RAPID IMMUNOPEROXIDASE MONOLAYER ASSAY (IPMA) FOR DETECTION AND TITRATION OF FOOT AND MOUTH DISEASE ANTIBODIES IN SHEEP IN COMPARISON WITH SNT AND ELISA

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ABSTRACT

This study describes the evaluation of immunoperoxidase monolayer assay (IPMA) for detecting antibodies against serotype O1/93 Foot and Mouth Disease virus in sera of infected, vaccinated and random field sera of sheep.The IPMA results were compared with that obtained by serum neutralization test (SNT) and indirect enzyme-linked immunosorbent assay (ELISA). All infected, vaccinated sheep sera tested positive by SNT and ELISA, were positive by IPMA with a mean titers of 1.35,1.77and 1.83 $log_{10}TCID_{50}$ one month post experimentally infected sheep or sheep vaccinated with inactivated monovalent gel adjuvant serotype O1/93 FMD virus vaccine. 30 out of 50 field sheep sera tested positive by both ELISA and IPMA. Out of the 30 positive sera 27 (90%) revealed neutralizing antibody titers of 0.6 to 1.5 $log_{10}TCID_{50}$. In experimentally infected or vaccinated sheep, antibodies against serotype O1/93 could be detected 5 to 7 days following infection or vaccination by ELISA and IPMA. The agreement between IPMA and ELISA was 100% but it was 90% between IPMA and SNT in field samples. The applicability of IPMA as specific and rapid for detection of FMD antibodies was discussed .

Keywords: Immunoperoxidaes monolayer assay (IPMA), ELISA, SNT, serotype O1/93 FMDV antibodies, infected, vaccinated and fields sheep.

INTRODUCTION

Because off the early response against Foot and Mouth disease (FMD) virus in sheep sera charactraized by low or even absence of neutralizing antibodies, and the clinical signs in sheep also occurred mainly subclinically, a rapid, specific and sensitive test is required for detection of antibody. ELISA could be detected early antibodies but the technique need automatic ELISA

reader to estimate the results. Immunoperoxidase monolayer assay (IPMA) is a visual method and have proven to be an easy method and available tool for diagnosis of several infectious diseases such as Bovine viral Diarrhea, Rinderpest, Cytomegalo virus, Influenza and Pseudorabies viral infection. IPMA permits the demonstration of antigens in various types of cells and fixed tissues. Most laboratories are routinely using IPMA for detection of antibodies especially early antibodies **(Afshar et al., 1989., Drew 1995, Horner et al., 1995, Yoon et al., 1995, Nodelijk et al., 1996, OIE, 1996, Sollman et al., 1997 and Deregt and prins1998)**. The aim of this study was to evaluate a newly developed IPMA which is based on ELISA assay in which Egyptian serotype O1/93 FMD for infected BHK21 clone 13 monolayer cultivated in 96 well flat plates were used as antigen to detect antibodies in sera of infected, vaccinated, random field sheep, atrial to detect the early antibodies in infected and vaccinated sheep. The results of IPMA were compared with the results of ELISA, SNT. Data on the prevalence of serotype O1/93 antibodies in random field sheep are presented.

MATERIAL AND METHODES

Serum samples:

A total of sixty serum samples were collected from experimently infected, vaccinated sheep of 1.5-2 years old five of each. Also fifty sera collected from apparently healthy sheep of 1-2 years old raised at sharquia governerate in endemic area with FMD. Sera were collected from expermintally infected and vaccinated at 3,5,6,7,15,30 days post infection and/or vaccination. The sera were inactivated at 56°C for 30 minutes and stored at - 20°C until used.

Immunoperoxidase monolayer assay (IPMA)

Immunoperoxidase monolayer assay (IPMA) was perfromed as described by Waris et al.,1990. Brielefly, BHK 21 clone 13 monolayer cells were grown to confluency in 96 well flat tissue culture plate (Nunc, Denmark). The confluency cells were inoculated with 100 TCID50 serotype O1/93 FMDV and incubated at 37°C for 24 hours. Plates washed once with 0.15 M phosphate buffer saline containing 0.1% Tween 20 and dried for 24 minutes in laminar flow and stored at -20°C in sealed plastic bag. Befor using the plate for testing antibodies, the plate were fixed in freshly prepared cold mixture of 50% acetone and 50% methanol at 4°C for 45 minutes. plates washed 3 times with washing buffer (PBS containing 0.1 tween 20 and 0.5% bovine serum Albumin BSA), incubated with tested serum (diluted two fold in PBS containing 0.1% tween 20 and 0.5% Bovine Serum Albumin (BSA) at 37°C for one hour then washed 5 times. Antisheep conjugated

with peroxidase in a dilution of 1:3000 was added and incubated at 37°C for one hour. The plates washed 5 times. Then substrate (filtrated mixture of 0.15 gm of 3.3 diamine benzidine tetrahydrochloride in 50 ml of 1% sodium acetate soln pH 5, 1ml of N sodium hydroxide and 0.6ml of 30% H202) was added and incubated at 37°C for 20 minutes. Positive reaction was seen as clear red-brown stained sheet of cells and no staining was observed in negative reaction. Positive and negative control sheep sera were included in each plate.

Virus neutralization test :

The micro neutralization test was applied using BHK-21 monolayers as described by **(Golding at el., 1976)** . Each serum sample was tested against serotype O1/93 FMD virus, and the titer was calcubated accrding to **Reed and Meunch (1938)**.

Indirect Enzyme Linked Immunosorbent Assay (Indirect ELISA):

The procedure was carried out as described by **Hamblin et al., (1986) and Shawky et al., (2000)**. The optimum dilution of the antigen used for coating was 1/160, sheep conjugate 1/3000.

RESULTS

Detection limit:

The geometrical mean titers (reciprocal of mean titers) against serotype O1/93 foot and mouth disease virus 30 days following experimental infected, vaccinated five sheep in each were 1.83,1.77,1.35 $\log_{10}$TCID$_{50}$ in infected and 1.52,1.41, 1.14 $\log_{10}$ TCID$_{50}$ in vaccinated tested by IPMA, ELISA and SNT respectively (Table 1,2&3). All infected and vaccinated sheep tested by IPMA revealed antibody titers ranged from 1.35 to 1.95 $\log_{10}$ TCID$_{50}$ with a percentage of (100%) at 30 days following infection or vaccination. The detection limits by IPMA were as high as that of ELISA (Table 1).

Experimentally infected and vaccinated sheep

The IPMA and iELISA first demonstrated antibodies against serotype O1/93 around 5 to 7 days post infection or vaccination with titers ranged between 0.6 to 0.9 $\log_{10}$ TCID$_{50}$ (Table1 &2). Whereas, neutralizing antibodies first detected at 7 to10 days post infection or vaccination with a titers ranged between 0.6 to 0.75 (Table 3).

Random sera collected from endemic area with FMD

The results of random fifty sera collected from apparently healthy sheep of 1-2 years old raised at Sharquiea Governorate in endemic area with FMD revealed that 30 sera (60%) had antibody titers ranged from 0.9 to 1.8 by IPMA and 0.75 to 1.65 $\log_{10}$ $TCID_{50}$ by IELISA. Whereas, 27 out of 30 (90%) tested positive by IPMA and IELISA had neutralizing antibody titers ranged from 0.6 to 1.5 $\log_{10}$ $TCID_{50}$ (Table 4). The correlation between IPMA positive and the results obtained by IELISA and SNT were demonstrated in table 5.

DISCUSSION

Immunoperoxddase monolayer assay (IPMA) has been reported as reliable, sensitive and specific technique for the detection of early developed viral antigen for rapid diagnosis of Cytomegalovirus, influenza, and respiratory syncytial infections **(Gleaves et al., 1984, Graham et al., 1985, Grenini et al., 1983, Waris et al., 1990,OIE, 1996 and Anon 1997)**. Based on the specificity, detection limit, results obtained to the response of early infection or vaccination and random field serum samples, the newly developed IMPA are reliable test for the detection of antibodies against serotype O1/93 FMD virus. The evaluation study in infected and vaccinated sheep revealed that, all positive sera tested by ELISA were also positive by IMPA with specificity 100%. The IPMA technique was able to detect antibody responses 5 to 7 days following experimental infected and vaccinated sheep. The results obtained from sheep sera collected from area endemic with FMD revealed that 30 out 50 (60%) sera had antibody titers ranged between 1.1 to 2.1 by IPMA and ELISA. Out of 30 positive sera 27 (90%) revealed neutralizing antibody titers ranged from 0.9 to 1.7. The decrease in positive percentage detected by SNT could be as results of sheep were sampled in early times after vaccination or infection. The early antibodies against serotype O1/93 FMDV detected at 5 to 7 days following infection and or vaccination by IPMA and ELISA techniques could not be detected by SNT. Our investigation revealed that IPMA and ELISA are the test of choice for detecting early antibodies against FMD virus in infected and vaccinated animals. The advantage of IPMA test is its results did not need automatic reader as that in ELISA **(Grenini et al., 1983, Gleaves et al., 1984)**. The need at least of two trained persons to be interpreted the results of IPMA to reduce the subjectivity of reading of test results is considered disadvantage of the test **(Wellenberg et al., 1999)**. Similar study were conducted by other authors which used IPMA for detection of antibodies **(Soliman et al., 1997, Wairs et al., 1990)** or comparison between IPMA and ELISA for detection of antibodies in infected and vaccinated animals **(Van Oirschot et al., 1986, Kelly et al., 1988 and Nodelijk et al., 1996)**. The present study revealed that Immunoperoxidase monolayer assay (IPMA) can be used for the screening

antibodies against foot and mouth disease virus as an alternative technique to ELISA. In conclusion, the IPMA is a reliable practical test for the screening FMD virus antibodies, and it could be used as a useful alternative technique to ELISA in infected, vaccinated and random field serum samples.

Table 1: Antibody titers against serotype O1/93 detected in sera of infected and vaccinated sheep tested by Immunoperoxidase monolayer assay (IPMA)

Days post infection and vaccination	Antibody titers against serotype O1/93 FMDV in infected and vaccinated sheep									
	Animal number									
	1		2		3		4		5	
	In.	Vac.	In.	Vac.	In.	Vac.	In.	Vac.	In.	Vac.
3 days	0.3*	0.0	0.15	0.3	0.0	0.0	0.15	0.30	0.30	0.15
4 days	0.45	0.30	0.30	0.3	0.15	0.0	0.3	0.30	0.45	0.15
5 days	0.6	0.3	0.6	0.6	0.75	0.45	0.45	0.30	0.6	0.3
6 days	0.6	0.45	0.6	0.6	0.6	0.45	0.6	0.45	0.9	0.45
7 days	0.9	0.6	0.9	0.9	0.75	0.6	0.75	0.6	0.9	0.75
15 days	1.35	1.2	1.5	1.2	1.5	1.2	1.5	1.2	1.35	1.2
30 days	1.5	1.35	1.8	1.5	1.95	1.75	1.95	1.5	1.95	1.5

In.: Infected sheep Vac.: vaccinated sheep

*: Antibody titers expressed as $\log_{10}$ $TCID_{50}$

Table 2: Antibody titers against serotype O1/93 detected in sera of infected and vaccinated sheep tested by indirect immunosorbent assay (ELISA)

Days post infection &or vaccination	Antibody titers against serotype O1/93 FMDV in infected and vaccinated sheep									
	Animal number									
	1		2		3		4		5	
	In.	Vac.	In.	Vac.	In.	Vac.	In.	Vac.	In.	Vac.
3 days	0.3*	0.0	0.15	0.3	0.0	0.0	0.15	0.30	0.30	0.15
4 days	0.45	0.30	0.30	0.3	0.15	0.0	0.3	0.30	0.45	0.15
5 days	0.6	0.3	0.6	0.6	0.6	0.15	0.45	0.30	0.6	0.3
6 days	0.6	0.45	0.6	0.6	0.6	0.3	0.6	0.45	0.9	0.45
7 days	0.9	0.6	0.9	0.9	0.75	0.6	0.75	0.6	0.9	0.75
15 days	1.2	1.2	1.5	1.2	1.5	0.9	1.5	1.2	1.35	1.2
30 days	1.5	1.35	1.8	1.35	1.95	1.5	1.95	1.35	1.65	1.5

In.: Infected sheep Vac.: vaccinated sheep

*: Antibody titers expressed as $\log_{10}$ $TCID_{50}$

Table 3: Antibody titers against serotype O1/93 detected in sera of infected and vaccinated sheep tested by serum neutralization test (SNT)

Days post infection. &or vaccination	Antibody titers against serotype O1/93 FMDV in infected and vaccinated sheep									
	Animal number									
	1		2		3		4		5	
	In.	Vac.	In.	Vac.	In.	Vac.	In.	Vac.	In.	Vac.
3 days	0.15*	0.0	0.15	0.15	0.0	0.0	0.15	0.0	0.0	0.0
4 days	0.15	0.0	0.30	0.3	0.15	0.0	0.3	0.0	0.45	0.15
5 days	0.15	0.3	0.45	0.45	0.6	0.15	0.45	0.30	0.45	0.3
6 days	0.3	0.3	0.45	0.45	0.6	0.3	0.45	0.45	0.45	0.3
7 days	0.6	0.45	0.6	0.6	0.75	0.6	0.6	0.6	0.6	0.45
15 days	1.05	0.9	1.2	1.05	1.05	0.9	1.05	0.9	1.05	0.9
30 days	1.2	1.05	1.35	1.2	1.35	1.2	1.5	1.05	1.35	1.2

In.: Infected sheep Vac.: vaccinated sheep

*: Antibody titers expressed as $\log_{10}$ $TCID_{50}$

Table 4: Antibody responses against serotype O1/93 FMD virus in random samples collected from Sharkia Governorate as determined by IPMA, iELISA and SNT

Type of serological test	Total No. of sera tested 50	
	Positive	Positive %
IPMA	30/50	60%
ELISA	30/50	60%
SNT	27/50	54%

Table 5: Correlation between positive IPMA, ELISA and SNT

Correlation between IPMA, ELISA and SNT		
Out of the total positive by IPMA	The positive % by ELISA	The positive % by SNT
30	30(100%)	27/30 (90%)

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إستخدام طريقة الأمينوبيروكسيدز على خلايا وحيدة الطبقة لتحديد ومعايرة الأجسام المناعية المضادة لڤيروس الحمى القلاعية ومقارنتها بطريقتى الأليزا والسيرم المتعادل

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توضح هذه الدراسة تقييم طريقه الأمينوبيروكسيدز على خلايا وحيدة الطبقة فى لتحديد ومعايرة الأجسام المناعية المضادة لڤيروس الحمى القلاعية $O_1/93$ فى مصل النعاج المحصنة والنعاج المصابة تجريبياً بالڤيروس وأيضاً فى أمصال النعاج المجمعة عشوائياً من الحقل.

ولقد تم مقارنة النتائج المتحصل عليها من الأمينوبيروكسيدز على خلايا وحيدة الطبقة مع النتائج المتحصل عليها من طريقتى الأليزا والسيرم المتعادل التى أجريت على عينات أخذت من النعاج بعد شهر من التحصين بلقاح الحمى القلاعية فوجد أن كل العينات الإيجابية لطريقتى الاليزا والسيرم المتعادل كانت أيضاً إيجابية باستخدام طريقة الأمينوبيروكسيدز بقوة عيارية (١ر٣٥ - ١ر٧٧ - ١ر٨٣) لو ١٠ $TCID_{50}$

٣٠ من ٥٠ عينة حقلية كانت إيجابية لطريقتى الأليزا والأمينوابيروكسيدز من ٣٠ عينة ٢٧ عينة (٩٠٪) أعطت نتائج بقوة عيارية (٦ر - ١ر٥) لو ١٠ $TCID_{50}$. فى حال النعاج المحصنة والمصابة تجريبياً وجد أن الأجسام المناعية المضادة لڤيروس الحمى القلاعية يمكن تحديدها من ٥-٧ أيام بعد التحصين أو الحقن باستخدام طريقتى الأليزا والأمينوبيروكسيدز. كانت نسبة التوافق بين طريقتى الأليزا والأمينوبيروكسيدز (١٠٠٪) بينما التوافق بين طريقتى السيرم المتعادل والأمينوبيروكسيدز (٩٠٪)، ولقد تمت مناقشة إمكانية تطبيق طريقة الأمينوبيروكسيدز كطريقة دقيقة وسريعة لتحديد الأجسام المناعية المضادة لڤيروس الحمى القلاعية.