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Preparation of Live Attenuated *Corynebacterium pseudotuberculosis* Vaccine against *Caseous Lymphadenitis* in Sheep

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Abstract

Caseous Lymphadenitis (CLA) is a chronic granulomatous disease commonly occur in small ruminants, caused by a gram positive *Corynebacterium pseudotuberculosis*. The present study aimed to identify the suitable attenuation method for *Corynebacterium pseudotuberculosis Ovis* and to evaluate the immunogenicity of the resulting live attenuated vaccine in sheep. Various attenuation methods were assessed, including; thermal treatment at 55 °C for 15 minutes, exposure to alkaline pH (pH11) and treatment with crystal violet (1/ 250.000 concentration). These methods resulted in significant alteration in the bacteria cultural and biochemical characteristics, as well as a reduction in its virulence by decreasing the invasive power of the ovine strain. The strain attenuated using crystal violet was selected for vaccine formulation. Ten native sheep were vaccinated with the crystal violet live attenuated *C. ovis* vaccine, humoral and cellular immune response were assessed. Results demonstrated that, the prepared live attenuated vaccine induce a robust immune response, indicating its potential efficacy. In conclusion, the developed live attenuated *C. ovis* vaccine is safe and eliciting a strong immune response in sheep which represents a promising strategy for controlling of the CLA infection in sheep with potential to reduce the economic impact of the disease in sheep industry.

Keywords: Attenuated vaccine, *C. ovis*, Gamma interferon, Lymphocyte proliferation, Sheep.

Introduction

Caseous lymphadenitis (CLA) is a chronic granulomatous disease commonly occur in small ruminants, caused by a gram-positive *Corynebacterium pseudotuberculosis.* CLA characterized by abscesses formation in lymph nodes and other internal organs such as lungs and liver. The disease responsible for significant economic losses in sheep industry due to decrease in the reproductive efficacy and reduction in meat and wool production of infected sheep. [1, 2]. Pseudotuberculosis is a chronic disease characterized by high morbidity and low mortality [3]. This disease is responsible for the formation of encapsulated abscesses containing thick, caseous pus. This may be present clinically in two forms: Visceral or internal form that affects the internal lymph nodes, and the superficial or external form that affects superficial lymph nodes and subcutaneous tissue [4, 1].

The etiological agent of CLA is *C. pseudotuberculosis*, which is a gram positive, intracellular, facultative anaerobic, and mycolic acid containing bacteria witch is phylogenetically related to *Mycobacterium tuberculosis*. This bacteria is highly resistant in the environment. Transmission occurs by direct contact with secretions from the abscess and shearing equipment [1]. The control of the disease is difficult because the bacteria is highly resistant to antibiotic therapy, the microorganism ability to survive in the environment for long time and also difficulties in detecting the subclinical infected animals [5]. So, animal's massive vaccination is still the best method for the disease

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control. There are different vaccines used for controlling this disease such as phospholipase D toxoid, formalin-killed bacterins, bacterial cell wall preparations and live attenuated bacteria [2].

The most effective method can trigger both types of immune response (cell mediated and humoral immunity) of animal was obtained by using live attenuated strain of *C. pseudotuberculosis* and express cytokine genes in case of caseous lymphadenitis in sheep [6, 7]. Different methods used for attenuation of C. *pseudotuberculosis* isolated from sheep, goats, cattle and buffaloes and a live attenuated vaccine was prepared which gave a satisfactory immune response in vaccinated Guinea pigs challenged with virulent strains [8]. Recently, scientists have been using IgG and IFN-γ as immunological markers for good quality CLA vaccines [2]. The present work aimed at evaluating the most effective methods for attenuation of *C. ovis* strain in order to prepare the live attenuated vaccine. Also evaluated its ability to elicit protective immune responses (humoral and cell mediated) in vaccinated sheep.

Material and Methods

Animals

Guinea pigs

Seven Guinea pigs (300 gm – 450 g), five of them were used to test the safety of the attenuated strain and the last two Guinea pigs were used for examination of pathogenicity and re-isolation of the organism.

Sheep

Ten native sheep of about 6- 8 months of age, apparently healthy, free from external abscess with no previous history of caseous lymphadenitis and had negative antibodies against caseous lymphadenitis using ELISA all the time of experiment the sheep were kept in good environmental conditions and were used for vaccination with attenuated living vaccine of *C. ovis*.

Bacterial strain

Lyophilized locally isolated strain of *C. ovis* was used for vaccine preparation. The isolate was identified bacteriologically and biochemically **[**8, 9]. Also, the *C. ovis* isolate was genotyped and identified by quadruplex PCR assay and was submitted into GenBank with accession numbers MN867024 [10].

Methods

Re-isolation of C. ovis

0.25 ml of 24 hours old *C. ovis* culture was injected subcutaneously in thigh region of two guinea pigs. P.M. lesions were recorded and re-isolation of the organism was done.

Attenuation methods

Different methods of attenuation Corynebacterium pseudotuberculosis strains were used following Roukaya 1982 [8].

Effect of heat (Thermal attenuation)

The resulting *C. ovis* was inoculated into brain heart infusion broth and cultured at 37°C for 24 hours. The obtained cultures placed in water bath adjusted at 55°C for different times (10,15 and 20 minutes) and incubated at 37°C for 48-72 hours, then left to cool gradually and cultured on 10% sheep blood agar and serum broth to check the growth of treated organism.

Effect of different Hydrogen Ion Concentrations (pH)

C. ovis re-isolated strain was inoculated in nutrient broth with different pH (4, 11 and 12) and incubated for 48 -72 hours at 37°C, then subcultured on 10% sheep blood agar and serum broth to check the growth.

Effect of different concentrations of crystal violet

C. ovis colonies was inoculated into different concentrations of crystal violet broth (1/300.000, 1/250.000 and 1/200.000) for 48 -72 hours at 37°C, then few drops were subcultured on sheep blood agar (10%) to check its growth.

Characterization of the treated strain

The treated strain by different methods of attenuation was subjected to the following tests in comparison to the original strain; Growth and turbidity in nutrient broth, Surface colony characters on sheep blood agar (10%) and Biochemical variation.

From these different attenuation methods, crystal violet attenuated method for *C. ovis* was chosen for preparing the vaccine.

Preparation of the C. ovis live attenuated vaccine by using crystal violet

The *C. ovis* strain was inoculated in the brain heart infusion broth media and incubated for 48 hours at 37°C, then the bacterial cells were harvested by centrifugation and washed three times by sterile phosphate buffer, the bacterial concentration was adjusted by to 7.5 x 10^8 bacterial cells /ml in a diluent containing 1/ 250.000 crystal violet and incubated for 24 hours at 37°C. Finally the treated bacterial cells were checked for the purity by subculturing on 10% sheep blood agar and incubated at 37°C for 48 hours. [8, 11]

Safety of the live attenuated vaccine

Following OIE manual [12] Five Guinea pigs were injected subcutaneously in the thigh region by 0.25 ml per Guinea pigs from 24 hours old treated

broth culture and observed for 21 days for any abnormalities then slaughtered for P.M. examination.

Vaccination Schedule of sheep

Ten sheep were vaccinated subcutaneously in the neck with 2 ml of *C. ovis* (7.5×10^8 bacterial cells/ml) suspended in 1/ 250.000 crystal violet. Two weeks later, all vaccinated sheep received a similar booster vaccination, the blood was collected after one, two, three, four, five, and six weeks to measure the cellular and humeral immune response of vaccinated sheep against *C. ovis*.

Evaluation of the efficiency of the prepared living attenuated C. ovis vaccine

Measuring of humoral immune response using Enzyme-linked immunosorbent assay (ELISA)

ELISA test was carried out for detection the *C. ovis* antibodies titer followed the administration of the attenuated *C. ovis* vaccine [13].

Measuring the cell mediated immune response using Lymphocyte proliferation activity (MTT reduction assay)

The lymphocyte proliferation activity was determined by measuring mitochondrial activity using the MTT (tetrazolium dye) reduction method [14].

Evaluating the cell mediated immune response by measuring the level of produced Gamma interferon (IFN-γ)

Quantification of IFN-γ mRNA expression by a real-time reverse transcription-PCR (RT.PCR) for analyzing the cellular immune responses of vaccinated sheep against *C. ovis* according to Hein ; Harrington [15, 7]. Peripheral blood mononuclear cells (PBMCs) were separated by density gradient centrifugation and washed twice with RPMI 1640 medium (Invitrogen, Burlington, Ontario, Canada).

RNA extraction and Quantitative real-time reverse transcription-PCR (qRT-PCR) sybr green based

Total RNA was isolated from PBMCs by using the QIAamp RNeasy mini kit (QIAGEN, Germany, GmbH) following the instructions of manufacturer's. QRT-PCR was performed using Oligonucleotide primers and probes targeting the IFN-γ and the B2M (Beta-2 Microglobulin) gene as a housekeeping gene. Primers used were supplied from Metabion (Germany) are listed in Table (1).Primers were utilized in a 25- µl reaction containing 12.5 µl of the 2x QuantiTect SYBR Green PCR Master Mix (Qiagen, Germany, GmbH), 0.25 µl of Revert Aid Reverse Transcriptase (200 U/µL) (Thermo Fisher), 0.5 µl of each primer of 20 pmol concentration, 8.25 µl of water, and 3 µl of RNA template. The reaction was performed in a Strata gene MX3005P real time PCR machine.

Analysis of Quantitative real time PCR (qRT-PCR) sybr green based

Amplification curves and CT values were determined by the Strata gene MX3005P software. To evaluate the variation of gene expression on the RNA of the various samples, the CT of each sample was compared to the positive control group Table (1). According to the "ΔΔCt" method stated by Yuan [16] using the following ratio: (2-ΔΔCt). Whereas:

$\Delta\Delta Ct = \Delta C$ treference $- \Delta C$ ttarget

ΔCt target = Ct control – Ct treatment

ΔCt reference = Ct control- Ct treatment

E: efficiency of amplification.

Statistical analysis

Data obtained in this study were analyzed using the Statistical Package for Social Sciences (SPSS) software (version 26.0 for Windows 10) [17]. Comparison of means using the ANOVA test was carried out to express the protective efficiency of the prepared living attenuated *C. ovis* vaccine.

Results

Effect of heat, hydrogen ion concentration and crystal violet on C. ovis characters of strain

Heat effects

The results of heat treatment showed complete inhibition of growth in nutrient broth after exposure to 55° C for 20 min, while the subinhibitory temperature and exposure time was 55 ° C for 15 min. The growth of the heated strain at 55 ° C for 15 min in the broth was scanty with sediment after 48 hours. The surface colonies on sheep blood agar (10%) appear after 48 hours as dew drops 0.2 mm in diameter and most colonies were non- haemolytic. Also, the heat treated stain showed clear biochemical variations (Table, 2).

Effect of pH values

The strain could not grow at pH 4 and 12, were the subinhibitory values was 11. The cultural characters were the same as the heated strain with small biochemical variation (Table, 2).

Effect of crystal violet

No growth could be developed in crystal violet concentration 1/ 200.000. While, the organism can grow at crystal violet concentration 1/ 300.000. Finally, at 1/250.000 crystal violet, few colonies are observed, the developed colonies are small in size, whitish in coloure and opaque, mucoid without haemolysis. Biochemically, the catalase test remained after treatment allows positive, while the other four reactions were altered to negative (Table, 2).

Biochemical variations

Comparatively, the change of the five biochemical reactions by the original strain of *C. ovis* was observed in the heated and crystal violet strain as most reactions changed to negative except catalase test. Moreover, the pH treated *C. ovis* exhibited slight variation (Table, 2).

Safety of the live attenuated vaccine

The prepared live attenuated *C. ovis* vaccine was confirmed to be safe without any mortality when inoculated in Guinea pig. The produced vaccine was sterile and doesn't show any growth on different media (nutrient agar, glycerin broth, Sabouraud's agar and thioglycolate media)

Measuring of humoral immune response using Enzyme-linked immunosorbent assay (ELISA)

The evaluation of humoral immune response was done by using ELISA, demonstrating that the mean antibodies titers of sheep immunized by live attenuated vaccine of *C. ovis* were significantly increased ($P \geq 0.05$) and reached their peak in the 2nd week post the second dose, as shown in Table 3)

Measuring the cell mediated immune response using Lymphocyte proliferation activity (MTT reduction assay)

It was found that the optical density of cell mediated immune response of sheep immunized with live attenuated vaccine of *C. ovis* showed a significant difference at $P \geq 0.05$ and reached its peak in the 2nd week post the second dose as shown in Table (4) and Figure (2).

Evaluating the cell mediated immune response by measuring the level of produced Gamma interferon (IFN-γ)

The two representative samples (B1 and B2) that show high immune response in both ELISA and MTT were examined by using the IFN-γ and the results showed that IFN-γ level when analyzed by qRT-PCR after 2nd dose of two weeks post vaccination was increased and reached Ct (Cycle threshold) to 19.31 and 19.70 in order and 22.84 for control as demonstrated in Table (5) and figures (3, 4 and 5).

Discussion

Caseous lymphadenitis is a contagious infectious disease, also known as pseudotuberculosis (cheesy gland), affecting small ruminants (goats and sheep) and is responsible for sever economic losses due to a decrease in meat and milk production, wool damage, as well as decrease the reproductive efficacy. The disease, characterized by abscess formation in internal organs, subcutaneous tissues and lymph nodes, the abscess contains yellowish- green thick pus. The causative agent of the disease is a Gram-positive, facultative intracellular organism *C. ovis* [18, 3, 19]. The *C. ovis* can survive in the environment for log time and the transmission occurs mainly via skin wound especially during the sheaving season or by inhalation causing lung infection [20, 21]. The disease control by using antibiotics is unuseful since the *C. ovis* bacteria protect by a thick capsule of abscesses [22]. So, effective vaccination considered as an essential way to disease compact. There are many types of commercial vaccines present, toxoid vaccines, live vaccines, bacterin vaccines, combined vaccines and DNA vaccines [18]. Most studies performed on formalin inactivated vaccines of *C. ovis*. The killed adjuvant vaccine has been used for control and prevention of disease, but the efficacy of the performed vaccines are variable [4]. The present study was aim to prepare and evaluate a live attenuated *C. ovis* vaccine from local field isolate.

The minimal death temperature of heat treated *C. ovis* was 55 °C for 20 minutes, while the subinhibitory temperature was 55 °C for 15 minutes. Our findings are inconsistence with those reported by Roukaya, and Hassan [8, 23].

The heat treated strain grows on blood agar as minute dew drop within 48 hours and most of these colonies were non – haemolytic, while the growth in the broth medium was scanty and sedimented after 48 hours. Also, the heat treated stain showed clear biochemical variations (Table, 2). The results of our study would tend to support the findings of pervious authors [8, 23]. Moreover, Merchant and Paker [24] recorded that the *Anthrax bacillus* lost its virulence when grown at 42 °C and such cultures were used to produce immunity against Anthrax.

pH treatment induced no growth at pH 4 and 12, while the subinhibitory values was 11. The growing colonies showed the same culture character of heated strain with small biochemical variation (Table, 2). This agree with Hassan [23] who found that, the growth of *C. ovis* was completely inhibited at pH 4 and 12 but the growth at 11, showed noticeable changes in the rate of growth, haemolytic activity and biochemical activity.

Goodman and Gilman [25] claimed that, the crystal and methyl violet dies are toxic to Gram – positive bacteria and too many fungi. The susceptibility of Gram – positive bacteria to these dyes presumably related to the characteristic of the cell. The dyes had both the bacteriostatic and bactericidal action.

In the present work no growth could be developed in crystal violet concentration 1/ 200.000. While, the organism can grow at crystal violet concentration 1/ 300.000 and Finally, at 1/250.000 crystal violet, few colonies are observed, the developed colonies are small in size, whitish in coloure and opaque, mucoid without haemolysis. Biochemically, the catalase test remained after treatment allows positive, while the other four reactions were altered to negative (Table, 2). The above results were inconsistent with those obtained by Wilson and Miles [26] who recorded

that, the crystal violet stain had a strong bacteriostatic and weak bactericidal action against bacteria particularly the Gram- positive group.

The present work aimed to prepare and evaluate the living attenuated vaccine of *C. oivs* by using 1/ 250.000 crystal violet concentration.

The humoral immune response of the sheep experimentally inoculated with the live attenuated vaccine of *C. ovis* was evaluated by using the ELISA test. As shown in Table (3) and Figure (1), the mean of antibodies titers against *C. ovis* of sheep immunized by the attenuated *C. ovis* vaccine was significantly increased (P≥ 0.05) and reached its peak at the 2nd week post the second dose. Our results are in agreement with that recorded by Vale [27] who showed higher levels of IgG2a and IgG1 against *C. pseudotuberculosis* in mice inoculated with the naturally attenuated T1 strain of *C. pseudotuberculosis*. In addition, Hodgson [6] found that, the sheep vaccinated with live Toxminus *C. pseudotuberculosis* vaccine elicits high antibodies response, which reached its maximum level at the 2nd week post vaccination. Moreover, Hodgson [20] noticed that, the strong antibody responses were developed rapidly in sheep immunized with live Toxminus *C. pseudotuberculosis*.

In this work, the optical density of cell mediated immune response of sheep inoculated with the prepared living attenuated vaccine of *C. ovis* was significant increased (P≥ 0.05) and reached its peak at the 2nd week post the second dose (Table, 4 and Figure, 2). These results come in inconsistent with those reported by Garg and Chandiramani, [28] showed that, the live attenuated *C. pseudotuberculosis* vaccine capable to induce cellular response and enhance the immunity of sheep. Moreover, Johnson, [29] demonstrated that, the use of live attenuated vaccine induced a cellular immunity that enhance the immune response. In addition Vale, [27] found that, the cellular immune response was elevated in mice inoculated with naturally attenuated *C. pseudotuberculosis* (T1) strain. Finally, Johnson, [29] reported that small dose of live *C. pseudotuberculosis* stimulated an immune response to prevent the development of abscesses in the lymph node and internal organs of goats.

Barakat, [30] suggested that, the solid immunity could not be obtained unless alive attenuated vaccine was used.

Roukaya [8] concerning the fact that, the crystal violet-attenuated vaccine provided effective protection in guinea pig challenge test.

Because IFN-γ enhances cellular immunity, it is thought that, its production is especially useful in the intracellular infections [31]. Harrington, [7] found that, the amplification curves of IFN-γ showed positive reactions for all tested animals and give different Cycles thresholds (Ct) ratios.

Reverse transcription PCR (RT-PCR) has made it easier to be an accurate measure the amounts of mRNA transcripts in samples from a variety of tissue types in recent years. Additionally, cytokine mRNA expression has been detected far before protein synthesis started and shows a strong correlation with cytokine protein levels [32].

Kim, [33] stated that, IFN-γ mRNA real-time PCR took less time to identify Cp infection than ELISA. Therefore, IFN-γ had the maximum sensitivity when used as a standalone diagnostic test.

During this study, IFN-γ levels analyzed by RT-PCR against the Beta-2 Microglobulin gene as a house keeping gene. The level of interferon gamma produced due to the vaccination after the 2nd dose showed a high level when estimated with RT-PCR and showed CT of 19.31 and 19.70. The change in expression level (fold change) of IFN-γ gene indicates that, the vaccine could upregulate the level of IFN-γ that can withstand Cp bacterial infection. Significant IFN-γ mRNA expression was detected from 30 min and peaked 4 h after stimulation with Cp antigens. This was defined as the optimal time point for IFN-γ mRNA real-time polymerase chain reaction (PCR).

Conclusion

In conclusion, the developed crystal violet live attenuated *C. ovis* vaccine is safe and elicits a strong immune response in sheep, which represents a promising strategy for controlling the CLA infection in sheep with the potential to reduce the economic impact of the disease in sheep industry. However, further investigations, "including challenge trials," are warranted to comprehensively evaluate its protective efficacy under field conditions.

Acknowledgments

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Funding statement

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

All guidelines of the Veterinary Serum and Vaccine Research Institute (VSVRI) for animal welfare were followed.

TABLE 1. Primers sequences, target genes, amplicon sizes and cycling conditions for SYBR green RT-PCR

Target gene	Primers sequences	Reverse transcription	Primary denaturation	Amplification (40 cycles)			Dissociation curve (1 cycle)		
				Secondary denaturation	Annealing	Extension	Secondary denaturation	Annealing	Final denaturation
ß2M	AGACACCCAC CAGAAGATGG TCCCCATTCTT CAGCAAATC	50˚C 30 min.	94˚C 5 min.	94˚C 15 sec.	60˚C 30 sec.	72˚C 30 sec.	94˚C 1 min.	60˚C 1 min.	94˚C 1 min.
IFNɣ	GCGCAAAGCC ATAAATGAAC CTCAGAAAGC GGAAGAGAAG								

TABLE 2. Variation in the biochemical characters of the subinhibitory values in comparison to original strain.

Characters / Biochemical reaction	Strain Original (control)	Heat 55° C for 15 min	pH 11	Crystal violet 1/250.000
Fermentation of glucose	+ Ve	- Ve	+ Ve	- Ve
Fermentation of sucrose	+ Ve	- Ve	- Ve	- Ve
Urea hydrolysis	+ Ve	- Ve	- Ve	- Ve
Nitrate reduction	- Ve	- Ve	- Ve	- Ve
Catalase test	+ Ve	+ Ve	+ Ve	+ Ve

TABLE 3. ELISA optical density for sheep vaccinated with *C. ovis* the live attenuated vaccine

Number of sheep	*ELISA Optical Density (OD)						
	Before vaccination	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
1	0.130	0.442	0.774	0.980	1.404	0.990	0.930
2	0.155	0.389	0.690	0.997	1.180	1.102	0.992
3	0.145	0.490	0.726	0.977	1.300	0.982	0.873
4	0.112	0.480	0.870	1.021	1.533	1.060	1.030
5	0.150	0.540	0.738	0.964	1.130	0.970	0.880
6	0.140	0.410	0.648	0.981	1.488	1.006	0.950
7	0.135	0.536	0.702	0.981	1.502	1.015	0.966
8	0.130	0.423	0.810	0.934	1.112	0.820	0.776
9	0.150	0.526	0.750	1.040	1.530	1.070	0.897
10	0.148	0.467	0.755	1.005	1.445	1.050	0.920
Overall mean	**0.139 ± 0.01**	**0.470 ± 0.05**	**0.746 ± 0.06**	**0.988 ± 0.03**	**1.362± 0.17**	**1.007 ± 0.08**	**0.921± 0.07**

Booster dose of vaccine

*ELISA: Enzyme-Linked Immunosorbent Assay.

TABLE 4. Lymphocyte cell proliferation assay (MTT) for sheep vaccinated with *C. ovis* live attenuated vaccine

Number of sheep	Optical density						
	Before vaccination	1week	2 week	3 weeks	4 weeks	5 weeks	6 weeks
1	0.177	0.520	0.530	0.590	0.595	0.450	0.330
2	0.158	0.560	0.566	0.620	0.634	0.545	0.360
3	0.132	0.405	0.520	0.588	0.590	0.437	0.235
4	0.169	0.562	0.580	0.640	0.642	0.480	0.220
5	0.143	0.530	0.556	0.603	0.610	0.510	0.315
6	0.110	0.490	0.530	0.590	0.590	0.423	0.233
7	0.147	0.488	0.522	0.570	0.580	0.405	0.246
8	0.159	0.534	0.546	0.614	0.620	0.518	0.386
9	0.086	0.480	0.530	0.608	0.600	0.510	0.234
10	0.132	0.460	0.544	0.610	0.630	0.465	0.214
Overall mean	**0.141 ±0.03**	**0.503±0 .05**	**0.542 ±0.02**	**0.603 ± 0.02**	**0.610± 0.02**	**0.474± 0.05**	**0.277± 0.07**

Booster dose of vaccine

TABLE 5. Showed the IFN-γ level after vaccination with 2nd dose of *C. ovis* live attenuated vaccine

Sample No.	ID	B2M	*IFN-γ	
		CT	CT	Fold change
A	Control	19. 52	22.84	
B1	Vaccinated	19.17	19.31	9.0631
B2	(2^{nd} dose)	19.43	19.70	8.2821
Mean		19.30	19.51	8.6638

***IFN-γ**: Gamma interferon, no: number, CT: Cycle threshold, B1: Representative sample 1, B2: Representative sample 2, A: control. Fold change: Expression ratio showing the change in the expression level of IFN-γ during study and was measured by CT.

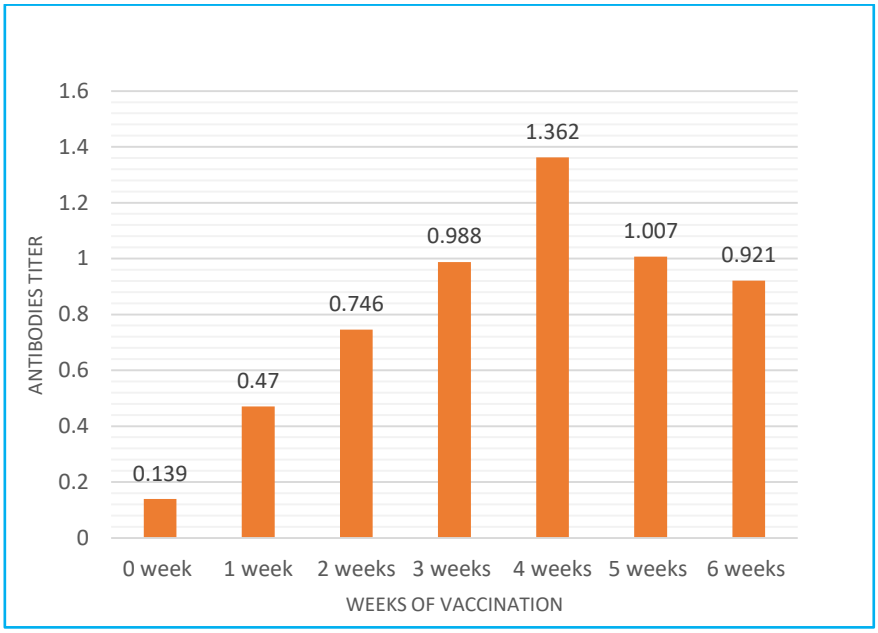

Fig. 1. Mean antibodies titer of sheep vaccinated with *C. ovis* live attenuated vaccine using ELISA

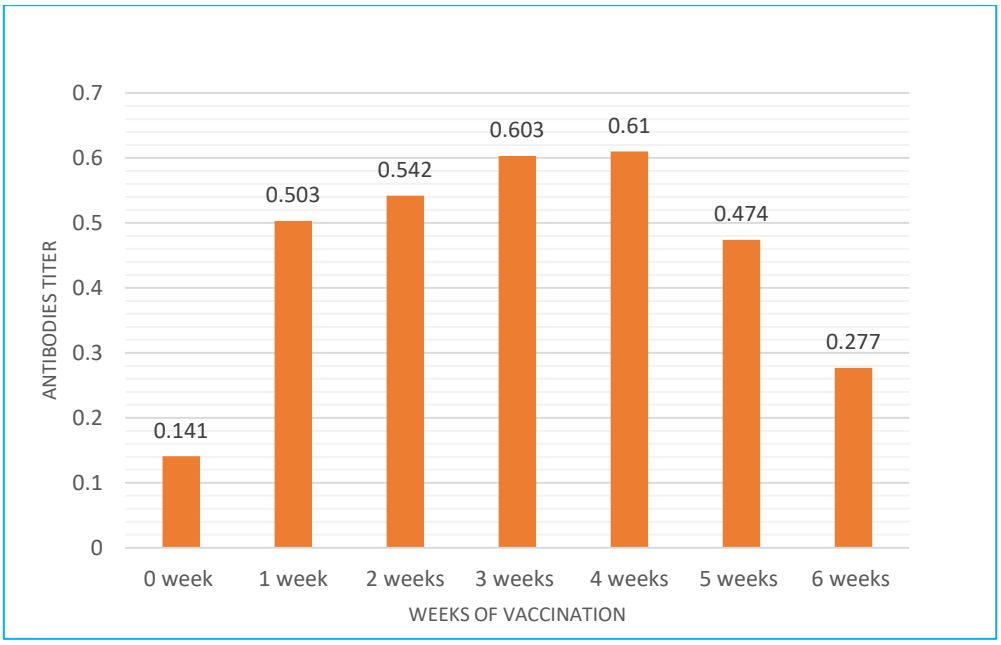

Fig. 2. The mean optical density of sheep sera immunized with *C. ovis* live attenuated vaccine using Lymphocyte cell proliferation assay (MTT)

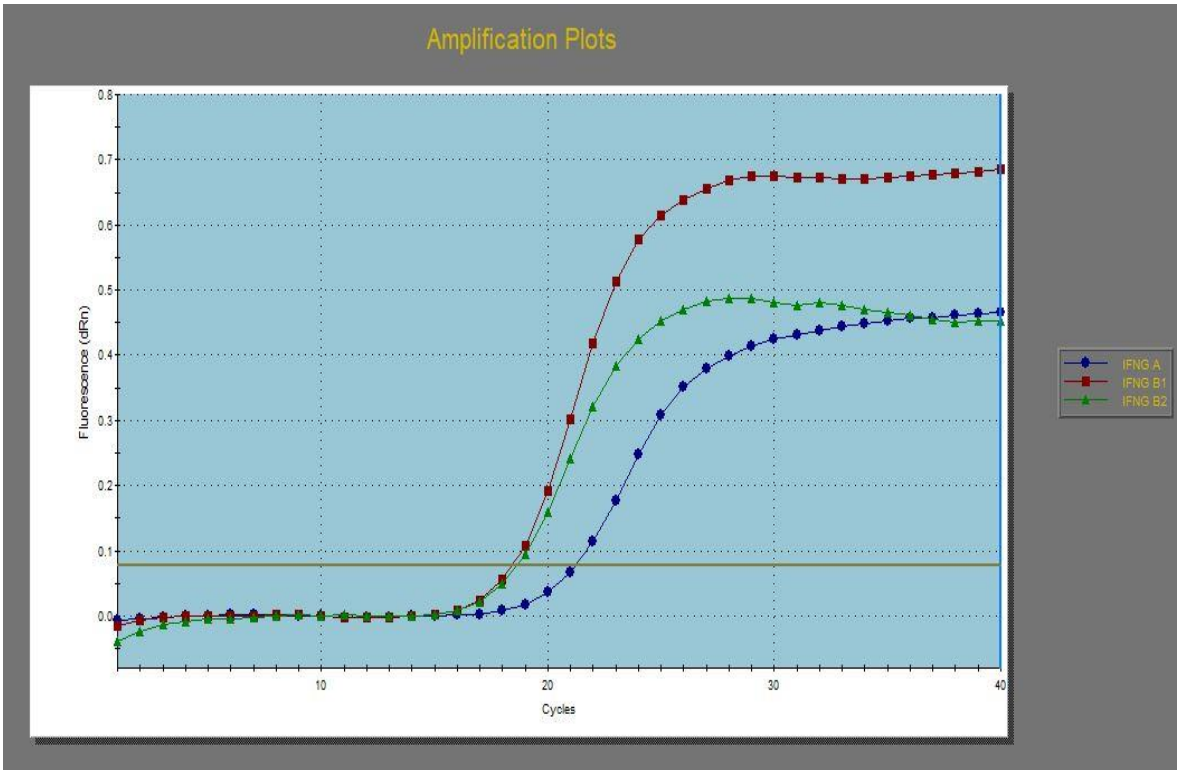

Fig. 3. Amplification curve of IFN-γ of two samples (B1 and B2) after 2nd dose of vaccine and control IFN-γ (A).

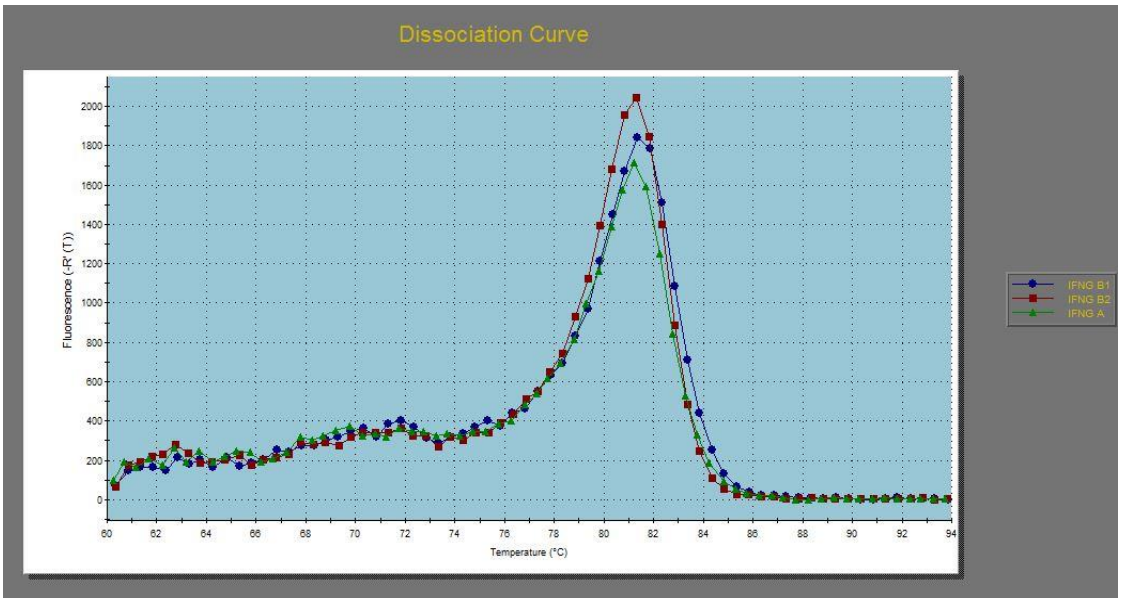

Fig. 4. Dissociation curve of IFN-γ of two samples (B1 and B2) after 2nd dose of vaccine and control IFN-γ (A)

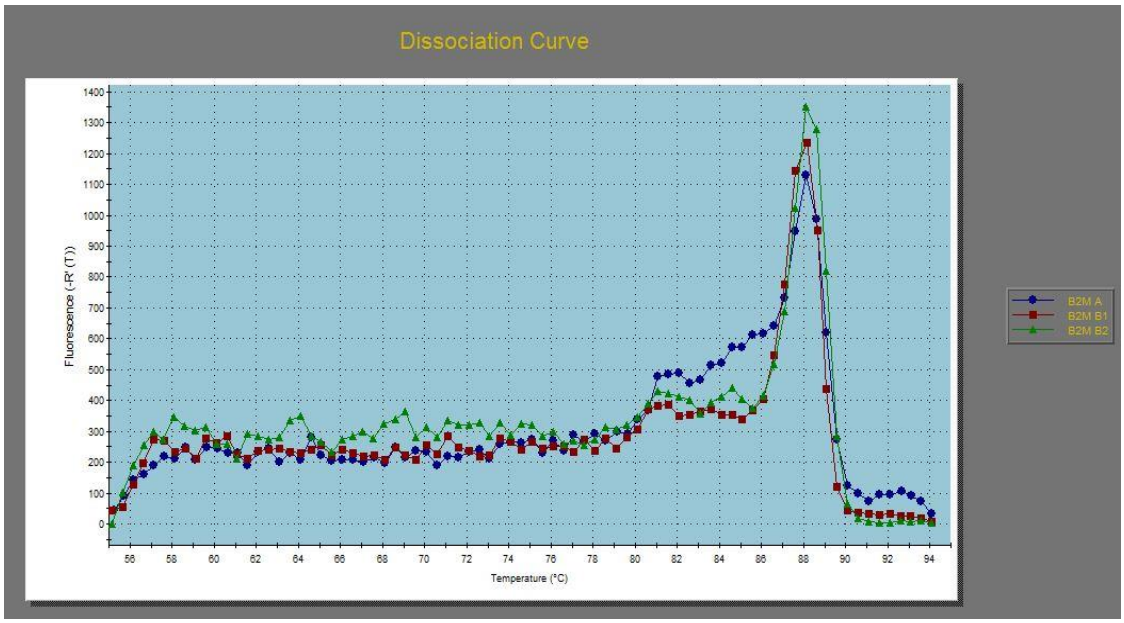

Fig. 5. Dissociation curve of housekeeping gene (B2M) of two samples (B1 and B2) after the 2nd dose of vaccination and control B2M (A).

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تحضير لقاح حي مُوهَّن من بكتيريا كورينيباكتيريوم الكاذبة ضد التهاب الغدد اللمفاوية الجبني في الأغنام

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الملخص

التهاب العقد اللمفاوية الجبني (CLA) هو مرض حبيبي مزمن شائع الحدوث لدى المجترات الصغيرة، ويسببه بكتيريا السل الكاذب موجبة الجرام .(Corynebacterium pseudotuberculosis) هدفت هذه الدراسة إلى تحديد طريقة التخفيف المناسبة لبكتيريا السل الكاذب(Corynebacterium pseudotuberculosis Ovis) ، وتقييم مناعة اللقاح الحي المُضعف الناتج في الأغنام. قُيّمت طرق تخفيف مُختلفة، بما في ذلك: المعالجة الحرارية عند درجة حرارة ٥٥ درجة مئوية لمدة ١٥ دقيقة، والتعرض لدرجة حموضة قلوية(pH11) ، والمعالجة باستخدام الكريستال البنفسجي (تركيز ١/٢٥٠,٠٠٠). أدت هذه الطرق إلى تغيير كبير في الخصائص الثقافية والكيميائية الحيوية للبكتيريا، بالإضافة إلى انخفاض في ضراوتها من خلال تقليل القدرة الغزوية لسلالة الأغنام. تم اختيار السلالة المُضعفة باستخدام الكريستال البنفسجي لتحضير اللقاح. تم تطعيم عشرة أغنام محلية بلقاح الكوليرا أوفيس الحي المُضعف باستخدام الكريستال البنفسجي، وتم تقييم الاستجابة المناعية الخلطية والخلوية. أظهرت النتائج أن اللقاح الحي المُضعف المُحضر يُحفز استجابة مناعية قوية، مما يُشير إلى فعاليته المُحتملة. ختامًا، يُعد لقاح الكوليرا أوفيس الحي المُضعف المُطور آمنًا ويُحفز استجابة مناعية قوية لدى الأغنام، مما يُمثل استراتيجية واعدة للسيطرة على عدوى التهاب الجلد الدهني المُترافق (CLA) لدى الأغنام، مع إمكانية تقليل الأثر الاقتصادي للمرض على قطاع تربية الأغنام.

الكلمات الدالة: لقاح مُضعف، الكوليرا أوفيس، إنترفيرون جاما، تكاثر الخلايا الليمفاوية، الأغنام.