h at 37 °C. Plates were then emptied and washed by filling (3 times for 3 min) with PBS containing a final concentration of 0·05% (w/v) Tween 20 (PBTS) Bovine sera under test were diluted in PBST containing 1% ovalbumin (final concentration) and added to the wells (100 μl/well). The dilutions of sera are described in the results. Plates were incubated for 2 h at 37 °C, then washed as described above. Purified FMD virus diluted in PBST to 2 μg/ml was added to each well (100 μl) and plates were incubated for 2 h at 37 °C. After washing, 100 μl of enzyme conjugated with anti FMD virus guinea-pig IgG was added to each well and the plates were incubated at 4 °C overnight. Plates were then washed and 200 μl of substrate solution was added to each well. After 30 min at room temperature the colour reaction was stopped by the addition of 50 μl of 3 M NaOH solution. The colour was read at 405 nm, using a Unicam spectrophotometer. For all tests, triplicate samples of each serum dilution described were examined.

2-mercaptoethanol treatment of sera

Samples of antisera obtained from infected or vaccinated bovines were added to an equal volume of a solution of 0·2 M 2-mercaptoethanol (2-ME) in PBS and incubated at 37 °C for 1 h. After dialysis of the treated sera against several changes of PBS, the samples were tested for antibody against FMD virus in the ELISA.

Adsorption of antibody

Immunoglobulins were removed from samples as described by Garland (1974), essentially by the addition of two volumes of polyvalent or class-specific anti-globulin IgG, followed by incubation at 4 °C for 16 h. Precipitates were removed by centrifugation at 50000 × g for 30 min. The absorbed samples were tested for various antibody activities in parallel with non-absorbent samples, to investigate the cross reactivity of the commercial anti bovine sub-class antibodies.

Serum neutralization tests

The neutralization antibody titres of the bovine sera obtained after infection were determined in microtitre plates, using the method described by Golding, Hedger & Talbot (1976).

RESULTS

The results in Figs 1 and 2 show the development of specific antiviral immuno-globulins in individual cattle sera after infection or vaccination and challenge. The level of neutralizing antibody from the whole serum of each animal is also shown. The activity of each anti-immunoglobulin class absorbed on to the microplates
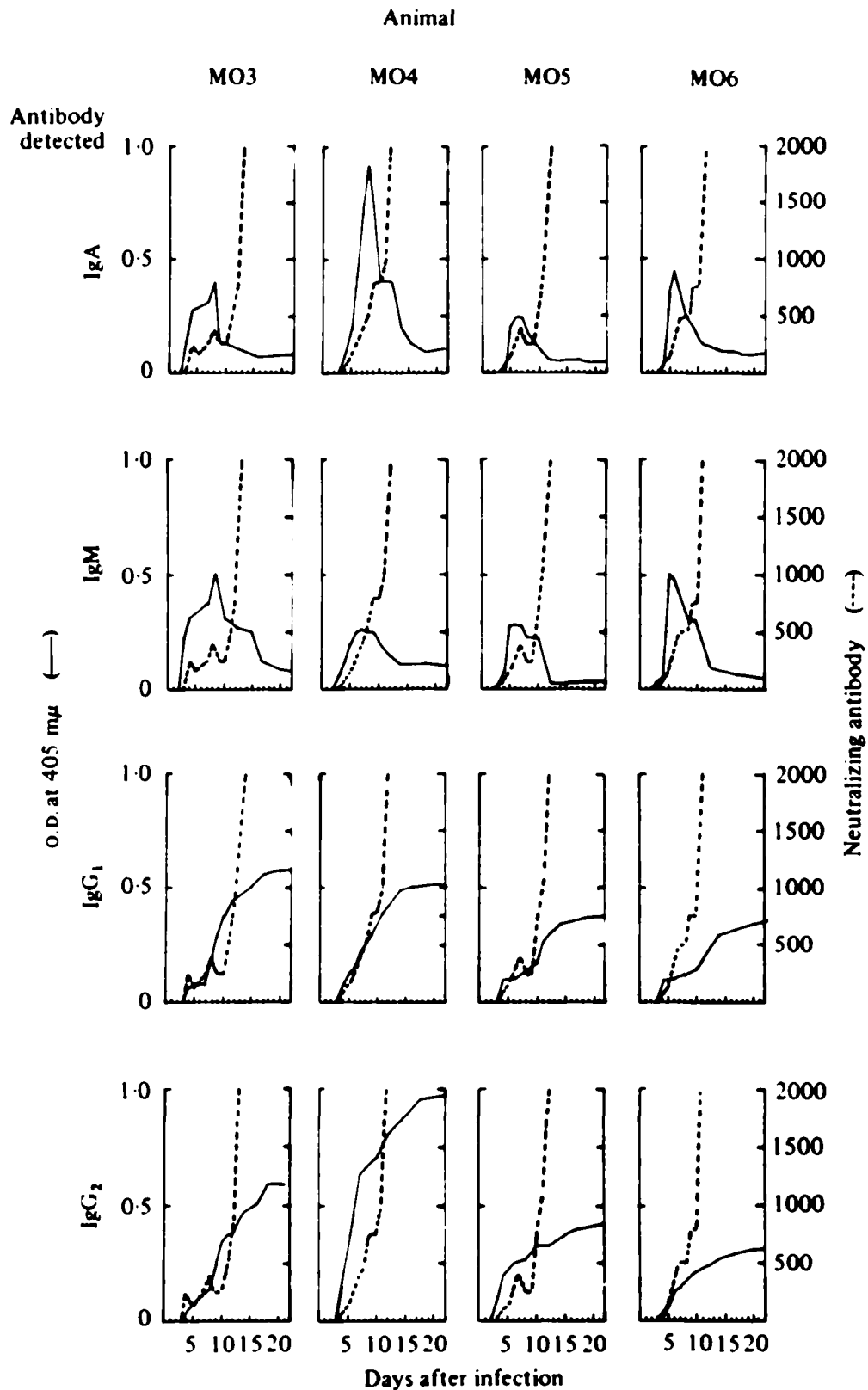
Animal

MO3   MO4   MO5   MO6

Fig. 1. Measurement of specific antibodies by ELISA (———), and neutralizing antibodies (- - -), in cattle after infection with type O_1/BFS/1860/FMD virus.

was not defined. Thus, direct comparison of the levels of immunoglobulin could only be made within and not between classes. The commercial antisera proved monospecific, as was confirmed in the cross-absorption tests. Tests on sera for specific IgM or IgA activity before or after treatment with 2-mercaptoethanol showed that treated sera did not react, although IgG_1 and IgG_2 could be measured.

Fig. 1 shows that specific antiviral IgM and IgA were detected between 3 and 4 days after infection. Both levels peaked at about 8 days and decreased to a constant amount by day 15. IgG_1 and IgG_2 antibodies were detected at 4–5 days and
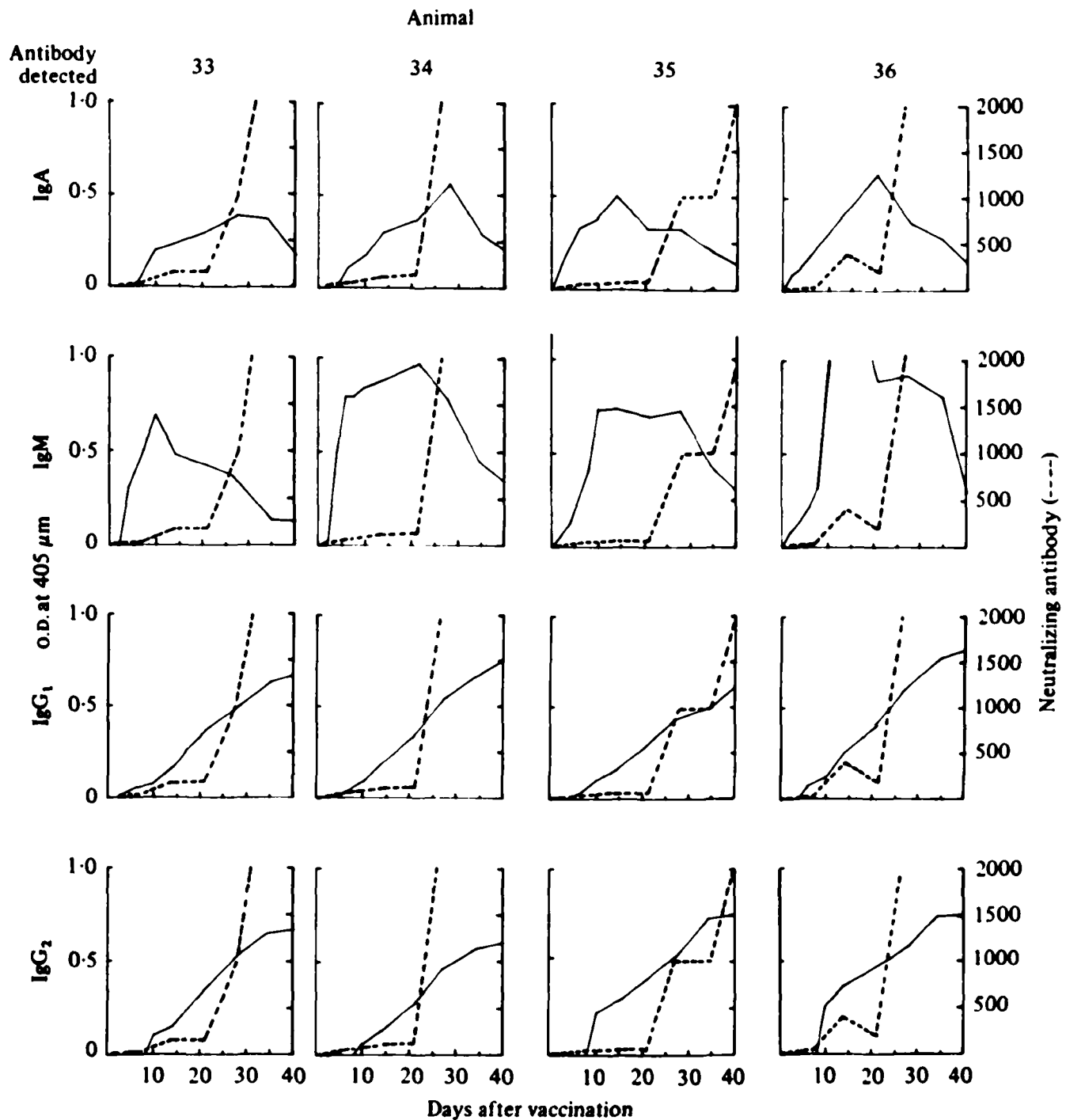
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Fig. 2. Measurement of specific antibodies by ELISA (———), and neutralizing antibodies (– – –), in cattle after vacination and challenge with type O_1/BFS/1860/FMD virus.

were produced at a constant rate until a plateau was reached at around 17 days. Neutralizing activity was detected at 4 days and showed biphasic development with a slow initial rise until 10 days, when a sharp increase in titre was observed which formed a plateau at about 18 days (not shown).

Fig. 2 shows that IgM and IgA were detectable between 2 and 4 days after vaccination. The levels, particularly of IgM, were higher than in infected animals and no clear peak was observed, there being a wide spread of activity with a gradual decline around day 20 even after virus challenge.

IgG_1 antibody was detected from day 4 and its level increased throughout sampling. IgG_2 was detected only after day 9 in all four cattle sera tested; its level increased sharply and reached a plateau at about 35 days. Neutralizing activity

was detected from day 4 and levels remained low until day 28, 7 days after virus challenge, when a large increase (5–10 fold) in titre over pre-challenge serum was measured. All the cattle at day 21 were protected with the relatively low levels of neutralizing antibody detected.

DISCUSSION

This work shows a useful adaptation of a solid phase ELISA for the rapid detection and quantification of immunoglobulin classes and subclasses and should be applicable to any antigen/antibody system.

Previous work presented by Brown, Cartwright & Newman (1964) showed that the neutralizing activity during the first 14 days after infection of bovines with FMD virus was linked mainly to the IgM class of antibody, a gradual changeover to IgG class being made between 14 and 21 days. Garland (1974) used column chromatography techniques to examine IgA, IgM, IgG_1 and IgG_2 antibodies after infection or vaccination with FMD.

The ELISA results confirm the differences between virus-specific IgM and IgA production in infected and vaccinated bovines. Generally, however, the antibody responses were detected at a similar time in this study except for IgG_2, which was delayed in vaccinated animals. The earlier detection is probably due to the increased sensitivity of the ELISA over previously reported methods in which IgG is first detected 7–8 days after infection. The earlier detection of specific IgG by ELISA also complicates the neutralizing antibody results since the early neutralizing activity cannot be wholly ascribed to that of IgM or IgA antibodies.

The commercially available anti heavy-chain immunoglobulin sera were used successfully in that they did not cross-react, as confirmed by the adsorption studies. Since only small quantities of anti-immunoglobulin sera are required, the use of commercial antiserum is both convenient and economical. Use of the double-sandwich ELISA in this way adds a versatile technique to the tests available for the specific detection of immunoglobulin subclasses and has a distinct advantage over indirect methods, which require enzyme conjugates of each specific anti-immunoglobulin.

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