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Dramatic Improvement in the Efficacy of Foot and Mouth Disease Vaccines by Co-administration of Echinacea

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SUMMARY

Background. Foot and mouth disease (FMD) is a highly contagious and economically devastating disease of cloven-hoofed animals. The goal of the present study was to increase the efficacy of FMD vaccines by co-administration of Echinacea (herbal medicine for enhancing the immune system).

Methods. Two dairy farms (I&II) containing 1620 cows and calves were firstly monitored by nonstructural proteins (NSPs) antibody test for identifying the previous or current infections. The non-infected calves of 4 months old were selected in the 2 farms and divided into 4 groups of 90 calves. Group A was vaccinated with imported FMD vaccine (Raksha-Ovac, India) alone. Group B was vaccinated with imported FMD vaccine associated with Echinacea. Group C was vaccinated with local FMD vaccine alone and, Group D was vaccinated with local FMD vaccine associated with Echinacea. The efficacy of such vaccine either alone or associated with Echinacea were evaluated by determination of the antibody titers in the sera of vaccinated animals by ELISA and SNT.

Results. The results of NSPs antibody test revealed that 12 animals out of 820 were infected in farm I while 10 out of 800 were infected in farm II. ELISA and SNT results showed that, the protective antibody level was higher in sera of group B than group A in farm I and in group D than group C in farm II. This level was generally higher in farm I than farm II and continued until the 32nd and 40th week post-vaccination in group A and B of farm I respectively while in farm II it

continued until the 20th and 24th week post-vaccination in group C and D, respectively.

Conclusion. The association of Echinacea significantly enhanced the efficacy of both FMD local and imported vaccines and this is the first paper, to our knowledge, that described the association of Echinacea with FMD vaccines in the veterinary field with its use in treatment of infected animals.

Keywords: FMD vaccines; Echinacea; Nonstructural proteins, Cattle.

INTRODUCTION

Foot and mouth disease (FMD) is a highly contagious disease of mammals and has a great potential for causing severe economic losses in susceptible cloven-hoofed animals and trade disruptions in animals and animal products. Due to its highly contagious nature and economic importance for many countries, FMD is the first disease for which the Office International des Epizooties (OIE) established an official list of free countries and zones. It is caused by FMD virus (FMDV) which has seven serotypes namely, O, A, C, SAT1, SAT2, SAT3 and Asia1, within which are numerous variants necessitating careful selection of vaccine strains because infection with one serotype does not confer immunity against another (OIE Terrestrial Manual, 2008). In Egypt, several outbreaks of FMD were caused by the new serotype, A, other than the endemic serotype, O_1, which were reported during 2006, and vaccination by bivalent vaccines containing strains O_1 and A was undertaken (OIE Disease Information, 2006 a & b). Typical cases of FMD are characterized by the appearance of vesicles on the feet, in and around the oral cavity, and on the mammary glands of females. Clinical findings can vary from mild to severe and fatalities may occur, especially in young animals due to a multifocal myocarditis. Mastitis is a common sequel of FMD in dairy cattle (Radostits et al., 2007).

Significant progress has been made in the development of diagnostic methods for detection of antibodies to FMDV non-structural proteins (NSPs), which can indicate the previous virus infection, irrespective of vaccination status (De Diego et al., 1997; Mackay et al., 1998). NSP 3ABC antibody is considered to be the most reliable indicator of present or past infection with FMDV in vaccinated animals (De Diego et al., 1997; Lu et al., 2007).

Many inactivated commercial FMD vaccines are multivalent to provide cover against the different serotypes likely to be encountered in a given field situation. Although vaccines inactivated chemically are able to control the disease successfully, these traditional vaccines provide only short-term protection (4-6 months) against infection by FMDV of the same serotype as the vaccine strain (Barterling 2002). The need for

improving the efficacy of such vaccine and prolonging the induced protection is necessary.

Echinacea, also known as purple coneflower, has become a top-selling medicinal herb and food supplement in Europe and the United States (Ernst 2002). There is evidence that Echinacea has the potential to treat a broad range of infectious diseases (Hartman et al., 2008). Meta-analysis on 234 articles suggested that standardized extracts of Echinacea were effective in the prevention of induced Rhinovirus colds (Schoop et al., 2006). It exerts many immunological functions both *in vivo* and *in vitro* and has the capacity to annihilate tumors and reduce viral infections (Melchart et al., 1995, Bauer 1996). In support of this approach, the present study was conducted to increase the efficacy of local bivalent inactivated FMD vaccine that contains FMD virus stains O_1/3/93 and A/Egypt/1/06 locally isolated in Egypt and imported (Raksha-Ovac) FMD vaccine that contains inactivated FMD virus stains O_1, A_{22} and A_{96} by co-administration of Echinacea in the field situation. In parallel, the previous or current infections with FMD virus in the dairy farms under study were diagnosed and controlled.

MATERIAL AND METHODS

Animals and clinical examination:

The present study was carried out between January 2008 and December 2008 in 2 separate private farms [I (n=820) & II (n=800)] of multi-aged dairy cows and calves (Table 1). Three hundreds sixty calves of 4 months old were selected for testing the efficacy of local and imported FMD vaccines, based on that the interference of maternal immunity could be avoided at this age. These calves were well identified by numbers and housed within the farms in separate groups. All these calves were clinically normal and their sera, before vaccination, were negative to ELISA antibody titers either to nonstructural or structural proteins of FMD virus (See results part). All animals were subjected to clinical examination starting from inspection to systematic examination of each body system. Detailed past and immediate history and management practices were also recorded. The infected animals (positive to NSPs ELISA antibody test, n=22) were isolated then subjected to thorough clinical examination in the morning and evening and closely monitored for one month to assess day-to-day changes. The infected animals in farm I (n=12) were treated with Echinacea only [Mulone (Atos Pharma, Egypt), 12 ml/day, orally for 5 successive days] while the infected animals in farm II (n=10) were not treated and used as a control (Table 1).

Samples:

Sera were separated from blood samples that were collected 11 times, one time pre-vaccination from all animals in 2 farms (n=1620) and 10 times, at 4 weeks intervals, post-vaccination from all selected calves (n=360). The serum samples were stored at -20°C until tested.

FMD virus:

FMD virus stains O_1/3/93 and A/Egypt/1/06 locally isolated in Egypt and of cattle origin were used in this study for ELISA and SNT.

Identification of the previous or current infections:

All pre-vaccination collected sera were tested by the nonstructural proteins (NSPs) antibody test using Chekit-FMD-3ABC bo-ov ELISA kit (Bommeli Diagnostics Liebefeold-Bern, Switzerland) for identifying the previous or current infections with any serotypes of the virus, whether or not the animal has also been vaccinated (Table 1). The test procedure was carried out as described by the manufacturer.

FMD vaccines:

Two FMD vaccines were used in this study (Table 2)

(i) Local bivalent inactivated FMD vaccine that contains FMD virus stains O_1/3/93 and A/Egypt/1/06 locally isolated in Egypt, inactivated by 2- bromoethylamine hydrobromide (BEI) and adjuvanted with aluminum hydroxide gel and purified saponin (Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt).

(ii) Imported FMD vaccine (Raksha-Ovac) that contains inactivated FMD virus stains O_1, A_{22} and A_{96} adjuvanted with mineral oil. Complete inactivation of the virus is ensured by the use of Aziridine compound (Indian Immunologicals Limited, Jubilee Hills, Hyderabad, India).

Vaccination schedules:

The selected calves in farm I (n=180) were divides into 2 groups, group A (n=90) that was vaccinated by imported FMD vaccine alone and group B (n=90) that was vaccinated with imported FMD vaccine associated with Echinacea [Mulone (Pure standardized Echinacea juice, Atos Pharma, Egypt), 12 ml/day, orally for 5 successive days (Table 2). In parallel, the selected calves in farm II (n=180) were divided into 2 groups, the group C (n=90) that was vaccinated with local FMD vaccine alone and the group D (n=90) that was vaccinated with local FMD vaccine associated with Echinacea (Mulone, 12 ml/day, orally for 5 successive days). Each group received two doses of such vaccine with 4 weeks interval (Table 2). At the same time, the group B and D in such farm received two co-administration period of Echinacea. All groups were vaccinated at the same time,

with the same vaccine batches. In addition, the rest of non-infected animals of other ages (Clinically normal and negative to NSPs antibody test) were vaccinated by the same type of vaccine in such farm without association of Echinacea.

Measurement of antibody titers:

(i) Indirect enzyme-linked immunosorbent assay (ELISA): It is used as serotype-specific serological test for measurement of antibody titers to structural proteins of FMD virus. It was performed as previously described by [Hamblin et al., 1986; Chenard et al., 2003]. Absorbance was measured at 492 nm. The antibody titer of each serum sample can be calculated from the following formula: Antibody titer = optical density (OD) sample – OD negative/ OD positive – OD negative. Ratio 1.0 or more than 1.0 means positive result. Cut off of FMDV is 1.5.

(ii) Serum neutralization test (SNT): It is also used as serotype-specific serological test for measurement of antibody titers to structural proteins of FMD virus but it can minimized the occurrence of false-positive results with ELISA. It was carried out according to (OIE Terrestrial Manual 2008). End-point titers were calculated as the reciprocal of the highest serum dilution to neutralize 100 $TCID_{50}$ of the virus. Results were expressed as the log_{10} SN titer±SD.

Both ELISA and SNT were carried out in FMD Department, Veterinary Serum and Vaccine Research Institute, Abassia, Cairo.

Statistical analysis:

Results were expressed as the mean±standard deviation (SD). ANOVA test was used to compare the antibody immune responses between different groups. P values of <0.05 were considered significant statistically.

RESULTS

Clinical examination & Identification of the previous or current infections :

The clinical examination before non-structural proteins (NSPs) ELISA antibody detection revealed that all animals were clinically normal. The NSPs ELISA antibody detection based on 3ABC polypeptides by using Chekit-FMD-3ABC bo-ov ELISA kit showed that 12 animals out of 820 (1.5%) were infected in farm I while 10 out of 800 (1.3%) were infected in farm II (Table 1). These infected animals were isolated then subjected to thorough clinical examination in the morning and evening and closely monitored for one month to assess day to day changes. Four infected animals out of 12 (33.33%) in farm I and 3 out of 10 (30%) in farm II showed mild clinical findings in the form of reduction of milk yield and short period of fever. All infected animals in farm I with were treated with Echinacea only [Mulone (Atos Pharma, Egypt), 12 ml/day, orally for 5 successive days] while the infected animals in farm II were not treated

and used as a control. A good symptomatic response was obtained with administration of Echinacea without observed side effects as proved by absence of clinical findings in farms I and its persistence in farm II (Table 1). After that, monthly clinical inspections of animals from all age groups on these farms were undertaken as part of the herd health-monitoring program. No clinical evidence of new FMD infection was present in any of these farms by the end of this study.

Serotype-specific antibody titers after vaccination as determined by ELISA:

The serotype-specific antibody titers in non-infected vaccinated calves of 4 months old were firstly measured by ELISA using FMD virus type O_1/3/1993 or A/Egypt/1/2006 (Table 3). The results indicated that the induced antibody titers were detected by the 4th week after the 1st dose with higher titers in group B and D in the farms I and II, respectively. Then the antibody titers increased gradually after the booster dose and reached the maximum by the 8th week with higher titers in group B and D. This maximum titer persisted until the 16th and 20th week in group A and B respectively of farm I while in farm II it persisted until the 12th week and 16th week in group C and D respectively. After that, the antibody titers decreased gradually till reaching the minimal protective level at the 32nd and 40th week post-vaccination in group A and B of farm I, respectively while in farm II it continued until the 20th and 24th week post-vaccination in group C and D respectively). No significant difference in titers between the two FMD virus strains used in ELISA (results not shown for A/Egypt/1/2006 strain).

Serotype-specific antibody titers after vaccination as determined by SNT :

The same tested sera by ELISA were retested by SNT using FMD virus type O1/3/1993 or A/Egypt/1/2006 (Table 4). The results obtained by SNT were correlated to that obtained by ELISA but the serotype-specific antibody titers were lower. Hence, The SNT titer of 1 in 16 ($\log_{10}$ = 1.2) Corresponds to an ELISA titer of 1in 32 ($\log_{10}$ = 1.5) No significant difference in titers between the two FMD virus strains used in SNT (results not shown for A/Egypt/1/2006 strain).

Table 1: Identification of the previous or current infections by using Chekit-FMD-3ABC bo-ov ELISA kit* and treatment of infected animals with Echinacea

Farm	No. of tested serum samples	No. of negative 3ABC	No. of positive 3ABC ** (%)	No. of positive 3ABC showed mild clinical findings (%)	Treatment with Echinacea
Farm I	820	808	12 (1.5%)	4 (33.33%)	Treated (absence of clinical signs)
Farm II	800	790	10 (1.3%)	3 (30%)	No treated (persistence of clinical signs)
Total	1620	1598	22 (1.4%)	7 (31.8%)	-

*The test is a blocking ELISA that measures the competition between test sera and a nonstructural proteins (NSPs) specific monoclonal antibody for the binding to the polypeptide 3ABC NSP of FMD virus.
**The 3ABC NSP antibody is considered to be the most reliable indicator of present or past infection with FMD virus in vaccinated animals.

Table 2: Vaccination schedules

Farm	Group (n)	Type of vaccine	Vaccination Regimen	Route
Farm I	A (n=90)	Imported FMD vaccine alone	First dose : 2ml; Booster dose: 2ml (4 weeks interval)	IM
	B (n=90)	Imported FMD vaccine associated with Echinacea	The same regimen but in association with Echinacea (12ml/day, orally for 5 successive days with 1st & 2nd doses of vaccine)	
Farm II	C (n=90)	Local FMD vaccine alone	First dose : 2ml: Booster dose: 2ml (4 weeks interval)	SC
	D (n=90)	Local FMD vaccine associated with Echinacea	The same regimen but in association with Echinacea (12ml/day, orally for 5 successive days with 1st & 2nd doses of vaccine)	

SC: Subcutaneous; IM: Intramuscular

Table 3 Mean antibody titers after vaccination as determined by ELISA

Period post 1st vaccination (Week)	Farm I		Farm II	
	Group A (Mean±SD) *	Group B (Mean±SD) *	Group C (Mean±SD) *	Group D (Mean±SD) *
0	0±0	0±0	0±0	0±0
4	1.7±0.2	1.95±0.3	1.5±0.22	1.87±0.23
8**	3±0.47	4±0.37	2.7±0.46	3.3±0.27
12	3±0.44	4±0.36	2.7±0.48	3.3±0.25
16	3±0.41	4±0.33	2.1±0.52	3.3±0.22
20	2.4±0.5	4±0.42	1.5±0.61	2.7±0.45
24	2.1±0.38	3.3±0.58	1.23±0.39	1.57±0.38
28	1.8±0.48	2.7±0.35	0.97±0.45	1.4±0.32
32	1.5±0.53	2.3±0.27	0.86±0.46	1.1±0.39
36	0.89±0.36	2.1±0.31	0.49±0.47	0.7±0.42
40	0.44±0.29	1.5±0.39	0.13±0.2	0.3±0.5
Overall mean±SD	2.0145±0.08[b]	2.93±0.07[a]	1.50±0.07[c]	1.98±0.08[b]

Group A, farm I: Calves vaccinated with imported FMD vaccine alone.
Group B, farm I: Calves vaccinated with imported FMD vaccine associated with Echinacea.
Group C, farm II: Calves vaccinated with local FMD vaccine alone.
Group D, farm II: Calves vaccinated with local FMD vaccine associated with Echinacea.
*: Results are expressed as the means Log_{10} titers ± SD.
* *: Titers after the booster vaccination started from the 8th week.
a, b, c: Means within the same row carrying different litters are significant at *(P <0.05)*

Table 4 Mean antibody titers after vaccination as determined by SNT

Period post 1st vaccination (Week)	Farm I		Farm II	
	Group A (Mean±SD)*	Group B (Mean±SD)*	Group C (Mean±SD)*	Group D (Mean±SD)*
0	0±0	0±0	0±0	0±0
4	1.3±0.4	1.58±0.37	1.1±0.51	1.43±0.39
8**	2.6±0.45	3.7±0.35	2.3±0.56	3.01±0.43
12	2.6±0.62	3.7±0.46	2.3±0.47	3.01±0.6
16	2.6±0.63	3.7±0.43	1.71±0.4	3.01±0.49
20	2.01±0.36	3.7±0.38	1.2±0.37	2.35±0.46
24	1.83±0.31	3.3±0.27	0.81±0.43	1.33±0.53
28	1.47±0.48	2.33±0.35	0.55±0.39	1.03±0.37
32	1.21±0.37	1.97±0.29	0.3±0.46	0.87±0.42
36	0.39±0.39	1.65±0.34	0.1±0.41	0.42±0.47
40	0.11±0.52	1.21±0.44	0.01±0.6	0.13±0.5
Overall mean±SD	1.75±0.07[b]	2.64±0.08[a]	1.12±0.07[c]	1.70±0.08[b]

Group A, farm I: Calves vaccinated with imported FMD vaccine alone
Group B, farm I: Calves vaccinated with imported FMD vaccine associated with Echinacea
Group C, farm II: Calves vaccinated with local FMD vaccine alone
Group D, farm II: Calves vaccinated with local FMD vaccine associated with Echinacea
*: Results are expressed as the means Log_{10} titers ± SD
** : Titers after the booster vaccination started from the 8th week.
a, b, c: Means within the same row carrying different litters are significant at *(P <0.05)*

DISCUTION

Actually, many developing countries vaccinate their animals with inactivated virus, which has proved to be effective and in future, a policy of "vaccinate-to-live" may be included in the repertoire of FMD control measures. In support of this approach, our study was aimed to increase the efficacy of local (contains inactivated FMD virus stains O_1/93 and AEGY/06 locally isolated in Egypt) and imported (Raksha-Ovac that contains inactivated FMD virus (FMDV) stains O_1, A_{22} and A_{96}) FMD vaccines by co-administration of Echinacea under the field conditions.

The present study identified the animal status in 2 dairy farms containing 1620 multi-aged dairy cows and calves and the results indicated that the detection of antibodies to NSP 3ABC was useful for identifying virus activity in the herd, differentiation between infected and vaccinated animals and evaluation of the efficiency of control measures adopted during or after the disease outbreak. This test is based on the assumption that semi-purified inactivated FMDV vaccines mainly contain capsid (structural) proteins, so they are less likely to elicit production of antibodies against non-structural proteins (NSPs). The NSPs antibody response is only related *in vivo* to the virus activity (De Diego *et al.*, 1997; Mackay *et al.*, 1998). The NSP 3ABC antibody is considered to be the most reliable indicator of present or past infection with FMDV in vaccinated animals, regardless of serotype of the virus involved (De Diego *et al.*, 1997, Lu *et al.*, 2007).

The infected animals in this study were treated with Echinacea only that gave a good symptomatic response. No clinical evidence of new FMD infection was present by the end of this study. This is the first report, to the best of our knowledge that used Echinacea in treatment of FMD in ruminants. It exerts many immunological functions both *in vivo* and *in vitro* and has the capacity to reduce viral infections (Melchart *et al.*, 1995; Bauer 1996). It also controls vesicular stomatitis virus that induced lesions resembling that of FMDV in ruminants (Binns *et al.*, 2002), enhances resistance to influenza viruses (Senchina *et al.*, 2005) and enhances phagocytosis when administered orally to mice (Currier and Miller 2002) and humans (Kim *et al.*, 2002) with anti-inflammatory activities (Raso *et al.*, 2002).

The selected calves for vaccination were at 4 months of their age to avoid the interference of maternal immunity and this was in agreement with Kitching *et al.*, (2007) who reported that cattle are normally vaccinated twice with inactivated FMD vaccine, starting at an age when any maternally derived immunity will no longer interfere with the development of active immunity (4-6 months). Although vaccines chemically inactivated are able to control the disease successfully, these traditional vaccines provide only short-term protection against infection by FMDV of the same serotype as the vaccine strain (Barterling 2002). The need for improving the efficacy of such vaccine and prolonging the induced protection is necessary. Therefore, we associated Echinacea with the two FMD vaccines used in this study, and then the efficacy of such vaccine was determined by measurement of the antibody titers in sera of vaccinated animals using ELISA and SNT. The obtained results showed that the association of Echinacea significantly enhanced the efficacy of both FMD local and

imported vaccines. The results obtained by SNT were correlated to that obtained by ELISA but the serotype-specific antibody titers were lower. SNT test depend on tissue cultures and are therefore more prone to variability than ELISA. It is also slower and subject to contamination (OIE Terrestrial Manual 2008). The relationship between ELISA and SNT titers was determined and the results showed that a SNT titer of 1 in 16 (Log_{10}=1.2) corresponds to an ELISA titer of 1 in 32 (Log_{10}=1.5). This finding approximately agreed with the observation of Hamblin *et al.*, 1986, who reported that a SNT titer of 1 in 16 (Log_{10}=1.2) corresponds to an ELISA titer of 1 in 40 (Log_{10}=1.6). Echinacea is a well-studied herb noted for stimulating the human immune system. In our study, the Echinacea increased the antibody titers that indicated the stimulation of humoral immunity. On the other hand, recent investigations have also shown that macrophage stimulation and the induction of cytokines are major parts of its mode of action (Bauer 2002).

In summary, this study showed some significant improvements to the efficacy of both FMD local and imported vaccines by the association of Echinacea and this is the first paper, to our knowledge, that described the association of Echinacea with FMD vaccines and used it in treatment of animals infected with FMD. Further studies are needed to clarify the mechnanism(s), which are responsible for the beneficial effect of Echinacea preparations and demonstrate the additional effect of Echinacea on sero-conversion against FMD virus strains after virus challenge.

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زيادة كفاءة لقاح مرض الحمي القلاعية بإستخدام الإكينيتسيا

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**قسم الفيرولوجي- كلية الطب البيطري - جامعة الزقازيق

الهدف من الدراسة: مرض الحمّى القلاعية (إف إم دي) هومرض وبائي للغاية ومدمر إقتصادياً ويصيب الحيوانات المشقوقة الحافر. لذلك كان الهدف من هذه الدراسة هو زيادة كفاءة اللقاحات المضادة لل إف إم دى وذلك باستخدام الإكينيتسيا (عشب طبي لزيادة كفاءة الجهاز المناعي).

الطرق: مزرعتا ألبان تحتويان علي 1620 بقرةً وعجل قد تم فحصهم باختبار الإليزا التشخيصي 3 أي بي سي (Chekit-FMD-3ABC bo-ov ELISA kit) وذلك لتشخيص الإصابات السابقة أو الحالية بفيروس إف إم دي في هاتين المزرعتين وللتفرقة بين تلك الحيوانات المصابة والمحصنة و ذلك بالكشف عن البروتين غير البنائي3 أي بي سي. تم اختيار العجول الغير مصابة فى المزرعتين والتى تبلغ من العمر 4 أشهر و قسمت إلى 4 مجموعات بكل منها 90 عجل.قد تم تحصين المجموعة (أ) بلقاح إف إم دى المستورد (راكشا – أوفاك ، الهند) وحده ، كما تم تحصين المجموعة (ب) بلقاح إف إم دى المستورد مصحوباً بالإكينيتسيا. أما المجموعة (ث) فقد تم تحصينها بلقاح إف إم دى المحلى وحده ، و المجموعة (د) تم تحصينها بلقاح إف إم دى المحلى مصحوباً بالإكينيتسيا. كفاءة كلا اللقاحين سواء لوحدهما أو مصحوبين بالإكينيتسيا تم تقييمهما بتحديد الأجسام المناعية الناتجة فى الحيوانات المحصنة باستخدام اختبار الإليزا واختبار المصل المتعادل.

النتائج: أظهرت نتائج اختبار الإليزا التشخيصي 3 أي بي سي عن اصابة 12 حيوان من أصل 820 في المزرعة الأولى و 10 من أصل 800 في المزرعة الثانية. في حين أسفرت نتائج اختبار الإليزا واختبار المصل المتعادل بعد التحصين عن زيادة معنوية في الأجسام المناعية للمجموعة (ب) عن المجموعة (أ) بكلا المزرعتين. هذه الزيادة في الأجسام المناعية كانت بصفة عامة أعلى في المزرعة الأولي عن الثانية واستمرت حتى الأسبوع الثاني والثلاثون والأسبوع الأربعون في المجموعة (أ) و(ب) على التوالي بالمزرعة الأولى بينما استمرت حتى الأسبوع العشرون والأسبوع الرابع والعشرون في المجموعة (أ) و(ب) على التوالي بالمزرعة الثانية.

الخلاصة: لقد أدى استخدام الإكينيتسيا الى زيادة كفاءة لقاحي مرض الحمى القلاعية المحلي والمستورد بشكل معنوي ويعتبر هذا أول بحث يستخدم الإكينيتسيا، الى حد معرفتنا، مع لقاحات مرض الحمى القلاعية وفي علاج الحيوانات المصابة بذات المرض بالحقل البيطرى.