Field Evaluation of Sheep Pox Vaccine in Sheep and Goats in Egypt

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Abstract

The Capripoxvirus (CaPVs) genus includes Sheep pox virus (SPV), Goat pox virus (GPV) and lumpy skin disease virus (LSDV) which poses cross protection and related diseases of major economic importance. In the present study, living attenuated sheep pox vaccines (Romanian strain) was used to vaccinate sheep and goat to be evaluated under field conditions. Twelve susceptible sheep and goats (7 sheep and 5 goats) were intra-dermally vaccinated with live attenuated SPV vaccine. The humeral immunity was checked by double antigen ELISA while non-specific cellular immune responses were detected by measuring lysozymes concentration, nitric oxide concentration, and interferon gamma (IFN-γ) concentration by realtime PCR. Humeral immune response evaluation in vaccinated sheep showed that the immune response increased gradually at the 7th and 14th DPV and reached the highest level on the 28th day with a mean of 2.4. While in goats, antibody response begins at the 7th DPV, and the peak response was at the 28th DPV (2.2±0.106). The lysozymes concentrations in vaccinated sheep showed a gradual increase till reach their peak on the 14th days (76.3±2.378 µg /ml), while in goat's serum, the lysozymes concentrations gradually increased till reached 65.30± 5.37 µg /ml at the 7th DPV then decreased at the 14th & 28th DPV. Nitric oxide concentration in sheep serum showed a gradual increase till reached its peak at 7th DPV and decreased at the 14th and 28th DPV, while in goat's nitric oxide concentration showed also a gradual increase till reached the peak at the 7th DPV then decreased to the lowest level at 14th and 28th DPV. IFN-γ was expressed in vaccinated sheep at the 2nd DPV then decreased at the 7th DPV, while in goats, IFN-γ were expressed at the 2nd DPV and decreased at the 7th DPV. In conclusion, ELISA,

lysozyme and nitric oxide concentration and Gamma interferon expression proved to be reliable and sensitive for the assessment of vaccine potency. Heterologous Capripox vaccine can be used efficiently for the control of pox disease in goats as efficiently as in sheep.

Keywords: Capripoxvirus, sheep pox vaccine, nitric oxide, lysozyme, ELISA, IFN-γ.

Introduction

There are three important poxviruses diseases include Sheeppox (SPP), goatpox (GTP), and lumpy skin disease (LSD) which affect sheep, goats, and cattle respectively ***(Babiuk et al., 2008; Carn, 1993).*** These responsible viruses are large, complex, double-stranded DNA viruses of the genus Capripoxvirus, subfamily Chordopoxvirinae, and family Poxviridae ***(Fauquet et al., 2005).*** Those show 96%–97% nucleotide identity ***(Tulman et al., 2002)***. All CaPVs members are serologically indistinguishable and provide heterologous cross-protection. Sometimes CaPV can be experimentally cross-infected ***(Diallo and Viljoen, 2007)***. SPP and GTP In endemic areas show a serious economic impact on small ruminant production systems, causing major productivity losses, mortality, damaging skins and hides, as well as inflicting international trade restrictions ***(Hosamani et al., 2004).*** So it is listed as a notifiable disease of high economic importance that outbreaks have to be notified immediately to the World Organization for Animal Health ***(OIE, 2017).*** Direct contact between diseased and non-infected animals is the main mode of virus transmission besides the indirect transmission routes ***(Balinsky et al., 2008).*** The main characteristic clinical signs of SPPV and GTPV infections are ocular and nasal discharge with pock-like lesions in the skin and mucosae of the respiratory and gastrointestinal tracts ***(Yan et al., 2012).*** SPV and GTPV are widespread throughout Northern and Central Africa, the Middle East, the Indian subcontinent, Central Asia, China, Vietnam, and Russia ***(Tuppurainen et al., 2017)***. Despite vaccination efforts, only a few countries have successfully eradicated these diseases. To date, available vaccines are live attenuated specific for small ruminants and cattle. In some countries, people use SPPV to vaccinate against GTPV and LSDV ***(Brenner et al., 2009).*** Cross-protection between SPPV and GTPV has been already demonstrated for various strains and vaccination with LSDV Kenyan Sheep and Goat pox (KSGP) strain has also been widely

used for many years to protect small ruminants against SPPV and GTPV***(Abutarbush, 2017; Tuppurainen et al., 2017)***. Poorly conditioned animals, overcrowding, poor feeding, general mismanagement, and abnormal uses of vaccination are considered the main causes of the distribution of SPV and GPV ***(Zangana and Abdullah, 2013).*** Few studies assessed the field evaluation of vaccine efficacy at the field conditions ***(Abutarbush and Tuppurainen, 2018)***. Due to the inability to distinguish clinically or serologically between CaPVs infections and incomplete protection against heterologous vaccines, some researchers recommended the annual regular evaluation of the currently used vaccines at the field conditions in addition to the need for more reliable tests for monitoring the humoral and cellular immune response and strain identification based on molecular methods ***(Hosamani et al., 2004).*** The aim of the present study was the field assessment of attenuated sheep pox vaccine at field conditions. To achieve this aim, 7 sheep and 5 goats were vaccinated intradermally with live attenuated SPV vaccine, and 6 animals were used as the non-vaccinated control group (3 sheep & 3 goats). Both humeral and cellular immune responses were evaluated by ELISA, nitric oxide detection, lysozyme concentration, and IFN gamma detection.

Material and Methods

1.Animals:

Ten sheep (1-2 years of age) participated in this research. 7 sheep were vaccinated intradermally with live attenuated SPV vaccine and 3 animals were used as a non-vaccinated control group with 8 goats (1-2 years of age), 5 goats were vaccinated intradermally with live attenuated SPV vaccine and 3 were used as a non-vaccinated control group. All tested animals proved to be seronegative to the SPV vaccine by ELISA test.

2.Vaccine

attenuated Sheep pox virus vaccine (Romanian strain) supplied from Servac, Veterinary Serum and Vaccine Research Institute (VSVRI), Egypt (batch NO.2038) dosage (0.5ml for sheep), kept at -20°C, package (20 doses for sheep), the route by I/D injection.

3.Serum samples

Blood samples were collected from all animals vaccinated with sheep pox Vaccine before vaccination and after vaccination in separate tubes. Sixty blood samples from sheep vaccinated with sheep pox vaccine and 48 blood samples from goats vaccinated with sheep pox vaccine. Serum sample of the sheep pox vaccine was collected in separate tubes before vaccination, the 2nd day, 7th day, 14th day, 28th day, 7th week, and 4th month of vaccination. 5 ml/ sample was collected from the jugular vein and then allowed to

clot without anticoagulant and then separated and kept frozen at -20˚c till humeral and cellular immunity evaluation.

4.ELISA (for detection of Capripoxvirus antibodies)

Double antigen ELISA (IDvet Company, FRANCE) is designed to detect antibodies against SPV and GPV was conducted according to the instructions of the manufacturers. The kits contain microplates coated with CPV purified antigen, a CPV purified antigen labeled with horseradish peroxidase conjugate, positive control, negative control, dilution buffer, wash concentrate(20x), a substrate solution (TMB), and stop solution (0.5M).

5.**Evaluation of nitric oxide concentration in serum**

100µl from each sample was transferred into the flat bottom 96 well ELISA plate and 100µl of Griess reagent was then incubated at 21˚C for 10 minutes, the absorbency of the sample and standards was measured at 570nm using an ELISA reader. The absorbency of tested samples was converted to micromolar of nitrite by comparison with absorbance values of sodium nitrite standard curve within a linear curve fit according to ***(Ramadan and Attia, 2003).***

6.Evaluation of lysozyme concentration in serum

The lysozyme diffuses through the agarose gel containing a suspension of Micrococcus Lysodeikticus, and a clear zone ring of lysis develops in the initially translucent agarose gel. All procedures and calculations performed according to ***(Ramadan and Attia, 2003; Schultz, 1987).***

7.SYBER Green real-time PCR for detection of IFN Gamma

RNA was extracted using RNA easy mini kit (Qiagen) Catalogue no.74104 according to the manufacturer's instructions. Quantitect SYBR green PCR kit used for the preparation of PCR master mix according to manufacturer's instructions. Samples were checked using RT-PCR for detection of IFN-γ using specific primers and probes ***(Harrington et al., 2007)*** Table (1) using cycling conditions according to manufacturer's instructions (table 2). Amplification curves and CT values were determined by the Stratagene MX3005P software. To estimate the variation of gene expression on the RNA of different samples, the CT of each sample was compared with that of the control group according to the"ΔΔCt" method stated by ***(Yuan et al., 2006).***

8.**Statistical analysis**

Data obtained from vaccinated subjects were compared with non-vaccinated groups by the student t-test and SPSS programs to determine the mean and standard deviation and statistical significance of differences between the two groups as described by ***(Snedecor and Cochran, 1982).*** P

values below 0.05 were regarded as statistically significant.

Results

Evaluation of the humeral immune response of sheep vaccinated with sheep pox vaccine by ELISA:

The immune response increased gradually to be detected by the 7th & 14th DPV with mean ELISA absorbance of 1.18±.1461 & 1.7±.1297 respectively, then reached the highest level at 28th DPV with a mean of 2.4±.11518 and declined to 1.27±.223 at the 7th week PV (Table 3).

Evaluation of the humeral immune response of goat vaccinated with sheep pox vaccine by ELISA:

Antibody response begins at the 7th DPV with a mean O.D of 1.29 ± 0.1347 and increased up to 1.60 ± 0.137 at the 14th DPV. The peak response was obtained at the 28th DPV 2.2±0.106 and decreased till reached 0.93 ± 0.2223 at the 7th week PV. When the ELISA O.D of vaccinated and non-vaccinated groups was compared, a significant increase in ELISA O.D showed (P< 0.05) at 7 weeks PV (Table 4).

Evaluation of nitric oxide concentration of sheep vaccinated with attenuated SPV vaccine:

Nitric oxide concentrations in sheep serum showed a gradual increase in mean till reached 8.8 ± 3225 µmol /ml at 7th DPV then decrease to the lowest level at 14th and 28th DPV (5.5±.2091 and 4.1±.175 16 µmol /ml). When vaccinated and non-vaccinated groups were compared, a significant increase in nitric oxide concentration showed (P< 0.05) at 7th DPV (Table 5).

Evaluation of nitric oxide concentration of goat vaccinated with attenuated SPV vaccine:

Nitric oxide concentrations in goat serum showed a gradual increase in mean till reached 7.4 ± 0.58258 µmol /ml at 7th DPV then decrease to the lowest level at 14th & 28th DPV (5.5±.296 and 3.9 ± 0.129 µmol /ml) (Table 6).

Evaluation of lysozymes concentration in serum of sheep vaccinated with sheep pox vaccine.

The mean concentrations of serum lysozymes showed a gradual increase in mean serum concentrations of lysozymes from 48.6±1.8714 µg/ml at the 7th DPV then reach its peak at the 14th DPV (76.3±2.378 µg /ml) then decrease to the lowest level at 28th DPV(30.8±3.3274 µg /ml) (Table 7).

Evaluation of lysozymes concentration in serum of goats vaccinated with sheep pox vaccine.

The mean concentrations of lysozymes revealed a gradual increase till reached 65.30± 5.37 µg /ml at the 7th DPV then decreased at the 14th DPV (54.39±2.836 µg /ml) and (31.40±0.64048) at 28th DPV. When lysozyme concentrations of vaccinated and non-vaccinated groups were

compared, significant increase showed ($P< 0.05$) at 7th DPV (Table 8).

Evaluation of interferon gamma expression in sera of sheep vaccinated with sheep pox vaccine:

IFN-γ was expressed at the 2nd DPV with a fold change ranging between 3.25 and 4.1 and decreased to an obviously low level at the 7th DPV with a fold change ranging between 1.10 and 1.74 (Table 9).

Evaluation of interferon gamma expression in sera of goats vaccinated with sheep pox vaccine:

IFN-γ were expressed at the 2nd DPV with a fold change ranging between 2.82 and 3.85 and decreased to an obviously low level at the 7th DPV with a fold change ranging between 1.07 and 1.74 (Table 10).

Table (1): *Primers and probes used in SYBER Green real-time PCR:*

Gene	Primer sequence (5-3)	Reference
IFN-γ	GCGCAAAGCCATAAATGAAC, F	*(Harrington et al., 2007)*
	CTCAGAAAGCGGAAGAGAAG, R	
	HEX-CAAAGTGATGAATGACCTGTGCCA-BHQ, P	

Probes contained either the 6-carboxyfluorescein (FAM) or hexachloro-6-carboxyfluorescein (HEX) reporter dye covalently attached at the 5′ end and the BHQ molecule covalently attached at the 3′ end. F, forward primer; R, reverse primer; P, probe.

Table (2): *Cycling conditions of real time PCR*

Reverse transcription	Primary denaturation	Amplification (40 cycles)		Dissociation curve (1 cycle)		
		Secondary denaturation	Annealing & Extension	Secondary denaturation	Annealing	Final denaturation
50˚c/30 min	94˚c/15 min	94˚c/ 15 sec	60˚c/45sec	94˚c/1 min	60˚c/1min	94˚c/1min

Table (3): *Mean ELISA O.D of sheep pox vaccinated sheep*

Groups	No.of animals	Days post-vaccination			
		7 days	14 days	28 days	7 weeks
Non-vaccinated	3	0.9±.033	0.5±.033	0.5±.11547	0.45±.0033
Vaccinated	7	1.18±.146	1.7±.1297	2.4±.11518	1.27±.223
P value		0.077	0.056	0.256	0.17

Table (4): *Mean ELISA O.D of sheep pox vaccinated goats*

Groups	No.of animals	Days post-vaccination			
		7 days	14 days	28 days	7 weeks
Non-vaccinated	3	0.90 ± 0.121	0.5±.033	0.50±.133	0.45±.00667
Vaccinated	5	1.29 ±0.134	1.60±.137	2.2±0.106	0.93±0.222
P value		0.397	0.107	0.940	0.046

Table (5): *Mean nitric oxide concentrations of sheep vaccinated with attenuated sheep pox vaccine*

Groups	No. of animals	Days post vaccination		
		7days	14 days	28 weeks
Non-vaccinated	3	2.4±.0333	3.2±.0577	2.5±.12019
Vaccinated	7	8.8±.3225	5.5±.2091	4.1±.17516
P value		0.046	0.167	0.111

Table (6): *Mean nitric oxide concentrations of goat vaccinated with attenuated sheep pox vaccine*

Groups	No. of animals	Days post vaccination		
		7days	14 days	28 weeks
Non-vaccinated	3	2.1±.06064	1.8±.033	1.5±.122
Vaccinated	5	7.4 ±.58258	5.5±.296	3.9±.129
P value		0.141	0.137	0.391

Table (7): *Mean lysozyme concentrations of sheep vaccinated with attenuated sheep pox vaccine.*

Groups	No. of animals	Days post vaccination		
		7days	14 days	28 weeks
Non-vaccinated	3	22.8±.29059	21.5±.18559	18.6±.03333
Vaccinated	7	48.6±1.8714	76.3±2.378	30.8±3.3274
P value		0.067	0.135	0.207

Table (8): *Mean lysozyme concentrations of goats vaccinated with attenuated sheep pox vaccine.*

Groups	No. of animals	Days post vaccination		
		7days	14 days	28 weeks
Non-vaccinated	3	19.80±0.1527	21.5±0.2333	18.6±0.03333
Vaccinated	5	65.30± 5.37	54.39±2.836	31.40±0.64048
P value		0.035	0.256	0.062

Table (9): *Interferon gamma expression in serum of sheep vaccinated with attenuated sheep pox vaccine.*

Animal	Before vaccination			2 days post-vaccination		7 days post-vaccination	
	Ct B2M	Ct sample	ΔΔ Ct	Ct sample	ΔΔ Ct	Ct sample	ΔΔCt
Non-vacci.	19.3	19.0	1.22	19.40	1.75	18.7	1.20
Vacci.1	18.7	19.00	1.01	18.32	3.50	17.2	1.30
Vacci.2	19.7	19.25	1.81	18.25	3.25	17.1	1.35
Vacci.3	18.2	18.30	0.89	18.77	3.31	17.3	1.22
Vacci.4	19.7	19.66	1.22	18.83	3.52	17.4	1.74
Vacci.5	19.4	19.70	1.34	18.80	3.85	17.9	1.10
Vacci.6	18.5	19.20	2.10	18.50	4.10	17.5	1.40
Vacci.7	19.7	19.60	0.75	18.8	3.70	17.9	1.30

Table (10): *Interferon-gamma expression in serum of goat vaccinated with sheep pox vaccine*

Animal	Before vaccination			2 days post vaccination		7 days post vaccination	
	Ct B2M	Ct sample	ΔΔ Ct	Ct sample	ΔΔ Ct	Ct sample	ΔΔCt
Non vacci	19.3	19.00	1.22	19.40	1.75	18.7	0.20
1	19.7	19.00	1.01	20.19	2.82	19.7	1.07
2	19.7	19.25	1.81	18.25	3.25	17.1	1.35
3	18.2	18.30	0.89	18.77	3.31	17.3	1.22
4	19.7	19.66	1.22	18.83	3.52	18.4	1.74
5	19.4	19.70	1.34	18.80	3.85	17.9	1.10

Discussion

Vaccination of susceptible animals is the basic route for the Prevention of Capripoxvirus infections in countries where the diseases exist ***(Achour et al., 2000)***. Despite that, the 3 capripoxviruses share major neutralization sites and cross-immunity has been reported, but not experimentally documented ***(Quinn et al., 2011; Tuppurainen et al., 2017).*** Vaccination has been considered to be the cheapest and most sustainable means of disease control in the enzootic situation like Egypt and the Middle East ***(Kallesh et al., 2009)*** especially the Romanian strain which are widely used in endemic countries to protect sheep and goats against Capripoxvirus disease As researchers confirmed that vaccination of goats with Romania strain induced cell-mediated immunity with a satisfactory lymphocyte proliferation ***(Abd-Elfatah et al., 2019).***

Sheep pox and goat pox are ancient diseases that are currently endemic in the Middle East, the Indian subcontinent, and Central and Northern Africa ***(Rao and Bandyopadhyay, 2000)***. Field evaluation of the immune response of such vaccines was carried out not only for sheep pox and goat-pox disease but also for several vaccines ***(Gibert et al., 2013).*** In this study, attenuated sheep pox vaccine was injected in sheep and goats. Both humeral and cellular immune responses of the two vaccines were evaluated. Enzyme-linked immunosorbent assay (ELISA) had already been proven to have great potential as a quantitative serological tool in the detection of antibodies against several viral infections including the poxviruses ***(Tuppurainen et al., 2017).*** Also, lysozymes, nitric oxide, and gamma interferon proved to have superiority in the evaluation of cellular immune response against virus infection ***(Norian and Azadmehr, 2017; Singh et al., 2011)***

Results documented in tables 4, 6, 8 & 10 revealed that heterologous sheep pox vaccine was used for the

vaccination of goats against goat pox virus disease, this is due to the cross-protection between CaPVs genus diseases as described by ***(Kitching and Taylor, 1985).*** Also, the results are parallel to that obtained by ***(Yogisharadhya et al., 2011)*** who mentioned that live attenuated vaccines are safe and can be used in pregnant animals and have been used for decades in Capripox endemic countries.

The antiviral activity of interferon may be mediated in at least two ways. First, IFNs can prevent the multiplication of viruses by inducing an antiviral state in host cells ***(Samuel, 1991).*** Second, they can activate other effector cells, such as macrophages, to confer antiviral protection. IFN-γ is the most potent macrophage activating factor and the only known cytokine with the capacity to induce iNOS in macrophages hence named immune interferon. Late expression of interferon-gamma after 7 DPV with sheep pox vaccine observed in Table 9 & Table 10 may be associated with the induction of nitric oxide by activated macrophages ***(Harris et al., 1995)***.

Data obtained from humeral immune response against sheep pox vaccine in sheep recognized in Table 3 showed that the immune response increased gradually to be detected by the 7th & 14th DPV as the mean ELISA absorbance were 1.18±.146 and 1.7±.1297 more than protective level (> 1) then reached to the highest level at 28th day with a mean of 2.4±.11518 and respectively then declined to 1.27±.223 at 7 weeks PV. The obtained results were documented by ***(Agag et al., 1992; Gomes et al., 2010)*** whose mentioned a significant rise of antibody titer was detected from the 21st to 42nd day post-inoculation also ***(Woods, 1988)*** reported that ELISA O.D ≥ 1 considered protective mean against Capripoxviruses. Cell-mediated immune responses of sheep vaccinated with living attenuated SPV vaccine were evaluated through the expression of IFN-γ by real-time PCR. Table 9 indicated that IFN-γ were expressed at 2nd DPV with a fold change ranged between 3.25 and 4.1 and decreased to an obvious low level at 7th DPV with fold change ranged between 1.10 and 1.74 compared to 1.2 fold change in non- vaccinated animals. These results agree with that obtained by ***(Khafagy et al., 2016).*** The study of sheep pox in goats revealed that vaccine could induce immunity against the disease within seven days of vaccination and reached a peak at 28 days as tested by ELISA test (table 4). The same results were obtained by ***(Baksi et al., 2016)***. Results of lysozyme, nitric oxide, and interferon-gamma expression in goats are nearly the same as obtained in sheep. Challenge test using a virulent field virus strain is commonly used for estimating the efficacy of live attenuated vaccine ***(Abbas et al., 2010)***. ELISA O.D can be used as

an alternative for measuring the protection against viral vaccine ***(Hamblin et al., 1987)***. In our study, we used ELISA O.D and IFN-γ to evaluate the protection level of living attenuated Capripox vaccine instead of challenge test as described by ***(Ramyar and Hessami, 1971)***. We can conclude that field evaluation of humeral and cellular immune response for sheep pox is very important in designing control strategies to sheep pox disease In Egypt. ELISA had been proven to have great potential as a quantitative serological tool in the detection of antibodies. Also, lysozymes, nitric oxide, and gamma interferon proved to have superiority in the evaluation and early monitoring of cellular immune response against the sheep-pox vaccine.

Conclusions

Based on the obtained results under field conditions, the humoral and cellular immune response of living attenuated sheep-pox vaccines were evaluated at the field conditions. ELISA and gamma interferon expression tests proved to be reliable and sensitive techniques for the assessment of vaccine potency. Significant differences between the two vaccines and the humeral and cellular immune tests were obtained. Furthermore, using of heterologous Capri pox vaccine is more efficient for the control of pox diseases in other animal species. Sheep-pox vaccine is efficiently used for the vaccination of goats against goat-pox disease.

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الملخص العربى

فيروسات جدرى الماغز تشمل فيروس جدرى الاغنام وفيروس جدرى الماعز ذوى الصلة المناعية والحماية المختلطه بين المرضين المسببين لامراض ذات اهمية اقتصادية لذلك حاولنا فى هذا البحث اثبات وجود الصلة بينهما باستخدام لقاح جدرى الاغنام الحى المضعف (العترة الرومانية) لتحصين كلا من الاغنام و الماعز لتقييم اللقاح على المستوى الحقلى. تم تحصيين عدد 12 حيوان (7 من الغنم و 5 من الماعز) المعرضة للمرض عن طريق حقن التحصيين داخل الادمه مع استخدام 6 حيوانات (3 من الماعز و 3 من الغنم) كمجموعه متحكمة غير محصنة. تم فحص المناعة الخلطية الناتجه عن التحصين باستخدام اختبار الاليزا مزدوج الانتجن فى حين تم تقييم المناعة الخلوية الغير متخصصة عن طريق تقييم تركيز الليزوزيوم و تركيز اكسيد النيتريك و الانترفيرون غاما باستخدام تفاعل البلمرة المتسلسل حقيقى الوقت.

المناعة الخلطية فى الاغنام باستخدام اختبار الاليزا اظهرت ارتفاع تدريجى فى اليوم السابع والرابع عشر بعد التحصين ووصلت الى اعلى درجاتها فى اليوم الثامن والعشرين بينما فى الاغنام بدأت المناعة فى الظهور عند اليوم السابع و وصلت اقصاها عند اليوم الثامن والعشرين اما تركيز الليزوزيوم فى الاغنام اظهر ايضا زيادة تدريجية فى المناعة حتى وصلت قمتها فى اليوم الرابع عشر فى حين تركيزه فى الماعز وصل اقصاه فى اليوم السابع ثم بدأ يقل تدريجيا فى اليوم الرابع عشر والثامن والعشرون بعد التحصين.
اكسيد النيتريك ايضا اظهر زياده تدريجية فى مناعة الاغنام حتى وصل قمته فى اليوم السابع بعد التحصين ثم بدأ يقل تدريجيا فيما بعد ذلك و كذلك اتبع نفس النمط فى الماعز المستخدمة فى التجربة وبالنسبة الى مستوى المناعة الخلوية والتى تم فحصها من خلال تعبير الانترفيرون غاما باستخدام تفاعل البلمرة المتسلسل الحقيقى لبوقت اظهر تواد فى اليوم الثانى والسابع بعد التحصين فى كلا من الاغنام والماعز المستخدمه فى التجربة.