

# SEROLOGICAL CHARACTERISATION OF FOOT-AND-MOUTH DISEASE TYPE "O" FIELD ISOLATES FROM PERU: 1992-1994

A.M. ESPINOZA  
Foot-and-Mouth Disease Laboratory,  
National Institute of Health,  
Lima, Peru



XA9848659

## Abstract

SEROLOGICAL CHARACTERISATION OF FOOT-AND-MOUTH DISEASE TYPE "O" FIELD ISOLATES FROM PERU: 1992-1994.

Nineteen field isolates of foot-and-mouth disease Virus (FMDV) recovered from bovine epithelial samples corresponding to outbreaks present in different regions of Peru, between 1992-1994 were studied. The relationship of the virus isolates to the O/Urubamba vaccine strain of Peru was determined by the calculation of the "r" values obtained by the liquid-phase blocking ELISA. All the isolates showed "r" values higher than 0.66 indicating that the vaccine strain should protect against the field strains. Characterization of the field isolates by a trapping ELISA using a panel of monoclonal antibodies against FMDV O/Switzerland and O/Caseros, showed slight differences in the profiles of the field isolates when compared with the O/Urubamba vaccine strain, but no differences were found among all the isolates.

## 1. INTRODUCTION

Foot-and-mouth disease is a very important disease of cattle, sheep, goats and pigs in Peru. The first laboratory diagnosis of the disease was reported in 1951. In Peru the FMDV types O, A, and C have been isolated. In addition the presence of Vesicular Stomatitis Virus (VSV) serotypes New Jersey and Indiana has been reported. In the last ten years no isolation of the FMDV type C nor VSV serotype Indiana has been reported. The use of improved techniques for the rapid diagnosis and characterization of isolates such as the enzyme linked immunosorbent assay (ELISA) have been extensively described [1 - 5].

The establishment of ELISA as a routine method for the diagnosis and serotyping of FMD and VS, as well as for the detection and titration of antibodies in order to assess the immune status of the cattle population has been an important development. ELISA has also been used for the epidemiological monitoring of new field strains and measuring the protection given by the vaccine strains [6].

During the years 1992 to 1994, several outbreaks of FMDV type "O" were reported from all regions of the country [7,8]. The vaccine being used for the National Control Programme contains the strains O/1, A/24 and C/3 formulated with oil adjuvant.

The objective of this study was the characterization of the new isolates of FMDV present in Peru and in particular their relationship to the reference vaccine strain used in the FMD National Control Programme.

## 2. MATERIAL AND METHODS

### 2.1. Viruses and cell cultures

Viruses originally isolated from bovine epithelial samples from different regions of Peru, are listed in Table I. The antigens were passaged once in primary bovine thyroid cells (BTY) and used for the serological characterization. The vaccine strain O/Urubamba, grown in BHK/21 cells was obtained from the FMD Vaccine Production Laboratory of the National Institute of Health.

### 2.2. Polyclonal antisera

Rabbit and guinea pig anti-FMDV polyclonal antisera were provided by the World Reference Laboratory (WRL) Pirbright, U.K. and used as described by Ferris [9].

Bovine post-vaccinal sera from cattle vaccinated during the regular FMD vaccination program in Peru were used as reference sera to calculate the "r" values.

Rabbit anti-mouse and rabbit anti-guinea pig immunoglobulins conjugated to horseradish peroxidase were obtained from a commercial source.

### 2.3. Monoclonal Antibodies

A panel of eight neutralizing Mabs produced against the FMDV strains O/1/Switzerland/65 (B2, C6, C8, C9 and D9) and O/1/Caseros (OC1, OC2, and OC3) were provided by Dr J. Crowther, WRL, Pirbright, U.K..

### 2.4. ELISA method

Each isolate and the vaccine strain were titrated by the indirect sandwich ELISA described by Hamblin et al [1]. A fixed concentration of the field virus equivalent to a concentration giving an optical density (OD) of 1.5 was reacted with bovine post-vaccinal sera previously titrated against the O/Urubamba vaccine strain using the liquid-phase blocking ELISA (LPBE). The ELISA employed to obtain the "r" values described by Hamblin et. al. [2]. The "r" value was calculated using the titer of the reference sera against the field virus/the titer of the reference sera against the homologous vaccine strain.

The sandwich ELISA was used to screen the reactivity of Mabs against the field strains captured using rabbit antisera coated plates. The antigens were previously titrated to give an OD values of 1.2. The various Mabs were added as duplicate samples at a concentration that was found to give the plateau maximum absorbance when titrated against O/BFS virus in the sandwich ELISA. The reactions of the Mabs were standardized with reference to the concentrations of the captured viruses; these were determined using a polyclonal guinea pig antibody system.

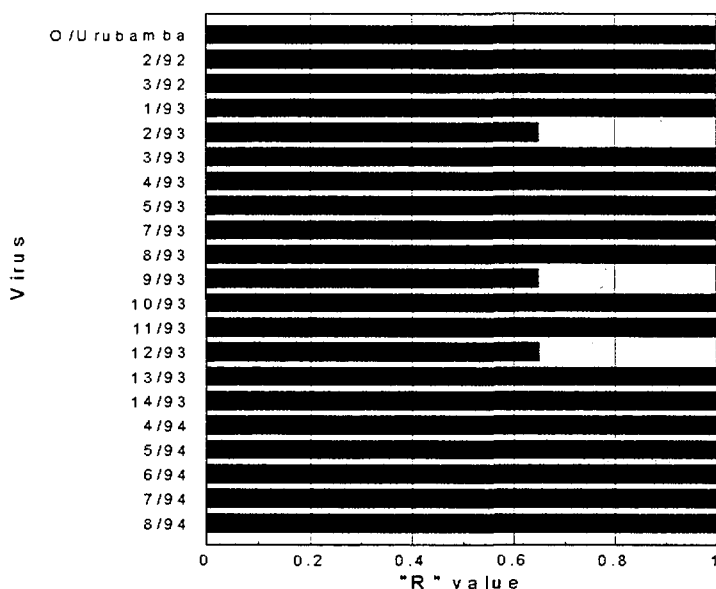


FIG. 1. "R" values of FMDV type "O" field isolates (Peru: 1992-1994).

### 3. RESULTS

The titers of the field strains and reference vaccine strain giving an OD of 1.5 are shown in Table 1. The reference sera were obtained from a pool of sera giving an end point titration of >128. The selected antigen dilutions were reacted with the reference sera and the titer obtained against each field strain is shown in Table I. The "r" values obtained are presented in Figure 1 and indicate that all the field isolates showed a close relationship to the reference vaccine strain giving values of 0.66-1.00.

The reactivity of the Mabs with the field strains and reference strain was carried out as described by Samuel et. al. [12], and the profiles were obtained using the software developed at the WRL (Figures 2, 3, 4, 5). These results confirmed the conclusions derived from the "r" values.

The profiles showed that the binding of the C6 Mab was greatly reduced in the field isolates when compared with the O/Urubamba vaccine strain, but all the isolates showed similar profiles.

TABLE I. FMDC TYPE "O" FIELD ISOLATES, PERU: 1992-1994

No/Year	Location	Antigen titer	Serum titer
2/92	Lima	1/6	384
3/92	Lambayeque	1/10	512
1/93	Piura	1/8	384
2/93	Huancavelica	1/8	256
3/93	Ayacucho	1/6	384
4/93	Pasco	1/8	384
5/93	Puno	1/6	384
7/93	Arequipa	1/6	384
8/93	Apurimac	1/6	384
9/93	Cusco	1/8	256
10/93	Puno	1/2	384
11/93	Lima	1/6	512
12/93	Tacna	1/8	256
13/93	Cusco	1/8	512
14/93	Cusco	1/4	512
4/94	Huancavelica	1/6	512
6/94	Arequipa	1/6	512
7/94	Arequipa	1/6	512
8/94	Ancash	1/8	384
O/Urubamba		1/6	384

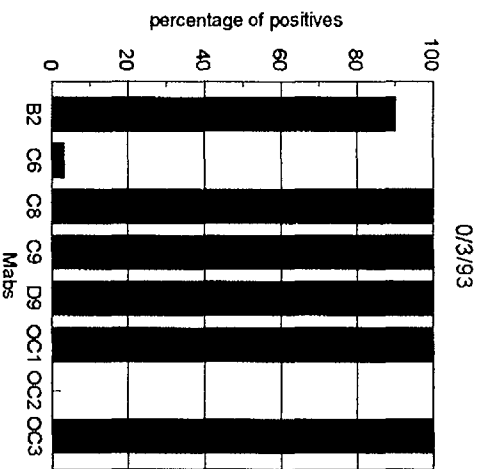
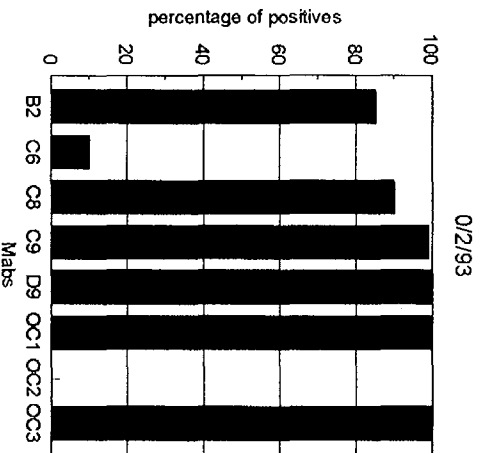
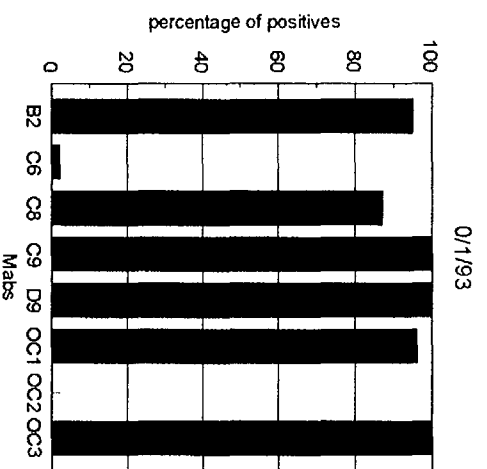
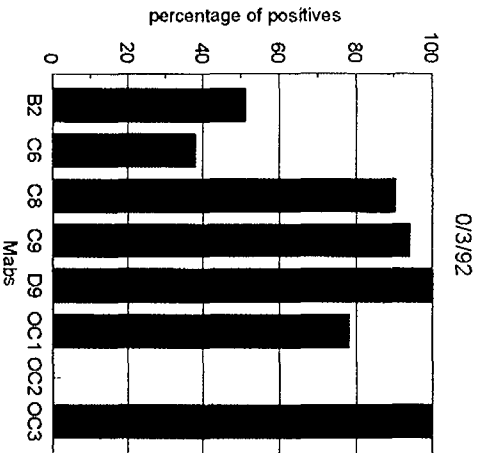
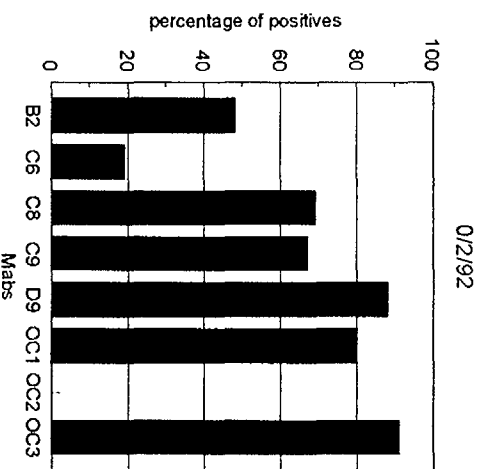
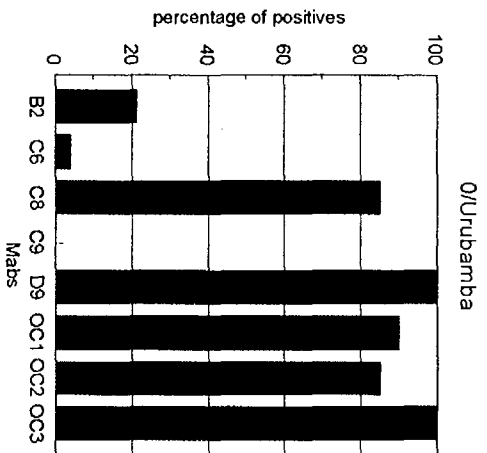


FIG. 2. Profile of field isolates of FMDV type "O" by a panel of O/BFS monoclonal Ab.

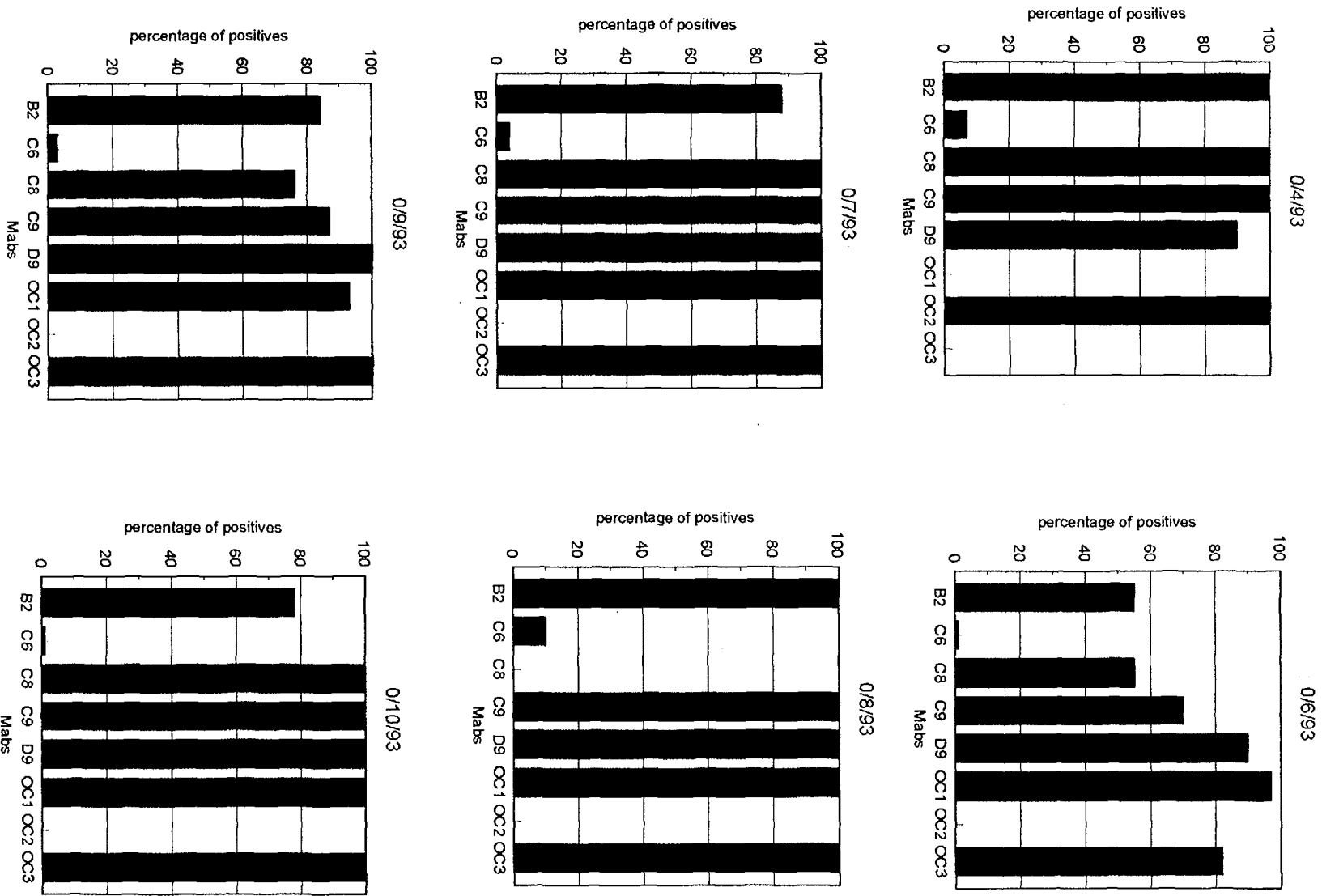


FIG. 3. Profile of field isolates of FMDV type "O" by a panel of O/BFS monoclonal Ab.

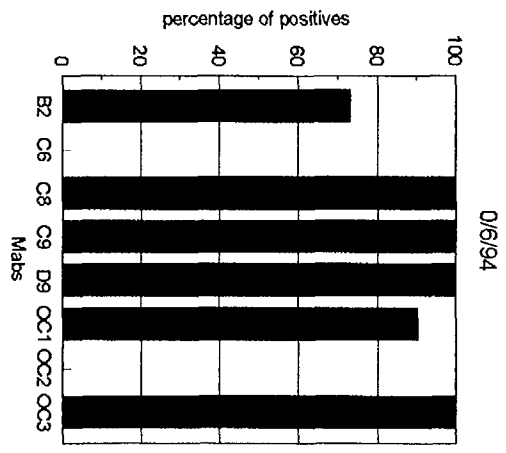
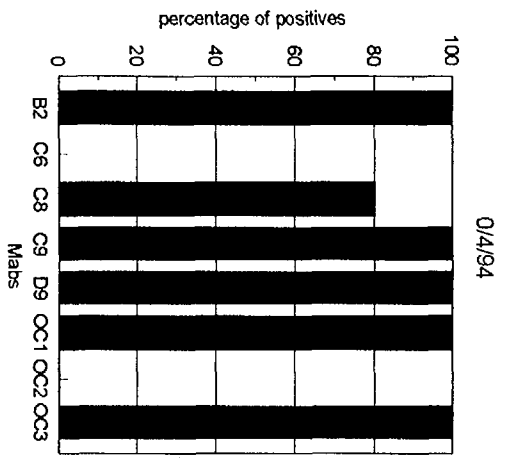
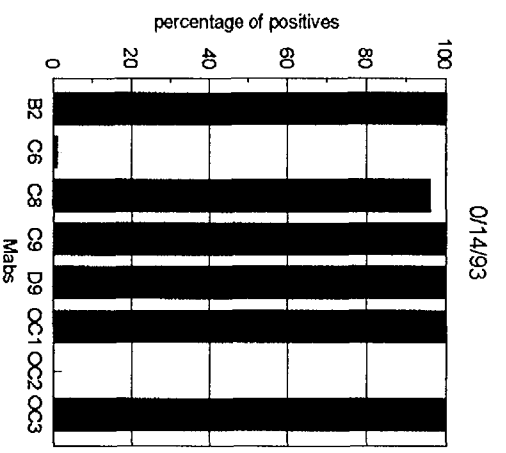
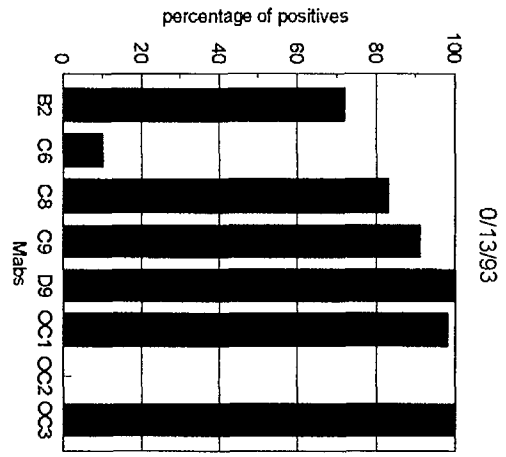
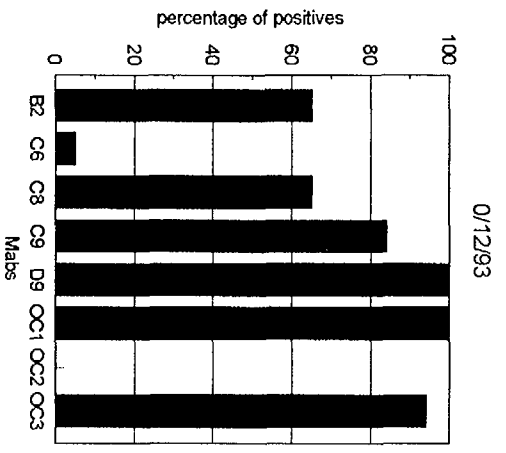
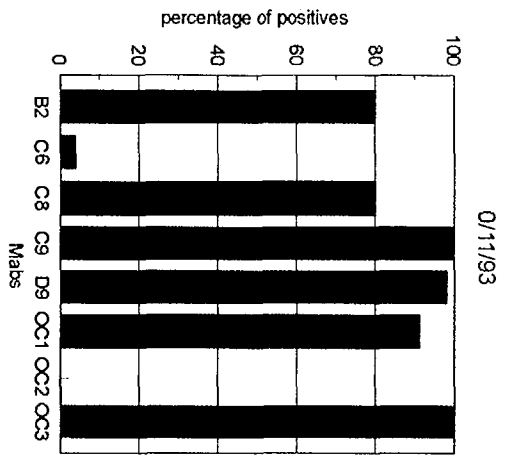


FIG. 4. Profile of field isolates of FMDV type "O" by a panel of O/BFS monoclonal Ab.

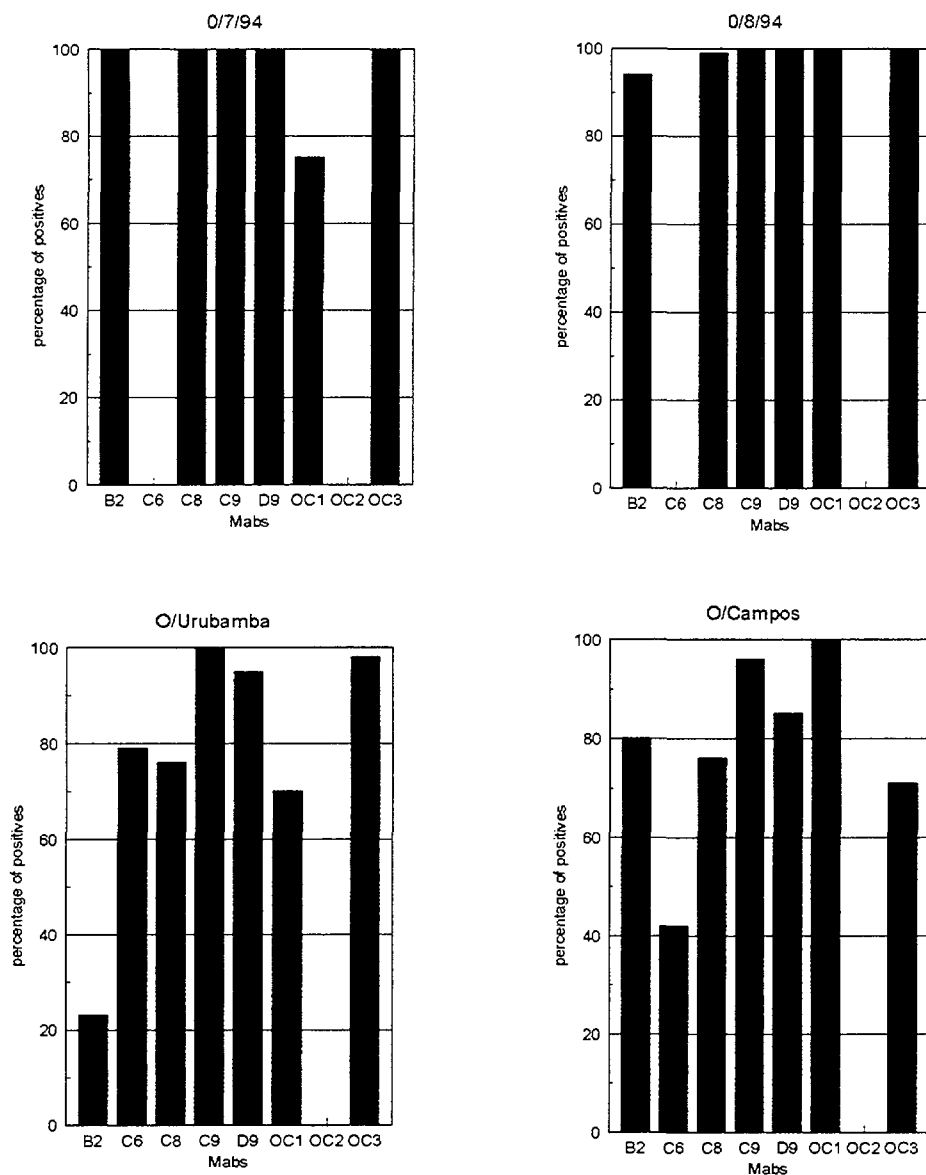


FIG. 5. Profile of field isolates of FMDV type "O" by a panel of O/BFS monoclonal Ab.

#### 4. DISCUSSION

The FMD diagnostic laboratory must identify and characterize FMD virus in samples submitted, and also advise the FMD National Control Programme on the appropriate vaccine strains to be used for control measures. The advantages of ELISA techniques have been emphasized by different authors [4,5,9]. The LPBE was developed to replace the Virus Neutralization Test (VNT) [1] and correlates directly with VNT and protection [10]. LPBE incorporated into a two-stage system provided a practical and rapid method to study the relationship field isolates and vaccine strains [3]. Interpretation of "r" values, according to Ferris and Donaldson [11] indicates that there were not significant differences between the field isolates and the vaccine strain.

Characterization of FMDV using Mabs has been extensively described [12,13]. Recently five antigenic sites for the FMDV type O have been identified by Mabs. [14]. In this study the panel of Mabs employed were raised against O/Switzerland and O/Caseros, and recognize the five immunodominant epitopes. Characterization of the strains under study would be improved if Mabs against O/Urubamba were available. The Mabs profiles of the field isolates showed a similar pattern. All the field isolates showed low binding with the C6 Mab, whereas high reactivity was shown between

the vaccine strain and the Mab. This slight difference suggests that the field strains did not originate from the vaccine or from an FMD vaccine production laboratory.

The consistency of Mab profiles among field isolates reflects that circulating virus in the field is conserved in cattle population with low immune status. Mab profile results provided a more detailed antigenic characterization and agreed with the "r" value findings. This study demonstrated that monitoring of field strains using ELISA provided valuable data for epidemiological surveillance and assessment of suitable vaccine strains.

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