

# SEROLOGICAL EVIDENCE OF THE CIRCULATION OF FOOT-AND-MOUTH DISEASE VIRUS IN BUFFALOES (*Bubalus bubalis*) FROM JESÚS MARÍA SEMPRUN AND CATATUMBO COUNTIES, ZULIA STATE, VENEZUELA

## *Evidencias Serológicas de Circulación Viral del Virus de Fiebre Aftosa en Bufalos (*Bubalus Bubalis*) de los Municipios Jesús María Semprun y Catatumbo, Estado Zulia, Venezuela*

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### ABSTRACT

Foot-and-mouth disease virus (FMDV) has a broad range of hosts and a high resistance to inactivation in the environment. Buffaloes (*Bubalus bubalis*) are considered major hosts within the natural ecology of FMDV. The objective of this research was to determine the seroprevalence of FMDV in Jesús María Semprun and Catatumbo Counties, Zulia State, Venezuela. A proportional stratified sampling was undertaken in September 2013. Blood samples were collected from 549 buffaloes (25 farms), and sera were used for 3ABC iELISA. Seroprevalences and odds ratios for risk factors were calculated. Likewise, a logistic regression model was created. Seroprevalences were 10.5 and 6.6% at animal level and 75 and 53.85% at herd level in Catatumbo and Jesús María Semprun Counties, respectively. A higher seroprevalence in animals older than 24 months (10.8%,  $P < 0.01$ ) was observed. Conversely, seroprevalence in animals younger than 24 months old did not show any significant differences between Counties ( $P > 0.05$ ). Animals older than 24 months and belonging to a farm where at least three susceptible species share pastures, were the factors that increased the odds of being in contact with FMDV. In conclusion, there is a low FMDV seroprevalence at buffalo level but a high seroprevalence at farm level in Catatumbo and Jesús María Semprun Counties. It is recommended to increase vaccination and epidemiological surveillance in buffalo farms in those Counties, especially since buffaloes are an important FMDV reservoir to cattle, which in turn are more prone to display clinical signs and hence outbreaks ensue.

**Key words:** FMDV; water buffaloes; viral activity; South America.

### RESUMEN

El virus de la fiebre aftosa (FMDV) posee un amplio rango de hospedadores y una alta resistencia a la inactivación en el ambiente. Los búfalos se consideran hospedadores preponderantes en la ecología del FMDV. El objetivo de esta investigación fue determinar la seroprevalencia del FMDV en búfalos de los municipios Jesús María Semprun y Catatumbo del estado Zulia, Venezuela. Se realizó un muestreo estratificado proporcional durante el mes de septiembre 2013. Se recolectaron muestras de sangre de 549 búfalos (25 fincas) y con el suero se realizó un ELISAi 3ABC. Se calcularon la seroprevalencia y las razones de desigualdad para los factores de riesgo. Así mismo se construyó un modelo de regresión logística. La seroprevalencia fue de 10,5% para el municipio Catatumbo y de 6,6% para el municipio Jesús María Semprun a nivel de búfalos, y de 75 y 53,85% para los mismos Municipios a nivel de fincas. Se observó que hubo una mayor seroprevalencia entre los animales mayores de 24 meses (10,8%;  $P < 0,01$ ). En cambio, la circulación viral no presentó diferencias significativas entre Municipios ( $P > 0,05$ ). El modelo de regresión logística determinó que los dos factores que aumentan la probabilidad de que un búfalo haya estado en contacto con el FMDV fueron el tener una edad mayor a los 24 meses y el pertenecer a una finca donde convivan al menos tres especies susceptibles al virus. En conclusión, en los municipios Catatumbo y Jesús María Semprun existe una baja seroprevalencia de fiebre aftosa a nivel de búfalos, pero alta a nivel de fincas. Se recomienda reforzar la vacunación antiaftosa y la vigilancia epidemiológica en las fincas bufalinas de esos Municipios, puesto que los búfalos son reservorios de ese virus para los bovinos, lo cuales son más propensos a mostrar signos clínicos y desencadenar brotes.

**Palabras clave:** Fiebre aftosa; búfalos de agua; actividad viral; América del Sur.

## INTRODUCTION

Foot-and-mouth disease (FMD) is caused by a virus from the family *Picornaviridae*, genus *Aphthovirus*, having a broad range of hosts and highly resistant to environment. Among hosts, major members are bovines (*Bos*, *Bubalus*, *Syncerus*), caprines (*Capra*), ovines (*Ovis*), and swine (*Sus scrofa*). Although a great number of species has been reported to be susceptible naturally or experimentally, domestic animals are deemed to be responsible for FMD endemicity, African buffalo (*Syncerus caffer*), which is a major link in the maintenance of viral serotypes circulating in Africa, being a relevant exception as wildlife [20].

Clinical FMD is characterized by the presence of vesicles and ulcers in the oral cavity, hooves, and teats. However, this clinical picture may vary depending on the affected animal species. In buffaloes (*Bubalus bubalis*), subclinical presentation is quite frequent or when it is clinical, it tends to be more conspicuous in hooves [13].

After animals are infected by FMD virus (FMDV), a proportion of those animals becomes carriers, because FMDV remains actively replicating in the pharyngeal region [2]. This viral persistence state depends on the species of the host: it has been reported to be around 3 weeks in swine and 5 years in African buffaloes [2]. In Asian buffaloes (*Bubalus bubalis*), FMDV persistence has been documented to be 1-2 years [11, 20].

Buffaloes are considered major hosts within FMDV ecology due to the fact that they undergo frequently a subclinical course, infection can be established at pharyngeal level even though there are neutralizing antibodies from previous infections or vaccinations, and there is the possibility of FMDV transmission to other animals sharing the same pastures or installations, such as cattle [2, 11, 12].

The general objective of this research was to determine the seroprevalence of FMDV in buffaloes (*Bubalus bubalis*) from Jesús María Semprun and Catatumbo Counties, Zulia State, Venezuela.

## MATERIALS AND METHODS

### Geographic location

This research was undertaken in two Counties from Zulia State, Venezuela: Catatumbo County, located between 09° 37' LN, 71° 43' LW and 08° 22' LN, 72° 29' LW, and with a sub-humid climate with precipitations of 1,000 mm per year. Temperature is high year round, with a mean of 27.8° C. The vegetation is characterized by an area of tropical dry forest from the shores of Lake Maracaibo to approximately isohyet 10 of 1,800 mm, the rest is represented by tropical wet forest [8].

The other studied County was Jesús María Semprun, located between 09° 29' LN and 72° 18' LW to 08° 30' LN and 73° 22' LW. Climate is varied, with mean precipitations of 1,453.6 mm

in Casigua, increasing gradually towards Sierra of Perijá up to 2,300 mm yearly at mountaintops. Mean temperature is 28° C (Casigua), lowering to 18° C at the tops of Sierra of Perijá. Life zones present in this county are a transition from tropical dry forest to montane wet forest [8].

### Sampling

In September 2013, a proportional stratified sampling in Catatumbo and Jesús María Semprun Counties, Zulia State, was undertaken. Results from the 2012 second cycle of FMD vaccinations were used in sample calculations. Epi Info® 7.0.9.7 (Centers for Disease Control and Prevention, 2012) was used to determine sample size per County (at both animal and farm levels) with 95% of confidence level, 5% of precision, and 25% of expected prevalence. The number of sampled farms and animals are displayed in TABLE I.

TABLE I  
SAMPLING OF BUFFALOES IN CATATUMBO AND  
JESÚS MARÍA SEMPRÚN COUNTIES OF ZULIA STATE,  
VENEZUELA

County	Population	Sample
<b>Catatumbo</b>		
Farms	12	12
Animals <24 months old	661	37
Animals >24 months old	3,434	239
<b>Jesús María Semprun</b>		
Farms	15	13
Animals <24 months old	1,660	130
Animals >24 months old	2,344	143

### Collection of serum samples

Local veterinarians were responsible for the collection of serum samples. Once the farm was located, animals were classified according to age: animals from 6 to 24 months old (prepuberal buffaloes) and animals older than 24 months (adult buffaloes). Proper identification (farm code and animal category) was written down in the Vacutainer® tubes used for blood collection, which was performed through either coccygeal or jugular vein. Tubes were placed with a 45° slope for 20 minutes (min) to enhance clotting. Afterwards, serum was separated and stored in another tube at -20°C until transport to the Faculty of Veterinary Sciences of the University of Zulia in Maracaibo.

### 3ABC iELISA

Samples were analyzed using IDEXX FMD 3ABC Bo-Ov® iELISA kit. Procedure was as follows: all reagents were allowed to come to room temperature before use; samples, positive, and negative controls were prediluted 1:100 using sample diluent provided in the kit. 100µL of prediluted samples, positive, and negative controls were dispensed into the appropriate wells of the microtiter plate. This plate was covered with a lid and incubated for 60 min at 37°C in a humid chamber. Each well was washed with 300µL wash solution three times. Afterwards, 100µL of

conjugate were dispensed into each well, and the microtiter plate was covered with a lid and incubated for 60 min at 37°C in a humid chamber. The washing step was repeated and 100µL of TMB substrate were added to each well. Incubation ensued for 15 min at room temperature. In order to stop the enzymatic reaction, 100µL of stop solution were added to each well. Optical densities (OD) were obtained using a spectrophotometer (ELx800, BioTek Instruments, USA) at 450nm. Positive control and samples OD were corrected by subtracting the negative control OD; then, the percentage of sample over positive control was calculated (S/P %). Samples with S/P % < 20 were considered negative, those with S/P % ≥ 20 and < 30 were considered suspect, and those with S/P % ≥ 30 were considered positive. Samples with borderline results (suspect) were retested.

**Field survey**

An epidemiological survey to farm managers was conducted. In that survey, the presence of the following risk factors was evaluated: keeping of records at farms, movements of animals, stock of buffaloes, and the presence of other susceptible species.

**Statistical analysis**

Statistical analysis comprised the construction of 2 x 2

contingency tables to calculate the corresponding statistics (Chi square or Fisher exact test depending on the case [1]) and odds ratios (OR) with 95% confidence intervals. True seroprevalences and 95% confidence intervals adjusted for the test specificity and sensitivity (specificity: 0.99, sensitivity: 0.332; [4]), were estimated from observed seroprevalences [17]. Moreover, models of logistic regression were created and the best model was chosen using AIC (Akaike Information Criterion) and VIF (Variance Inflation Factor) [1, 5]. Data were analyzed using R statistical program, version 3.2.2 (08-14-2015, The R Foundation for Statistical Computing) with packages MASS, tidyr, dplyr, car, Hmisc, and abd.

**RESULTS AND DISCUSSION**

Seroprevalences were 10.5 and 6.6% at animal level and 75 and 53.85% at herd level in Catatumbo and Jesús María Semprun Counties, respectively. There were no statistically significant differences between Counties (P>0.05, TABLE II). Estimated true seroprevalences are also shown in TABLE II. Due to the small number of studied farms (even though 83.3% of all buffaloes farms in the Counties were sampled), significant differences were not detected at farm level, so henceforth only results at animal level will be commented.

TABLE II

**RISK FACTORS ASSOCIATED WITH THE PRESENCE OF ANTI-FMDV ANTIBODIES IN BUFFALOES FROM CATATUMBO AND JESÚS MARÍA SEMPRUN COUNTIES, ZULIA STATE, VENEZUELA**

Factor	Levels	Observed prevalences		True prevalences (95% CI)		Missing data	P value	OR (95% CI)	
		No	Yes	Yes <sup>1</sup>					
<b>County</b>	Catatumbo	247 (89.5%)	29 (10.5%)	29.5%	(19.5-42.7%)				
	JMS	255 (93.4%)	18 (6.6%)	17.4%	(9.5-28.4%)		0.1372		
<b>Age</b>	<24 months old	161 (96.4%)	6 (3.6%)	8.1%	(1.8-20.4%)				
	>24 months old	341 (89.2%)	41 (10.8%)	30.1%	(21.4-41%)		0.009733	3.226	(1.342-7.755)
<b>Records</b>	Farms do not keep records	91 (88.3%)	12 (11.7%)	33.2%	(16.9-56.4%)				
	Farms keep records	340 (94.7%)	19 (5.3%)	13.4%	(6.9-22.1%)	87	0.04036	0.423	(0.198-0.905)
<b>Susceptible species</b>	Only buffaloes	43 (95.5%)	2 (4.5%)	10.6%	(0-43%)				
	Buffaloes and cattle	178 (95.2%)	9 (4.8%)	11.8%	(4.4-24.5%)			1.087	(0.226-5.215)
	Buffalos and two more susceptible species.	34 (82.9%)	7 (17.1%)	50%	(20.9-94.2%)	276	0.0213	4.426	(0.863-22.7)
<b>Movement of animals</b>	Farms where animals are not moved	68 (93.15%)	5 (6.85%)	18%	(5.4-42.8%)				
	Farms where animals are moved	398 (90.66%)	41 (9.34%)	25.8%	(18.2-35.6%)	37	0.6398		
<b>Stock of buffaloes</b>	≤100 buffaloes	31 (72.09%)	12 (27.91%)	83.5%	(45.2-100%)				
	>100- <500 buffaloes	403 (93.07%)	30 (6.93%)	18.3%	(11.7-27.1%)			0.192	(0.089-0.412)
	≥500 buffaloes	68 (93.15%)	5 (6.85%)	18%	(5.4-42.8%)		0.0002239	0.189	(0.061-0.585)

<sup>1</sup> 95% confidence intervals adjusted for test sensitivity and specificity; JMS: Jesús María Semprun county

Results from this study differ from those obtained by Boscán-Duque et al. [3], who found seroprevalences of 54.76 and 0% in bovines at farm level for Catatumbo and Jesús María Semprun Counties, respectively. Those differences may be caused by sampling, because they took samples from 3.18% of the total of farms, whereas 83.3% were sampled in this research. However, those differences are most probably produced by the species studied in each research. Buffaloes have been demonstrated to transmit FMDV as efficiently as cattle [12], even though they often display a subclinical disease. On the other hand, the development of typical FMD lesions are not required for an animal to be infectious, since it has been proved that animals with high levels of neutralizing antibodies may maintain a persistent infection at the pharynx [2,11, 12].

In Pakistan, prevalences of 19.2% FMDV-infected buffaloes at farms without overt FMD and of 53.9% at farms with animals with scarring FMD lesions, have been reported. At farms with animals with recent FMD lesions, FMDV prevalence was 87% [9], which illustrates how fast and widespread FMDV transmission can be within buffalo herds.

FMDV has been described to have 7 serotypes (A, O, C, SAT-1, SAT-2, SAT-3, and Asia-1), which do not cross-react with each other [10, 18]. By contrast, due to the great antigenic variability in this virus, there are several subtypes within each serotype with varying degrees of homology [9]. That is the reason why diagnosis of FMDV-infected animals (previous or ongoing infections) is directed quite frequently to the detection of antibodies against non structural proteins (NSP's). Since these proteins have not been subjected to the selective pressure of host immune system, therefore they are mostly conserved among strains [19].

However, the usefulness of the techniques for detection of antibodies against NSP's (such as 3ABC iELISA) depends on the degree of purification of viral particles (146S) present in vaccines, because if vaccines used in a herd do not meet internationally established standards for FMD vaccines as for purification, it is possible that they would induce the generation of anti-NSP's antibodies in vaccinated animals [15].

Concerning age groups, a higher seroprevalence in buffaloes older than 24 months (10.8%,  $P < 0.01$ , TABLE II) was observed. Likewise, a buffalo older than 24 months has 3 times higher odds of having antibodies against FMDV than a buffalo younger than 24 months (OR= 3.226, 95% CI= 1.342-7.755). Animals 6-12 months old have been previously used to indirectly assess viral circulation of FMDV [7]. In this study, seroprevalences in prepuberal animals (6-24 months old) did not show any significant differences ( $P > 0.05$ ) between Counties.

The older an animal in an endemic area is, the higher the probability that it has been in contact with one or more FMDV strains, even different serotypes, as it has been demonstrated in this research. Molla et al. [14] reported in Southwestern Ethiopia seroprevalences of 1.98, 6.93 and of 13.6% for cattle younger than 2 years old, 2-4 years old, and older than 4 years old, respectively.

**TABLE III**  
**LOGISTIC REGRESSION FOR THE PRESENCE OF ANTI-FMDV ANTIBODIES IN BUFFALOES FROM CATATUMBO AND JESÚS MARÍA SEMPRUN COUNTIES, ZULIA STATE, VENEZUELA**

Variables	Coefficients	OR (95% CI)
Intercept	-4.1096	
>24 months old	2.1637	8.702 (2.37-41.4)
>100 - <500 buffaloes	-0.9669	0.380 (0.013-10.51)
Presence of buffaloes and cattle	0.4052	1.499 (0.26-28.39)
Presence of buffaloes and two more susceptible species	2.8318	16.976 (2.4-354.72)

OR were calculated by exponentiating the coefficients of the variables.

Buffaloes from farms at which records were not kept showed a higher seroprevalence (11.7%,  $P < 0.05$ , TABLE II) than those from farms at which records were kept. This makes keeping records a protective factor since buffaloes from farms keeping records had 2.38 times lower odds of having anti-FMDV antibodies than those from farms not keeping records. Concerning the presence of several FMDV susceptible species (at least three species, buffaloes included), it entails a higher seroprevalence (17.1%,  $P < 0.05$ , TABLE II) and greater odds of having had contact with the virus (OR=4.426, 95% CI= 0.863-22.7).

Several FMDV susceptible species' sharing the same ecological niche fosters FMDV maintenance and the establishment of endemicity, because there is a higher probability of the contact of animals with different degrees of susceptibility and viral excretion kinetics (amplifier species such as swine or highly susceptible to respiratory infection such as cattle), besides the appearance of carriers whose characteristics are also species-dependent [2, 11, 12, 20].

As for movements of animals and their relationship with FMDV seroprevalence, there were no significant differences between buffaloes from farms moving their animals and those not moving them ( $P > 0.05$ , TABLE II). Conversely, buffaloes from farms with a stock smaller or equal to 100 animals were observed to have a higher seroprevalence (27.91%,  $P < 0.001$ , TABLE II), whereas the rest of the categories had much lower odds of having anti-FMDV antibodies (OR≈0.19).

Other studies have found that farms with great animal stock are a risk factor for FMD [3, 6, 16] due to higher animal densities, which would facilitate faster viral transmission. The fact that this research had opposite results may be related to deficiencies in health management, such as inexistent biosecurity, inefficient vaccination and disease controls, which are common on small farms in Venezuela. However, further research is needed to prove this hypothesis.

The model of logistic regression that generated the lowest AIC value (123.2) along with low VIF values (1.20-1.34) is displayed in TABLE III. After evaluation of this model, it is inferred that two factors increase considerably the odds that a buffalo has been in contact with FMDV in the studied Counties; these are being older than 24 months and belonging to a farm where at least three FMDV susceptible species share pastures. The model does not include the lack of records because it shows collinearity with the presence of other FMDV susceptible species at farms.

## CONCLUSIONS

There is a low FMDV seroprevalence at buffalo level but a high seroprevalence at farm level in Catatumbo and Jesús María Semprun Counties, Zulia State. Taking into account that there is viral circulation in young animals and that it is frequent to encounter farms with several susceptible species sharing pastures, it would be worthwhile to increase vaccination and epidemiological surveillance in buffaloes' farms in those counties, especially since buffaloes are an important FMDV reservoir to cattle, which in turn are more prone to display clinical signs and hence outbreaks ensue.

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