A comparative serological study for the quantification of antibodies against foot-and-mouth disease virus in water buffalo sera

Abstract

Two methods of enzyme-linked immunosorbent assay – Sandwich ELISA (SW-ELISA) and Liquid-phase blocking sandwich ELISA (LPB-ELISA) – and the conventional serum neutralization test (SNT) were used for the quantification of antibodies against the vaccinal strains O, Campos, A24, Cruzeiro and C3 Indaiatuba of the foot-and-mouth disease virus (FMDV), in 143 samples of water buffaloes sera. The correlations among these tests were investigated. The overall correlation coefficients (r) between SW-ELISA and SNT were 0.8447, 0.8547 and 0.8905; between LPB-ELISA and SNT, r=0.9053, 0.8251 and 0.9019, respectively for the strains O, A24 and C3. These results demonstrate that SNT can be replaced, with advantages, by any of the two studied ELISA methods in the quantification of antibodies against FMDV in water buffaloes sera. The overall correlation coefficients between the two ELISA were highly significant, that is, r=0.9165, 0.8635 and 0.9419 for O, A24 and C3, respectively; although the LPB-ELISA has obtained a better performance than the SW-ELISA in relation to the strains O and C3, the situation was reversed in relation to A24. These assays are very sensitive, less variable and are developed more quickly, besides not being dependent upon living systems (cells or suckling mice), as the SNT or protection serum test.

Introduction

Foot-and-mouth disease (FMD) is a highly important infectious disease of cloven-hoofed animals (cattle, swine, sheep, goat and buffaloes) because of the economic losses it causes and the restrictions imposed on international livestock trade. FMD virus isolation and antibody studies have shown that the Water buffalo (Bubalus bubalis) (12,19,27,28), as the African buffalo (Syncerus caffer) (17,18,25,33) can

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maintain the infection in an inapparent form for long periods of time without presenting signs of the disease. Programs for the control of FMD are based on the vaccination and monitoring of post vaccination protection, which is usually done by serum neutralization test (SNT) \((8,9,13,20,30)\) or more recently by immunoassay (ELISA) \((1,2,14,15,20,22,23,29,30)\) due to high sensitivity, specificity and the rapidity with which it can be performed. In fact, a precise and rapid method for the detection and quantification of serum antibody levels in large number of animals is necessary to critically evaluate vaccination and management practices of any program of FMD control. Testing representative samples of a livestock at a regular intervals, could reveal problems associated with a faulty vaccination program as evidenced by a failure of the buffaloes to respond with antibodies and provides a means to make key disease management decisions.

In the present study, two methods of enzyme-linked immunosorbent assay - Sandwich ELISA (SW-ELISA) and Liquid-phase sandwich blocking ELISA (LPB-ELISA) - and serum neutralization test (SNT) were used for the quantification of antibodies against foot-and-mouth disease virus (FMDV) in water buffaloes sera vaccinated or naturally infected. These tests were compared to their sensitivity, reproducibility and facility to be performed.

**Materials and Methods**

**Virus** - FMDV \(O_1\) Campos, \(A_{24}\) Cruzeiro and \(C_3\) Indaial were grown in BHK-21 cells and the supernatant inactivated with binary ethylenimine \((4,10)\). The 146S antigen was purified from these infected cell supernatants by continuous sucrose gradients ultracentrifugation \((3,6)\). Purified virus concentration was determined \((5)\) and stored at \(-70^\circ C\) at siliconised glass vials.

**Field buffalo sera** - A total of 143 serum samples collected during the years of 1993 to 1994 at various premises from São Paulo State - Brazil was used to assess antibody responses in the SW-ELISA, LPB-ELISA and SNT tests. The majority of serum come from animals systematically immunized with commercially produced trivalent \((O_1\) Campos, \(A_{24}\) Cruzeiro and \(C_3\) Indaial inactivated with AEI) FMD vaccine; some whose vaccination status was not known (the nature, frequency, and exact timing of any vaccination) and sera also were obtained from non-vaccinated animals. A pool of 10 sera from buffaloes systematically vaccinated and selected on the basis of high SNT titres was used as positive reference serum. The negative reference serum consisted of a pool of 10 sera from buffaloes that had never been vaccinated, in which the disease had never been reported, and which had no detectable SNT antibody to the 3 FMDV serotypes, as also demonstrated by probang \((17,18,25)\) and by Double Immunodiffusion (DID) test to VIA antigen \((24, 25)\). All sera were previously inactivated at \(56^\circ C\) for 30 min and also treated with trichloroacetic acid at 0.5% to eliminate nonspecific reactions, before being tested, a condition which produced a better distinction between positive and negative sera in this test \((7)\).

**Rabbit capture anti-FMDV antisera** - Type-specific antiserum to each viral strain was prepared in rabbits by 2 subcutaneously inoculations of 50mg of purified 146S FMDV emulsified in
Freund’s complete adjuvant. The second inoculation was given after 4 weeks and followed by bleeding 10 days later (16). The serum was divided into aliquots, inactivated at 56°C for 30 min and stored frozen at -20°C.

**Anti-buffalo IgG peroxidase conjugate** – Consisted of rabbit IgG anti buffalo IgG, purified by gel filtration in sephadex G200 conjugate to horse-radish peroxidase (32). The conjugate was titred against purified buffalo IgG by an direct ELISA method and the working dilution was determined (1).

**Enzyme substrate** – A mixture of 0.006% H$_2$O$_2$ and 0.4mg/ml of o-phenylenediamine (OPD) of 0.1M Na$_2$HPO$_4$ and 0.1M citric acid buffer, pH 5.0, was used as substrate and chromogen. The substrate reaction was stopped by adding 50ml of 2M HCl solution.

**Sandwich-ELISA (SW-ELISA)** – The SW-ELISA test was performed as described (23), with the reagent dilutions previously determined. The activity of each serum was determined through the calculation of correction factor of OD from the sample in relation to the positive and negative controls (26), according to the formula:

$$P/S = \frac{Xa - Xn}{Xp - Xn}$$

where P/S (Positive Sample) = correction factor of OD from the sample in relation to the positive and negative controls.

**Blocking-ELISA (LPB-ELISA)** – The test was carried out as described elsewhere (2).

**Serum neutralization test (SNT)** – The SNT procedure were assayed in flat-bottomed microplate wells on IB-RS-2 clone17 cells line (9). The virus strains used were homologous to the three vaccine strains. Antibody titres were expressed as the logarithm of the reciprocal of the final dilution of serum in the virus/serum mixture that neutralized an estimated 100TCID$_{50}$ at the 50% endpoint (2). For purposes of this study, SNT titres £1,2 were considered negative results, and titers >1,2 were considered positive results. Each serum test was run in duplicate.

**Reproducibility of ELISA** – The reproducibility of results obtained for the SW-ELISA and LPB-ELISA were determined using the strain O$_1$ Campos against 3 sera from the groups (one negative and two positive sera) and titrating them 45 times on different occasions.

**Comparison of the anti-FMDV serum antibodies titres obtained by the SW-ELISA, LPB-ELISA and SNT** – Antibodies titres were compared by the usual measurement of precision of the relation of the variables X (SNT) and Y (SW-ELISA), X (SNT) and Y (LPB-ELISA) and X (SW-ELISA) and Y (LPB-ELISA), and by linear regression determined between the 2 variables. Student’s t-test was used to evaluate the correlation coefficients and the agreement among these 3 tests. The cutoff in ELISA was determined by chart analysis of the copositivity and conegativity among the ELISA methods and SNT (21). The cutoff recorded was the point plotted on the abscissa from the cross of the curves of the copositivity and conegativity.

**RESULTS**

The correlation coefficients (r) between the titres of antibody anti foot-
and-mouth disease viruses \([O_1 \text{ Campos } (a), A_{24} \text{ Cruzeiro } (b) \text{ and } C_3 \text{ Indaial } (c)]\)

obtained from the SW-ELISA and SNT for a total of 143 samples were 0.8447, 0.8547 and 0.8905; from the LPB-ELISA and SNT, 0.9053, 0.8252 and 0.9019, respectively for the strains \(O_1\), \(A_{24}\) and \(C_3\) \((p<0.0005)\) are shown in Fig.1 and 2. The coposity and conegativity results were determined, with the cutoff recorded for SW-ELISA as 0.083, 0.160 and 0.115; for LPB-ELISA, 0.93, 1.39 and 0.99, respectively for the strains \(O_1\), \(A_{24}\) and \(C_3\) as showed in Fig. 3 and 4. Using the determination of the cutoff,

it was possible to calculate the agreement between SW-ELISA and SNT, which was 89.5%, 91% and 92.3%; between LPB-ELISA and SNT, these values were 95%, 84% and 95%, respectively for \(O_1\), \(A_{24}\) and \(C_3\). The correlation coefficients between SW-ELISA and LPB-ELISA were highly significant 0.9165 for \(O_1\) Campos, 0.8635 for \(A_{24}\) Cruzeiro and 0.9419 for \(C_3\) Indaial \((p<0.0005)\) as showed in Fig. 5. The agreement between SW-ELISA and LPB-ELISA was 99% for the three tested strains.

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**Fig. 1-** Regression and correlation coefficients between anti foot-and-mouth disease virus \([O_1 \text{ Campos}[a], A_{24} \text{ Cruzeiro}[b], \text{ and } C_3 \text{ Indaial}[c])\) antibody titre obtained from the Sandwich ELISA (SW-ELISA) and serum neutralisation test (SNT).

**Fig. 2-** Regression and correlation coefficients between anti foot-and-mouth disease virus \([O_1 \text{ Campos}[a], A_{24} \text{ Cruzeiro}[b], \text{ and } C_3 \text{ Indaial}[c])\) antibody titre obtained from the Liquid-phase blocking (LPB-ELISA) and serum neutralisation test (SNT).
Fig. 3- Graphic representation of the copositivity and conegativity between Sandwich-ELISA and SNT. Cutoff values in the SW-ELISA (anti foot-and-mouth disease virus O1 Campos[a], A24 Cruziero[b], and C3 Indaiat[c] antibody titres expressed as P/S).

Fig. 4- Graphic representation of the copositivity and conegativity between LPB-ELISA and SNT. Cutoff values in LPB-ELISA (anti foot-and-mouth disease virus O1 Campos[a], A24 Cruziero[b], and C3 Indaiat[c] antibody titres expressed as logarithm).
Fig. 5- Regression and correlation coefficients between anti foot-and-mouth disease virus (O, Campos [a], A, Cruzeiro [b] and C, Indaiatuba [c]) antibody titres obtained from the Liquid-phase blocking ELISA (LPB-ELISA) and Sandwich-ELISA (SW-ELISA).

DISCUSSION

The implementation of effective foot-and-mouth disease (FMD) control programs requires, in addition to rigorous zoo-sanitary measures and an appropriate vaccination schedule, specific and reproducible tests for the monitoring of humoral immune response to FMDV in vaccinated and / or non-vaccinated animals. Extensive water buffalo breeding in endemic or former endemic areas makes general FMD vaccination and its control very difficult, and too many buffalo could remain unprotected. It has been shown that during FMD outbreaks the clinical disease is clear in cattle and pigs but signs of disease can range from a mild or inapparent infection in Water buffalo (12,19,27,28) and in African buffalo (17,18,25,33), as well as in sheep and goats (11).

This study was prompted by a need for a simplified and cheaper test to assess the immune response of water buffalo to FMDV vaccine. Although serum neutralization test (SNT) has been used successfully for many years for the quantification of antibodies against FMDV in several animals species, recently, several studies have shown in cattle, sheep and pigs that enzyme-
immunoassays may replace the SNT for this purpose (14, 15, 20, 23, 30, 31). However, there are only few reports about the application of ELISA for the detection or measuring buffalo anti FMDV antibodies. Thus, two methods of enzyme-linked immunoassay - Sandwich ELISA (SW-ELISA) and Liquid-phase sandwich blocking ELISA (LPB-ELISA) were applied for the quantification of antibodies against the strains O₁ Campos, A₂₄ Cruzeiro and C₃ Indaiatuba of the FMDV, in 143 samples of buffaloes sera.

The correlation coefficients (r) between the titres of antibodies to FMDV strains O₁ Campos, A₂₄ Cruzeiro and C₃ Indaiatuba, obtained respectively from the SW-ELISA and SNT and LPB-ELISA and SNT (Fig. 1, 2 and 5), which included samples from vaccinated and non-vaccinated buffaloes, were higher and similar to those previously published (23, 29, 30). Our comparative study of the two ELISA procedure and the SNT is based on individual titres from each test analyzed in a pair-wise fashion and using least-square linear regression. Both the assays appear to be of similar sensitivity to the SNT, though the LPB-ELISA reached a slightly better performance than that of the SW-ELISA in relation to the titration of antibodies against the strains O₁ and C₃, the situation was reversed in relation to A₂₄ (Fig. 1 and 2). However, none ELISA used here was clearly superior and both were able to titre anti-FMDV antibodies from buffaloes, differing from the results recorded previously (23), which showed a marked advantage of the LPB-ELISA, when sera collected from FMD vaccinated cattle were analysed. In view of this, the SW-ELISA could be suggested as a useful alternative to monitoring in large scale antibodies against FMDV in buffaloes sera, because it can be run with a single serum dilution, being more rapid and economic than the LPB-ELISA.

Because water buffalo have also been shown to become carriers after exposure to infection with FMDV, the use of protection against challenge exposure to evaluate the cut-off values is practically impossible. In this case, an alternative procedure based on drawing co-positivity and co-negativity curves (Fig. 3 and 4) allowed determination of high levels of agreement between the LPB-ELISA, SW-ELISA and SNT antibody titres against FMDV (Fig. 5), as showed previously with LPB-ELISA (2).

The results emphasize also the usefulness of the SW-ELISA for the detection and quantification of antibodies from water buffalo sera to FMDV, as reported formerly in cattle (14, 15, 20, 23, 30, 31) and once for buffalo (2) sera.

References


22. McCULLOUGH, K.C., PARKINSON, D. (1984). The standardization of "spot-test" ELISA for the rapid screening of sera and hybridoma cell products II. The determination of binding capacity, binding ratio and


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