Evaluation study for detection of NSP of FMDV in sheep and Goat by ELISA

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Abstract

In the present study ELISA to detect NSP of FMDV by as good /best diagnostic tool for differentiation between the vaccinated and infected animals. The present article based on this fact for diagnosis of FMD in sheep and goat to clarify the current situation screening of FMDV. The results revealed that a total of 972 sera samples were collected from 15 Egyptian governorates from sheep and goat (646 and 326) respectively The seropositive samples were 159 out of 646 (24.61%) in sheep while 68 out of 326 (20.86%) in goat. The over all seropositive in sheep and goat were (23-35%) ,On the other hand, the proportion of seropositive in sheep and goat in relation to the age and distribution in governorates villages were as follows the high percentage of seropositive was 21(19.27%) , 90 (22.67%) and 55(24.34%) among sheep and goat in age range between 1-3 years respectively; the fully discussion of results and evaluation of current situation are presented in the study.

Key words: *FMD, sheep, goat, Egyptian governorates* .

Introduction

Foot-and – Mouth Disease (FMD) is the most important disease of the international organization of epizooties (OIE) List A, and one of the most contagious diseases among domestic animals **(OIE/ FAO/ WHO,1996).** It is an acute viral disease of cloven- hoofed animals including cattle, pigs, sheep and goats. The course of the disease in cattle and pigs is characterized by vesicular lesion in and around the mouth and on feet causing lameness and on teats. In sheep and goats, however, the clinical signs can be very mild or absent. FMD can spread unnoticed even to experienced veterinarians **(Sharma, 1981).** Foot-and-mouth disease virus (FMDV; genus Aphthovirus, family Picornaviridae) causes an acute vesicular disease of ruminants and pigs that is considered to be the most economically important disease of livestock. There are seven known immunologically distinct serotypes [O, A, C, SAT (Southern African Territories) 1, SAT 2, SAT 3 and Asia 1], with a spectrum of different strains within each serotype. Type O FMDV is the most commonly isolated serotype (**Knowles and Samuel, 2003**)..

Although the minimum infectious doses of airborne virus for cattle and sheep are similar, the threshold concentration of virus required to infect sheep is thought to be higher because of their lower inhalation rate **(Sørenson *et al*., 2000).** The incubation period for natural infection is normally between 3 and 8 days **(Kitching & Mackay, 1994),** but can be less than 24 h following experimental inoculation **(Sellers *et al*., 1977).** The mortality rate for adult sheep infected by the virus is usually negligible, but mortality in lambs can reach 90% **(Chevskii *et al*., 1964).** The evidence that carrier sheep can be a source of infection for other livestock species is only circumstantial **(Stougaard, 1983).**Diagnosis of FMD is made by the demonstration of FMD viral antigen or nucleic acid in samples of tissue or fluid. Detection of specific humoral antibody can also be used for diagnosis. The detection of antibody to non-structural proteins (NSP) of FMDV has been used to identify past or pre-

sent infection **(DeDiego *et al*., 1997; Brocchi *et al*., 1998; Dekker and Gijsen, 1998; Malirat *et al*., 1998; Sorensen *et al*. 1998** and **Muller *et al*., 2010)** .Antibodies to 3ABC were detected from day 10 after experimental infection of sheep. During the course of infection, immune response to NSP appears later than response to structural proteins of the virus. Following experimental infection antibodies to the 3-ABC antigen could not be detected earlier than 10 DPI in sheep **(Chung *et al*., 2002)**. Perhaps the most reliable single NSP indicator is the poly-protein 3 ABC, antibodies to which appear to provide conclusive evidence of previous infection **(Makay *et al*., 1998)** whether or not the animals have also been vaccinated. **Sorenson *et al*. (1998)** stated that antibodies against 3 ABC have been detected up to 395 days post infection in both cattle and sheep. **Kitching and Hughes (2002)** reported that the 3-ABC antibodies persist more than 12 months. The severity of infection may influence serum levels of NSP antibodies and also the duration for which this antibody is detectable. The aim of this work is to evaluate the usefulness of the CHEKIT-FMD-3-ABC ELISA as a diagnostic tool to differentiate between FMDV-vaccinated and FMDV-infected Egyptian sheep and goats.

Material and Methods

Sample collection, transportation and preparation sheep and goat blood samples were randomly collected from fifteen Egyptian Governorates (age of animals ranged from one to 3 years).

A total of 972 serum samples were separated from collected blood samples of diseased animals (sheep and goat) and kept at -20ºC until used

Method :

ELISA was done for monitoring the immune status of these animals and determination of antibodies against FMDV non structure protein which was done by using ELISA PrioCHEK FMD NS according to **(Sorensen et. al., 1998)** done according to the PrioCHEK FMD NS manufacture.

Interpretation of percent inhibition

-PI= <50% negative (antibodies against the NS protein of FMDV are absent in the sample)

Results

Table 1. FMD-NSP test results in goats and sheep (N=972) animals.

Result	**Goats**	**Sheep**	**Total**
No. of samples	258	487	745
Seronegative			
Percentage%	79.14	75.65	76.65
No. of samples			227
Seronegative	68	159	
Percentage%	20.86	23.39	
Total	326	646	972

Table 2. FMD-NSP test results in relation to age class (N = 972) small ruminants.

Result	Age Class			Missing	Total
	١	٢	٣		
Seronegative	88	307	171	179	745
Percentage%	80.73	77.33	75.66	74.58	76.65
Seropositive	21	90	55	61	227
Percentage%	19.27	22.67	24.34	25.42	23.35
Total	109	397	226	240	972

Table 3. FMD-NSP test results by Egyptian governorates (N=972) small ruminants

Governorate	-ve	+ve
Aswan	40 100	0 0
Behaira	66 70.21	28 29.79
Dkahlya	69 86.25	11 13.75
El-Wadey El-Gadid	77 96.25	3 3.75
El- Fayoum	17 42.50	23 57.50
El-Sharqya	95 70.90	39 29.10
Garbia	44 73.33	16 26.67
Kafer El-Sheikh	59 62.77	35 37.23
Marsa Matrouh	67 82.72	14 17.28
Menia	41 75.93	13 24.07
Monufya	39 70.91	16 29.09
North Sainay	37 92.50	3 7.50
Qalubia	31 77.50	9 22.50
Red sea	38 95.00	2 5.00
Sohag	25 62.50	15 37.50
Total	745 76.65	227 23.35

+ve = the serum sample positive non structure protein for FMD-NSP

- ve= the serum sample negative for non structure protein for FMD-NSP

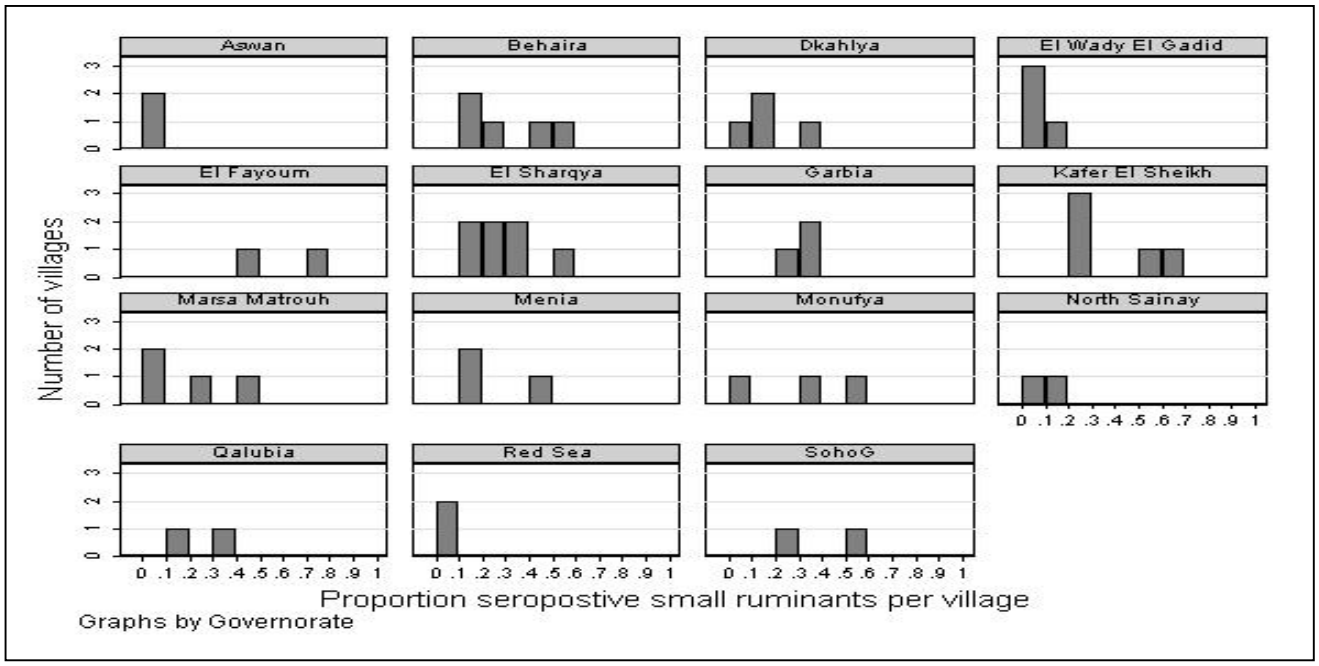

Figure 1. Histogram within-village proportion of FMD-NSP positive small ruminants per governorate in Egypt.

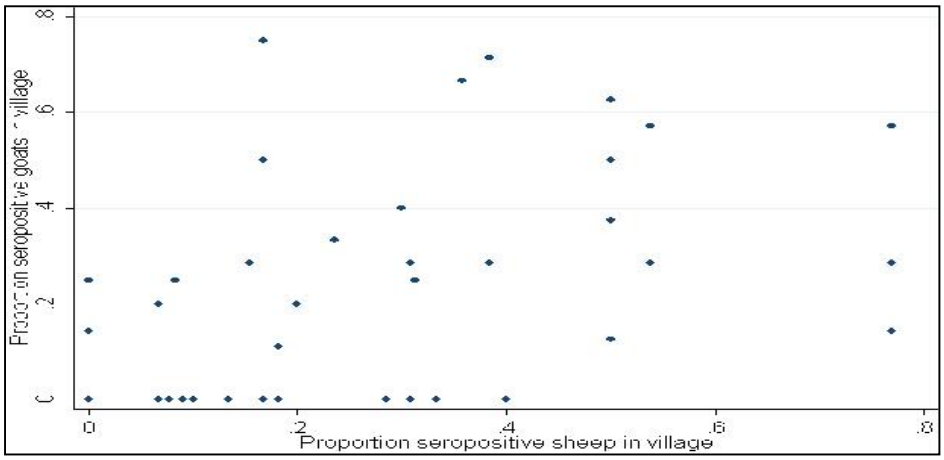

Figure 2 Scatter plot of proportion FMD-NSP positive sheep and goats per village (N = 50 villages)

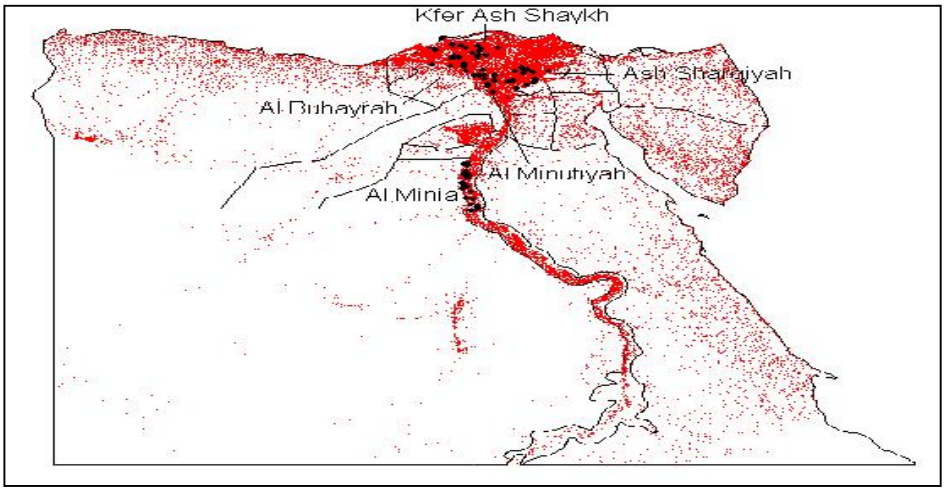

Figure3. Geographical map of governorates in Egypt with FMD-NSP seroproprotions in sheep and goats.

Discussion

Identification of currently- or previously- infected sheep with FMDV is very important to any control strategy for the eradication of FMD, that the apparently healthy sheep may be source of new outbreak. The only way to efficiently identify carrier sheep is by detection of antibodies against non-structural proteins of FMDV, such as 3-ABC. NSP antibodies only develop following infection of animals with FMD virus and not after vaccination with purified, inactivated vaccine. To evaluate the use of an NSP antibody detection assay in differentiating between vaccination and infection **(Mackay *et al*., 1998 and Chung *et al*., 2002),** samples were collected from different governments and tested for the presence of FMD NSP

The data in this research was received from 15 governorates, 47 districts from 50 villages. Between 12 and 21 samples per village, about 972 sheep and goats sera samples either from sheep (66.64%) or goats (33.54%). In addition, the age class was given for 742 records and this agree to **(Balinda *et al*., 2009)** In 2007, mean prevalence estimates of antibodies towards FMDV NSP was 14% in goats and 22% in sheep in Kasese district, while Bushenyi was still free. The difference between these two districts probably reflects different levels of FMDV may be challenge attributed to the variation in exposure rates which again in part may be as a result of the different husbandry practices.

Table(1) show seropositive of sheep 24.61% (159 out of 646) and goat 20.82% (68 out of 326) where the seropositive overall percentage was 23.35%(227 out of 972) the results which agree with previously reported by others **(Sorensen *et al*. ,1998 and Paton *et al*., 2009)**

Table (2) showed the FMD-NSP test results by goats and sheep (N=972 animals). The proportion seropositive seems to increase by age where the seropositive in small ruminants class (1,2 and 3 years) was 21 out of 109 (19.27%), 90 out of 397 (22.67%) and 55 out of 226 (24.34%) respectively indicate the high proportion of seropositive between class 2 and 3.The missing data 61 out of 240 (25.42%) however was statistically non-significant **(Davies, 2002 and Balinda *et al*., 2009).**

That animals have been infected with replicating virus develop antibodies to the non-structural proteins of FMD virus and these may be detected using 3ABC ELISA and this test has been fully validated for use in small ruminants **(OIE 2001)** (**Ranabijuli *et al*., 2010).**

Table 3 and figure 1 clarifies the FMD-NSP test results by Egyptian governorates (N=972) small ruminants with a Histogram within-village. Proportion of FMD-NSP positive small ruminants per governorate in Egypt(Figure 1)

shows no significance difference among village or the village level then used the Scatter plot of the proportion FMD-NSP positive sheep and goats per village (N = 50 villages) **(FAO, 2010).**

Comparison of geographical presentation of FMD-NSP antibodies in sheep/goats (FAO-TCP1) indicate that regions away from the Nile have lower seroproportions and that there are stark differences between Nile delta governorates as shown in the results

which help in Geographical and Epidemiological mapping of governorates in Egypt .With FMD-NSP seroproprotions in sheep and goats in **(Figure 3)** the Egyptian governorates map pointed to the seroprevalence in highly affected governorates like El Fayoum 57.5% , Sohag 37.5% ,Kafer El-Sheikh 37.23% , Behaira 29.79% , El- Shargya 29.1% and Monufya 29.09% .The results provide that border governorates still are the lowest level in serpositive among small ruminant in table (3) Aswan 0% , EL- Wady El- Gadid 3.75% , Red Sea 5% North Sina 7.5% and Marsa Matrouh 17.28%. Where the infectious diseases tend to be distributed in spatial patterns associated in part with the physical and biological factors that influence risk of disease occurrence. This agree with **(Nick et. al. 2007)** thus the disease mapping and spatial distributions analysis to explore the nature of such distributions has been widely used in risk modeling to identify high-risk areas or geographically-related risk areas and this agree with Spatial epidemiology in which study spatial variation in disease risk or incidence **(Ostfeld *et al*.,2005)** and study in Nepal was to quantify associations between hypothesized epidemiological factors and the spatial distribution of foot-and-mouth disease (FMD). Spatial clustering of reports of at least one FMD case by Village in 2004 was examined by use of the spatial scan statistic**(Bimal *et al*., 2010).**

Small ruminants have a role in the transmission of FMD disease during the early stages of either clinical or sub-clinical the period of greatest risk for transmission is up to seven days after contact with the infection **(Barnetti and Cox , 1999).**

Although there have been numerous examples in the past where small ruminants have been responsible for the introduction of FMD into previously disease-free countries. The difficulty in making a clinical diagnosis should encourage the development of more rapid screening tests to assist in future control programmes **(Kitching and Hughes, 2002).** The Differentiating between vaccinated and infected sheep and goats may help in the future FMD control strategy (**Neitzert *et al*., 1991 and Lubroth and Brown , 1995**).

Conclusion

NSP is one of diagnostic tool in sheep and goat Our recommendation to applied PrioCHICK screening program in small ruminant in large scale.

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دراسة لتقييم الكشف عن البروتينات اللابنائية لفيروس الحمى القلاعية فى الاغنام والماعز باستخدام اختبار الاليزا

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الملخص العربى

تهدف الدراسة الى تقيم الوضع الوبائى فى المجترات الصغيرة المحلية الاغنام والماعز بهدف الكشف عن وجود الاجسام المناعية المضادة للبروتين اللابنائى لفيروس الحمى القلاعية والدال على العدوى الطبيعية بأختبار "PrioCHECK" الاليزا وذلك لتتبع البؤر المرضية فى الاغنام والماعز والتى تعتبر مصدر العدوى أضافة الى انها عائل قابل للعدوى ولا تبدو عليه الاعراض ظاهريا واضحة مما يصعب التشخيص الاكلينيكى للحيوانات المصابة وقد اهتمت الدراسة بتجميع عدد ٩٧٢ عينة سيرم من الاغنام والماعز (٣٢٦,٦٤٦) على التوالى لفحصها بأختبار الاليزا (PrioCHECK) وكانت النتائج عدد ايجابى ١٥٩% (٢٤.٦١) من الاغنم وعدد ايجابى ٦٨ (٢٠.٨٦%) من الماعز على التوالى وقد تم تجميع العينات من عدد ١٥ محافظة ممثلة فى (٥٠ قرية) على مستوى الجمهورية تشمل الدلتا وصعيد مصر وتم ربط هذه النتائج على المستوى العمرى للحيوانات وكانت النتيجة عدد ايجابى٢١ (١٩.٢٧ %)، ٩٠(٢٢.٦٧ %)، ٥٥ (٢٤.٣٤ %) للاعمار من سنة الى ثلاث سنوات على التوالى وايضا على مستوى المحافظات والقرى وكانت النتائج بنسب مختلفة كما موضح بالخريطة الوبائية الناتجة من هذه الدراسة .