Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Immunomodulatory Effect of Levamisole, BCG, and Vit E+Se on the Immune Response of Ewes to Sheep pox Vaccine

Asmaa A. Darwish[1], Mona A. Mahmoud[1], Gehad R. Donia[1], Amira A. El-sayed[2] and Ayatollah I. Bassiouny[2]

[1] *Animal and Poultry Health Department, Animal Production and Poultry Division, Desert Research Center (DRC), Cairo, Egypt.*

[2] *Agriculture Research Center (ARC), Veterinary Serum and Vaccine Research Institute (VSVRI), Pox Department, Cairo, Egypt.*

Abstract

SHEEP POX infection poses a significant socio-economic threat to sheep production worldwide. Vaccination is the most effective preventive measure against this disease. This study examines the immunomodulatory effects of various immunostimulants on the immune response of Barki ewes to the live-attenuated sheep pox Vaccine. Sixty Barki ewes were vaccinated with the live-attenuated sheep pox vaccine and divided into four groups: Pox (vaccine only), BCG (vaccine + 0.1 ml BCG), E+Se (vaccine + 0.5 ml/10 kg Vitamin E + Selenium) and Lev (vaccine + 1 ml Levamisole 10%/50 kg). Blood samples were collected to assess humoral immunity (NI), innate immunity, and clinicopathological parameters. The E+Se group showed the best NI, minimal innate immunological alterations, normal hemogram, liver, and kidney function tests, and improved total protein, globulin levels, and neutrophil phagocytic index. The Lev group had NI values close to the Pox group, while the BCG group had lower NI than the Pox group. Innate immunological alterations and haemato-biochemical changes were more pronounced in the Lev and BCG groups compared to the Pox group and in the BCG group compared to the Lev group. Conclusion: The E+Se injection before the live-attenuated sheep pox vaccine significantly enhanced the ewes' humoral immune response and reduced vaccination side effects.

Keywords: Sheep pox vaccine, Neutralization index, Clinicopathological alterations, Levamisole, BCG, E+Se.

Introduction

Sheep pox is an extremely contagious viral ailment that has a significant impact on the sheep industry in Egypt, leading to substantial economic losses. The disease manifests in various detrimental effects, including high morbidity and mortality rates among infected sheep, diminished milk and meat production, increased rates of abortion, inferior wool quality, and compromised skin conditions. Moreover, it also imposes restrictions on the international trade of animals and animal products. Sheep pox is particularly fatal in newly introduced animals, whereas local inbred animals from the endemic regions may exhibit milder symptoms. The virus responsible for sheep pox is a large, brick-shaped virus classified within the subfamily Chordopoxvirinae, family Poxviridae, and the genus Capripoxvirus. Its survival time in the external environment is approximately six months, while it can persist on the wool or hair of infected animals for up to three months following infection. Sunlight, drying, freezing, thawing, and detergents can inactivate the virus, although it remains viable for several months when lyophilized [1-3].

Vaccination is the only reliable method to prevent contagious disease outbreaks including sheeppox. However, outbreaks have been observed in both vaccinated and unvaccinated animals due to vaccine failure stemming from rapid vaccine deterioration and animals' poor immune response. Consequently, there is a pressing need to enhance sheep's immune response to vaccination. One approach involves the

*Corresponding authors: Asmaa A Darwish, E-mail: asmaa_vet25@yahoo.com, Tel.: 01148161026
(Received 09 June 2024, accepted 28 July 2024)
DOI: 10.21608/EJVS.2024.296483.2165

concurrent administration of an immunostimulant alongside the vaccine [4-12]. In addition, these immunostimulants may reduce the unwanted clinicopathological alterations (anemia, raised hepatic and renal function values, and oxidative stress), usually accompanied the vaccination process due to the innate immune response [13].

In the last few decades, different immunostimulants garnered significant attention from researchers in both human and animal medicine such as levamisole, Bacillus Calmette-Guérin (BCG) vaccine, and vitamin E and selenium combination (E+Se). They have remarkable effects on the immune system. They work non-specifically to enhance both innate and adaptive immunity, resulting in increased production of antibodies, activation, and proliferation of T-cells, phagocytosis, and chemotaxis by monocytes, macrophages, and neutrophils. They approved their efficiency in healthy animal protection against different diseases. They also increase the recovery rates of diseased animals and shorten the disease duration [14]. Moreover, prior research has indicated the immunoenhancing effects of these immunostimulants on animal responses to various vaccines. They have been shown to augment the immunogenicity of sheep and goats towards different vaccines, including those against foot-and-mouth disease (FMD), peste des petites ruminants (PPR), Brucella vaccine Rev.1, and Clostridium vaccines [4-12].

This research endeavour focuses on examining and contrasting the immunomodulatory impact of levamisole, BCG, and a combination of vitamin E and selenium (E+Se) on the immune response of Barki ewes to the Sheeppox Vaccine. This investigation specifically delves into their influence on certain haematological and biochemical changes observed in vaccinated sheep.

Material and Methods

Sixty apparently-healthy Barki ewes aged (3-5 years), weight 50-55 Kg, were kept under an adequate nutrition system and housing conditions, in closed pens at sustainable development Centre of Matrouh resources (SDCMR).

All ewes were injected with sheep pox vaccine (Live-attenuated sheep pox virus (SPV) vaccine (Rominan strain) was supplied from the Pox Research Department. Veterinary Serum and Vaccine Research Institute, Egypt (VSVRI) (batch no. 8). The vaccination of animals were performed as described by OIE [15] with 0.5 ml of field dose containing 10^3 $TCID_{50}$ of tissue culture Rominan SPV, inoculated intradermally in the tail.

The vaccinated ewes were divided into 4 groups as follows:

Pox group: 15 ewes didn`t receive any treatment with the pox vaccine.

BCG group: 15 were injected (immediately before vaccination) S/C with 0.1 ml of BCG vaccine supplied by the veterinary serum and vaccine research institute, El Sekka El Beda St., Abbasia, Cairo, Egypt.

E+Se group: 15 ewes were injected (immediately before vaccination) subcutaneously for one time, with 0.5 ml /10 kg B. W. E+Se (ADWIA Co, Egypt). Each ml contains 150 mg vit E and 1.67 mg Se.

Levamisole group (Lev group): 15 ewes were injected (started immediately before vaccination) subcutaneously for 3 consecutive days, with l ml of levapan® 10% (Parma swede-Egypt) /50kg B.Wt (100 mg of levamisole).

All doses and routes of administration are recommended by the manufacturing company.

Blood samples

Blood samples were collected from the jugular vein of all ewes using clean sterile vacutainer tubes 2 hr before receiving vaccination and treatment (0 day) and 7, 14, 21, 28, and 35 days after vaccination, and divided into three parts, 1st part was placed in a tube containing EDTA and were used instantly for evaluation of different haematological parameters [red blood cells count (RBCs), haemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total leukocytic count (TLC) and differential leukocytic count, (DLC) manually according to the method described by Feldman et al. [16]. Whereas, the 2nd part was placed in a tube containing heparin and was used immediately for estimation of neutrophils phagocytic activity and index according to the method described by Wilkinson [17], using *Candida albicans*. The 3rd part was placed in a clean, plain tube and was left to coagulate then was centrifuged at 3000 r.p.m for 20 minutes and serum was separated in clean *Eppendorf* tubes that were stored at -80º C until used for estimation of humoral and innate immunological parameters and biochemical parameters.

Humoral immunity

Virus Neutralization Test (VNT):

All collected sera were screened against SPV according to the method described by OIE [15] using Cell culture African green monkey Kidney cell line (Vero), kindly supplied by the Pox Research Department, Veterinary Serum and Vaccine Research Institute, Egypt, and Eagl's minimum essential medium (MEM) with neonatal calf serum (10% for growth medium and 2% for maintenance medium).

The neutralization index (NI) was calculated according to Reed and Muench [18].

- NI = **VT** (virus titer) - **SVT** (serum virus titer).

The innate immunological parameters and biochemical parameters:

Serum cytokines (IL-1β, IL-6, TNF-α) levels were measured in serum by ELISA kits of Ray Biotechcompany following the manufacturer's instruction. Serum caeruloplasmin (Cp) concentrations were estimated by a turbidimetric method using Elabscience USA® kits. Serum concentrations of total antioxidant capacity (TAC), serum liver enzymatic activity (alanine transaminase (ALT), aspartate transaminase (AST)), kidney function test (urea (BUN), creatinine (Cr)), total protein (TP), albumin (Alb) were detected spectrophotometrically using commercial kits of Biodiagnostics company® following the manual instructions.

Statistical analysis

Means of different statistical parameters between the different animal groups were compared by two-way ANOVA test by SPSS version 24 at 0.05 level of probability [19].

Results

The immunological parameters

Humoral immunity: NI of the four vaccinated groups significantly (P<0.05) increased from the 7th day and reached its highest value on the 35th day with a mean of 2.83 in the E+Se group, 2.68 in the Pox and the Lev groups and 2.58 in BCG group (Fig. 1).

Innate immunity: IL-1β, IL-6, TNF-α, and Cp displayed significant increases (P<0.05) in the four groups, peaking on the 14th day, followed by a significant decline toward baseline values by the 35th day for IL-1β, TNF-α, and Cp in Pox, E+Se, and Lev groups. However, IL-6 in the four groups, and IL-1β, TNF-α, and Cp in the BCG group remained higher than the 0-day values till the end of the experiment. In contrast, TAC values significantly decreased (P<0.05) in the Pox, Lev, and BCG groups, reaching their lowest point on the 14th day, followed by a significant increase back to baseline values by the 35th day in the three groups. Interestingly, TAC non-significantly (P≥0.05) in the E+Se group changed throughout the experiment (Fig. 1).

Haematological parameters: the red blood cell parameters and indices (RBCs, Hb, PCV, MCH, MCV, and MCHC) significantly (P<0.05) decreased in the Pox, Lev, and BCG till the 14th day (except RBCs reached its bottom on 7th day) then gradually increased approaching their initial values on the 28th day for RBCs and 35th day for Hb, PCV, MCH, MCV, and MCHC in the three groups. No significant changes (P≥0.05) were detected in the E+Se group hemogram (Fig. 2).

On the other hand, a significant leukocytosis accompanied by a significant (P<0.05) neutrophilia, lymphocytosis, and increased neutrophils phagocytic activity and index were detected in groups from the 7th day, achieving their peaks on the 14th day (except lymphocytosis reached its peak on 7th day) then start decreasing till the research end but didn`t return to their basic values in the pox, Lev, and BCG Groups. While E+Se group approached the 0-day values for TLC, neutrophils, lymphocytes, neutrophils phagocytic activity, and index on the 28th day (Figures 3, 4).

Biochemical parameters

Liver and kidney function tests: ALT, AST, blood urea, Cr significantly (P<0.05) increased in the Pox, Lev, and BCG groups reaching their peaks on14th day for urea, Cr, ALT, AST in the three groups, and then they significantly (P<0.05) decreased approaching their initial values at 21st day for urea, Cr, AST in the three groups and at 35th for ALT in the three groups. While E+Se group showed non-significant (P≥0.05) changes in liver and kidney function tests (Fig. 4).

Protein profile

A significant (P<0.05) hypoproteinemia and hypoalbuminemia and decreased A/G were observed in the Pox group till the 14th day, then significantly (P<0.05) increased, returned to the 0-day value on the 35th day for TP, on the 28th day for Alb. A/G decreased till the 7th day then increased and exceeded its original values on the 28th, and 35th day. Globulin (Glob) significantly (P<0.05) increased achieving two peaks on the 7th and the 21st days then returned to normality on the 35th day (Fig. 5).

A significant (P<0.05) hyperproteinemia was observed in BCG, E+Se, Lev groups till the 21st day in BCG and Lev groups significantly (P<0.05) declined approaching the baseline values on the 35th day while hyperproteinemia continued with the E+Se group till the research end. Albumin and A/G significantly (P<0.05) decreased in BCG and Lev groups till the 14th day then significantly (P<0.05) increased approaching their initial values at the 35th in the Lev group but it was still lower than the 0day values in the BCG group. While albumin values non-significantly (P<0.05) altered in the E+Se group and A/G significantly (P<0.05) decreased throughout the research and didn`t reach its baseline values (Fig. 5).

Globulin significantly (P<0.05) raised in BCG and Lev groups till the 14th day then significantly (P<0.05) decreased achieving its original values in the Lev group at the 35th day and higher than its 0-day values in the BCG group. While, E+Se showed significant (P<0.05) hyperglobulinemia (peaked on the 7th day), and continued till the research ended without achieving its original values (Fig. 5).

The comparison between the immustimulants groups and the Pox group cleared that: the E+Se group had a higher level of NI throughout the research than the Pox group and the BCG and Lev groups. While the Lev group (compared to the Pox group) nearly had the same level of NI along the research. The BCG group (compared to the pox group) had a lower level of NI throughout the study. While, all the above-described innate immunological and clinicopathological alterations were higher and persisted for a longer time in the BCG and Lev groups than the Pox group, and in the BCG group than the Lev group. In addition, the BCG and Lev groups (in contrast to the Pox group) showed significant ($P<0.05$) hyperproteinemia (Figures 1-5).

On the contrary, the E+Se group demonstrated the lowest degree of innate immunological and clinicopathological alterations among the four groups, except neutrophils phagocytic index (significantly ($P<0.05$) increased than Pox and Lev groups till the 14th day then significantly decreased lower than them), globulin (significantly ($P<0.05$) exceed the Pox group globulin values till the end of the research) and TP (the E+Se group displayed significant ($P<0.05$) higher levels than the Pox and Lev groups and extended to the research end). TAC, hemogram, liver, and kidney function tests, and albumin, non-significantly ($P\geq0.05$) changed in the E+Se group (Figures 1-5).

Discussion

SPV infection remains a significant socio-economic concern for sheep farming in Egypt, marked by its high morbidity and mortality rates. It poses a substantial threat to successful sheep production globally. Vaccination stands out as the most straightforward and effective preventive measure against infectious diseases, considered both humane and cost-effective [1-3].

In the current study, the sheeppox live-attenuated vaccine successfully elicited a humoral immune response represented by the gradual elevation of the NI observed in the Pox group, which started from the 7th day and increased gradually till the research end on the 35th day with a mean of 2.68. Whereas, the effectiveness of the vaccine hinges on its ability to elicit an immune response that closely resembles natural infection to a moderate extent and the associated antibody titers elevation [20].

These results are comparable to those reported by Elsayed et al. [21], who evaluated the humoral immune response of sheep under field conditions using a double antigen ELISA against alive-attenuated sheeppox vaccine (Romanian strain). Their study noted a significant rise in the humoral immune response starting from the 7th to the 14th day post-vaccination and peaking on the 28th day with a mean NI of 2.4. Likewise, Oreiby et al. [22] emphasize on the effectiveness of the Romanian strain vaccine as a control strategy against sheeppox outbreaks in highly susceptible populations.

This successful humoral immune response was assigned to the robust cytokine signature noticed in the Pox group, up to the 14th day following vaccination. Similar patterns have been observed previously with live-attenuated viral vaccines, inactivated viral vaccines, and bacterial vaccines [13, 23-28]. Cytokines, being immunoregulatory proteins, typically surge post-infection or vaccination. They play a crucial role in orchestrating the humoral immune response, including the formation of antibodies. Early elevation of cytokine levels following vaccination enhances the body's reaction to the vaccine and serves as a predictor for higher levels of immunoglobulins [29]. Moreover, their activity post-vaccination is associated with local and systemic reactions, such as fever and fatigue, commonly observed after vaccination [26]. These pro-inflammatory cytokines trigger a marked hepatic acute phase response, as evidenced by the marked increase in Cp levels observed in the Pox group up to the 14th day. Cp, a copper-containing protein, serves as a sensitive indicator of tissue damage and various diseases. This acute phase response has been documented post-vaccination in different animal species [13, 30-33].

Another consequence of the activation of the aforementioned pro-inflammatory cytokines is the release of free radicals from various immune cells. Free radicals are integral to the innate immune response, serving to defend the host against invasive bacteria by oxidizing vital components of the pathogens. They play a crucial role in both infection and vaccination by facilitating an effective immune response. However, their activity is regulated by enzymatic and non-enzymatic antioxidants, which protect body cells from their harmful effects. In the current dataset, the production of free radicals surpasses the neutralizing capacity of antioxidants, leading to pronounced oxidative stress in the Pox group, as represented by the decreased TAC observed up to the 14th day. This oxidative stress phenomenon with vaccination has been documented in various studies [13, 21, 28, 34-37].

The outstanding oxidative stress played a key role in the notable haematological and biochemical changes observed after the vaccination process in the Pox group. Free radicals, targeting the liver, kidneys, and RBCs, induce their damage and destruction. Consequently, a slight elevation in liver and kidney function tests, along with decreased levels of RBCs, Hb, and PCV, was noted in the Pox group up to the 14th day, consistent with findings from previous studies [13, 34-37].

Moreover, pro-inflammatory cytokines exert an inhibitory effect on bone marrow erythropoiesis by disrupting erythropoietin synthesis, intestinal iron

absorption, and iron macrophage recycling, consequently exacerbating the microcytic hypochromic anemia observed in the Pox group up to the 14th day [38]. This data contrasts with previous authors who reported normal erythrogram in vaccinated animals [28, 33, 39]. However, it aligns with the findings of Prentice et al. [40] and Darwish et al. [13] who obtained diminished red cell parameters in vaccinated infants and sheep. Such discrepancies may be attributed to differences in animal species, antigen type, and vaccine formulation.

On the contrary, pro-inflammatory cytokines play a role in enhancing the maturation, proliferation, and release of neutrophils and lymphocytes from the bone marrow and lymph nodes into circulation. This phenomenon elucidates the observed neutrophilia, increased neutrophilic phagocytic activity and index, and lymphocytosis, leading to total leukocytosis observed in the Pox group throughout the study. Neutrophils are integral to innate immunity, as they generate reactive oxygen species and play a significant role in the Toll-like receptor 4 (TLR4)-dependent innate clearance of pathogens, particularly bacteria, due to their granule-rich content of digestive and hydrolytic enzymes [35]. Additionally, viral proteins may contribute to lymphocytosis in the Pox group by acting as mitogens or superantigens on certain lymphocyte subpopulations [39]. Leukocytosis, with or without neutrophilia and enhanced phagocytic activity and index, and lymphocytosis, have been documented previously after vaccination in different animal species [13, 30, 33, 39].

The protein profiles of the Pox group reflected the immunological changes occurring post-vaccination. The increased production of cytokines and acute phase proteins (APPs), such as α and β globulins, directly contribute to the observed hyperglobulinemia in the vaccinated groups up to the 14th day. Subsequently, following the decrease in cytokines and APPs levels after the 14th day, this hyperglobulinemia endured because of the antibody production against the vaccine (γ globulin) [22, 33, 39].

Likewise, the acute phase response and oxidative stress described above are responsible for the notable hypoalbuminemia observed in the Pox group. Albumin, being a negative acute phase reactant, sees decreased production by the liver under the stimulation of pro-inflammatory cytokines, which prioritizes the synthesis of positive APPs instead [22, 33, 39]. Additionally, its antioxidant properties render it susceptible to attack by circulating free radicals.

A state of total hypoproteinemia and decreased A/G ratio were documented in the Pox group until the 14th day and 7th day respectively. This resulted from the prior changes in the albumin and globulin levels, as total protein and A/G ratios primarily rely on the combined levels of albumin and globulins, as well as the variance between them [33, 39].

Concerning the studied immunostimulants' effect on the animal's immune response to the pox vaccine, Levamisole injection had a negligible effect on the ewes' humoral immune response to the live-attenuated pox vaccine (Both the Pox and Lev groups had closed levels of NI throughout the research). While BCG negatively affected the ewes' humoral response to the live-attenuated pox vaccine (the BCG group had a lower level of NI than the Pox group). These findings contradict prior studies advocating for the use of levamisole and BCG injections to enhance humoral immune responses to viral and bacterial vaccines in various animal species [4-11]. This disparity may be attributed to differences in animal species, immunostimulant dosage, and vaccine type.

Contrariwise, the innate immune response, and the subsequent haematological and biochemical alterations were more prominent in the Lev and BCG groups than the Pox group, and in the BCG group than the Lev group. Additionally, the Lev and BCG displayed pronounced hyperproteinemia compared to the Pox group. The ability of BCG and levamisole to enhance innate immunity has been extensively documented across human and animal species, primarily through their capacity to induce the expression of pro-inflammatory cytokines [27, 41, 42]. Subsequently, BCG and Lev groups (compared to the Pox group) suffered from a more pronounced degree of haemato-biochemical alterations, especially hyperglobulinemia (which led to outstanding hyperproteinemia in both groups compared to the Pox group).

BCG also leverages a potent mechanism called trained immunity, where it triggers the production of pro-inflammatory cytokines via mycobacterium lipoproteins, LPS, and CpG oligonucleotide, potentially maintaining its effects for up to three months [27]. Taking into consideration, that BCG itself is a vaccine and the presence of two antigens in the BCG group magnifies the innate immune response and other related alterations (than the Pox and Lev groups) but at the same time, it distracts the immune system leading to a reduced level of NI in the BCG group (than the Pox and Lev groups) [33]. Besides, bacterial antigens are stronger than viral antigens in innate immune response stimulation [39, 43, 44]. This clarified why the innate immune response and its consequences (especially hyperproteinemia) were maximized in the BCG group (rather than the Lev and Pox groups).

In contrast to Levamisole and BCG, the E+Se combination markedly enhanced the ewes' humoral immune response to the live-attenuated pox vaccine (the E+Se group had the highest value of NI among the four groups). Similar observations were reported before in birds vaccinated against infectious bronchitis, goats vaccinated against *C. perfringens* type D infection, and sheep vaccinated against *C. tetani* and *C. perfringens* [6, 8, 12, 45]. On the other hand, the E+Se group had the minimum level of innate immunological and related haematological and biochemical alterations among the four groups. This result was because of the potent anti-inflammatory action of vitamin E, which suppresses the activity of the pro-inflammatory cytokines. Besides the antioxidant effect of Vitamin E and/or Se, they restrain the free radicals activity and protect the body cells from its harmful oxidative effect. Thus, the E+Se group displayed normal TAC, hemogram, albumin, and liver and kidney function tests and enhanced neutrophils phagocytic index [46-49]. Rationally, the obtained hyperproteinemia in the E+Se group (compared to the Pox and Lev groups) here was because of its normal levels of albumin (due to the E+Se antioxidant effect) and the hyperglobulinemia (due to the enhanced antibody production (γ globulin)).

Conclusion

Among the studied immunostimulants, the E+Se combination improved the ewes' humoral immune response to the live-attenuated sheeppox vaccine and kept normal TAC, hemogram, albumin, and liver and kidney function tests, with enhanced TP and globulin levels and neutrophils phagocytic index.

Acknowledgements

The authors acknowledge staff members of Animal Health and Poultry Department, Desert Research Center, Egypt.

Funding statement

This study didn't receive any funding support

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study follows the ethical approval of the Animal and Poultry Health Department, Desert Research Center (DRC), Mataria, Cairo, Egypt.

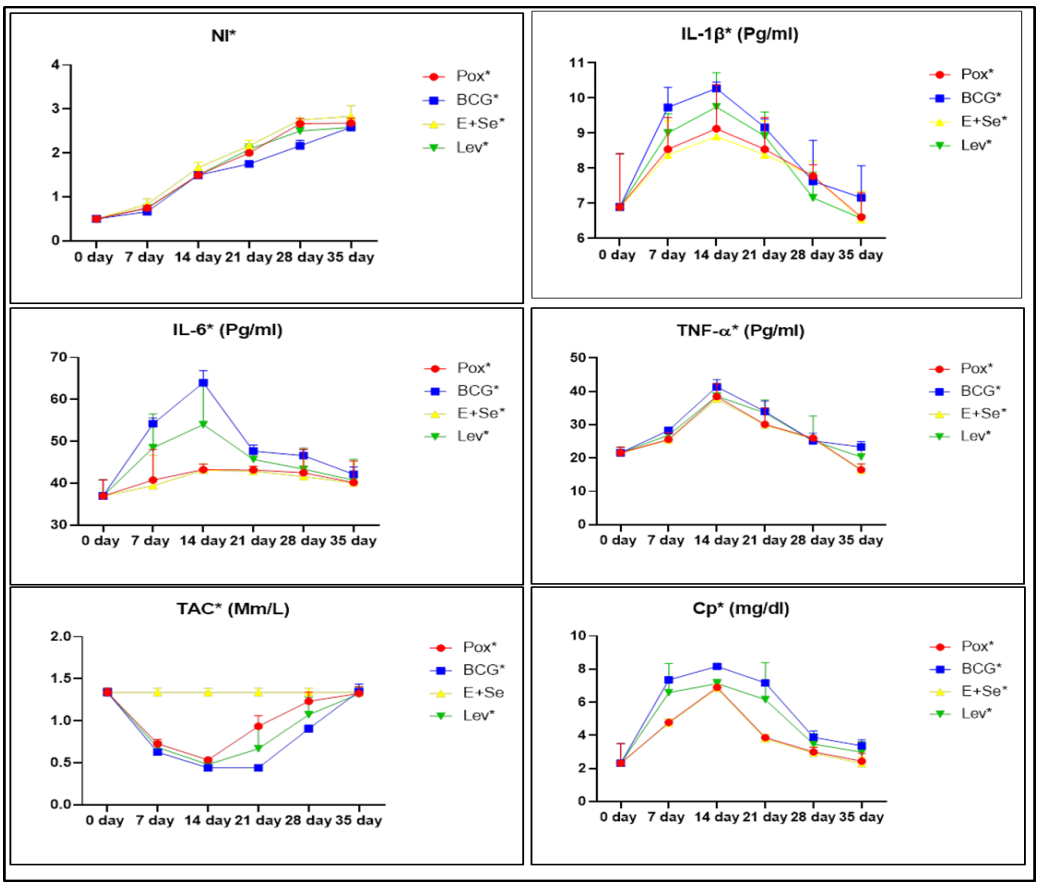

Fig. 1. Comparison between the humoral and innate immune response of the four studied groups throughout the research, (*) on the parameter means significant between the groups, (*) on the group means the vaccine or vaccine+immunstimulant effect is significant throughout the research. Considered significant when (P<0.05).

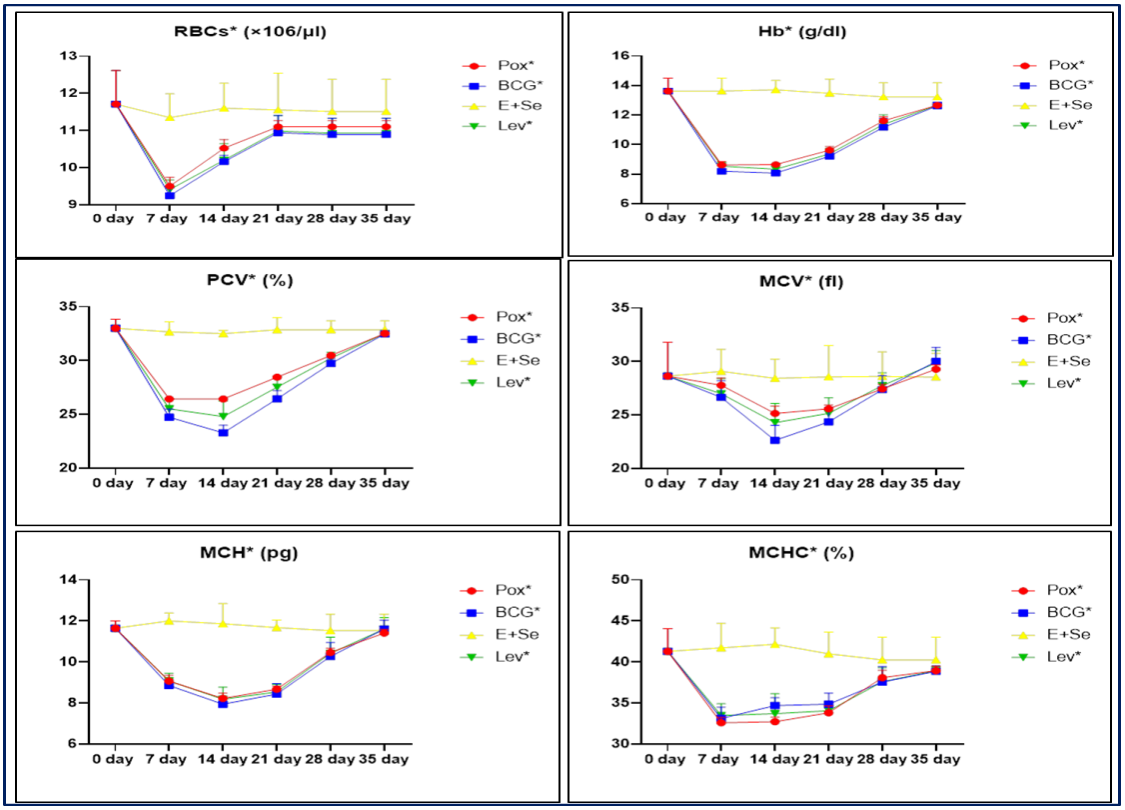

Fig. 2. Comparison between the erthyrogram of the four studied groups throughout the research, (*) on the parameter means significant between the groups, (*) on the group means the vaccine or vaccine+immunstimulant effect is significant throughout the research. Considered significant when (P<0.05).

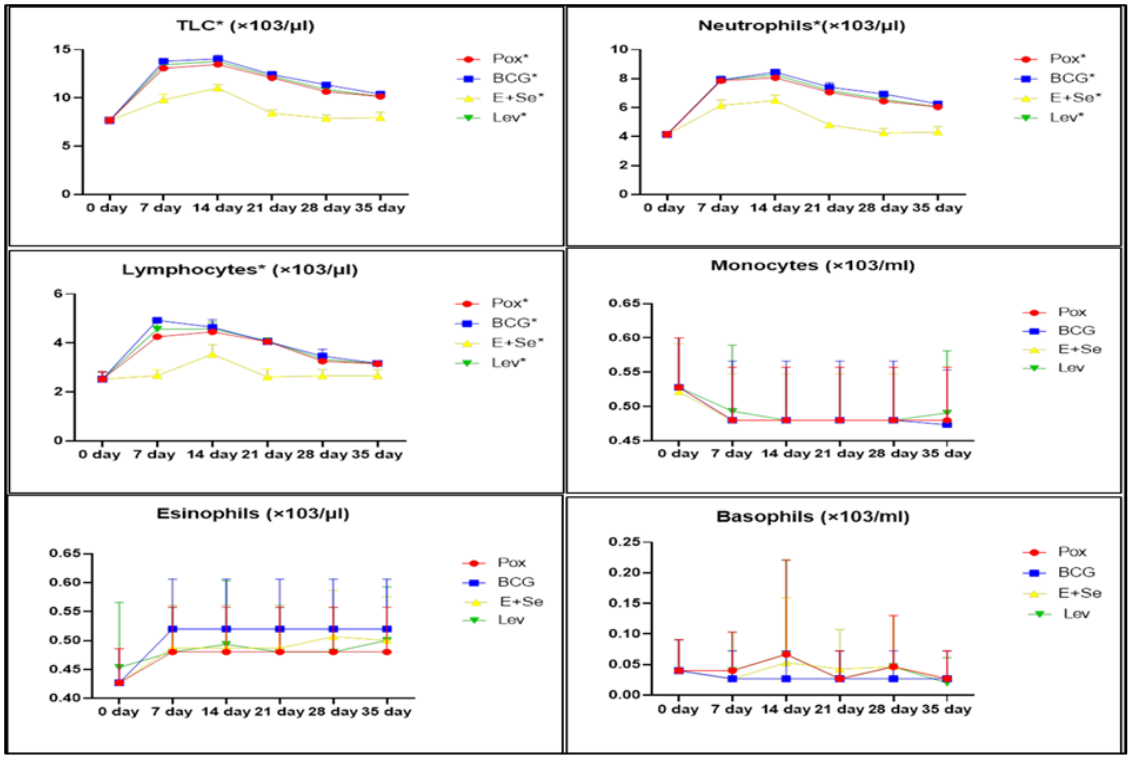

Fig. 3. Comparison between the leukogram of the four studied groups throughout the research, (*) on the parameter means significant between the groups, (*) on the group means the vaccine or vaccine+immunstimulant effect is significant throughout the research. Considered significant when (P<0.05).

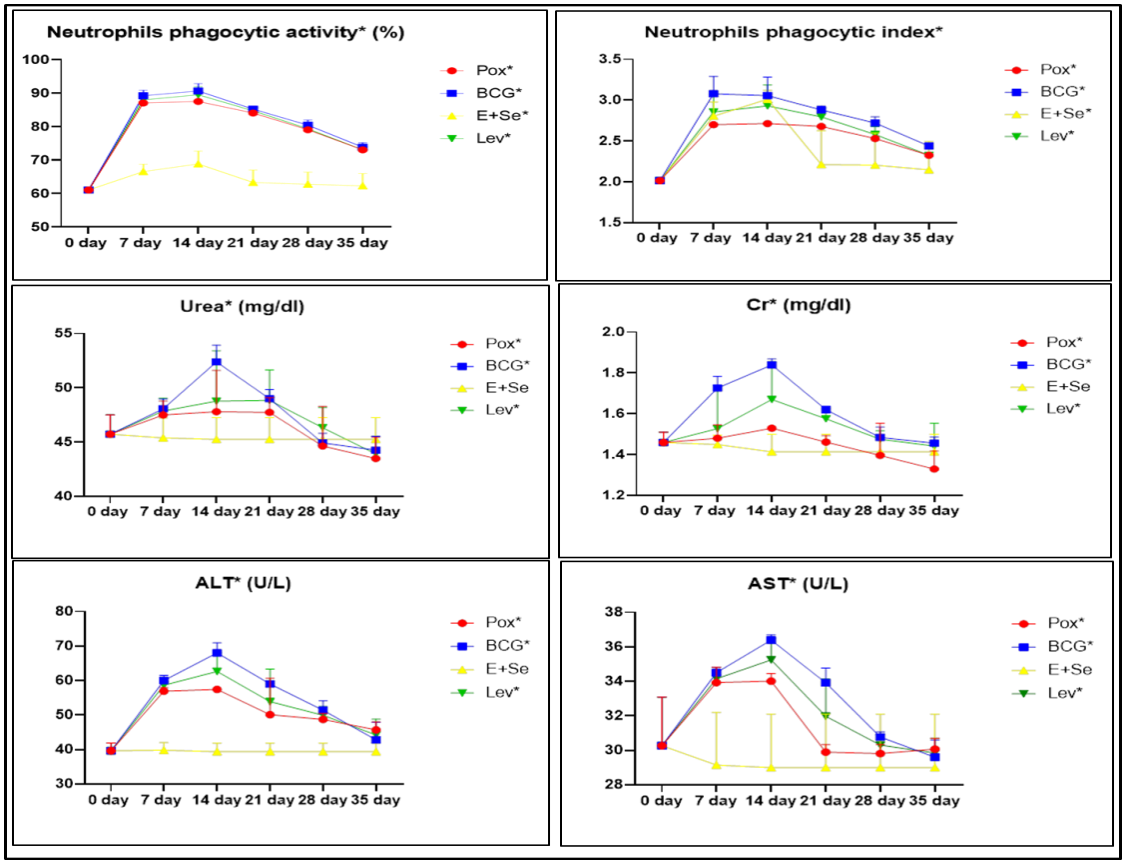

Fig. 4. Comparison between the neutrophils phagocytic activity and index and the kidney and liver function tests of the four studied groups throughout the research, (*) on the parameter means significant between the groups, (*) on the group means the vaccine or vaccine+immunstimulant effect is significant throughout the research. Considered significant when (P<0.05).

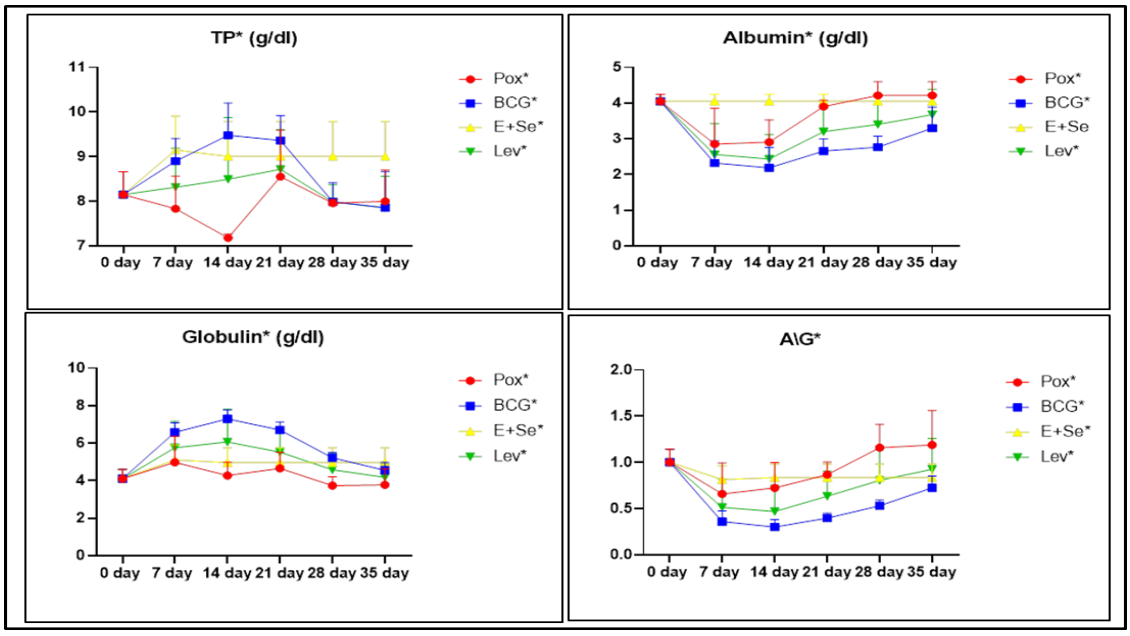

Fig. 5. Comparison between the protein profile of the four studied groups throughout the research, (*) on the parameter means significant between the groups, (*) on the group means the vaccine or vaccine+immunstimulant effect is significant throughout the research. Considered significant when (P<0.05).

References

1. Abu-EL-Saad, A.S., Abdel-Moneim, A.S. Modulation of macrophage functions by sheeppox virus provides clues to understand interaction of the virus with host immune system. *Virol.*, **J 2**, 22 (2005). https://doi.org/10.1186/1743-422X-2-22
2. Fares, M., Fawzy, M., Shahin, M. and Elshahidy, M. 'A Comprehensive Review of Sheep and Goat Pox Viruses: Perspective of Their Epidemiology and Economic Importance in Egypt'. *Suez Canal Veterinary Medical Journal. SCVMJ*, **24**(2), 313-328(2019). doi: 10.21608/scvmj.2019.69875.
3. Hamdi, J.; Munyanduki, H.; Omari Tadlaoui, K.; El Harrak, M. and Fassi Fihri, O. Capripoxvirus Infections in Ruminants: A Review. *Microorganisms*, **9**, **902**(2021). https://doi.org/10.3390/microorganisms9050902.
4. El-Ayouby, S.M.S., Salib, O.R. and El-Deen, H.K. Use of BCG vaccine for enhancement the immune response of sheep to Rev.1 Vaccination. *J. Vet. Med. Res.*, **18**(1), 6-11(2008).doi: https://dx.doi.org/10.21608/jvmr.2008.77836
5. Shawky, M.E., Mohamed, A.A., Daoud, H.M. and Farouk, E.M. Immunological Effect of Levamisole as Immunostimulant In Vaccination With Bivalent Oil Adjuvant Foot And Mouth Disease Vaccine In Sheep. *Zag. Vet. J.*, **40** (6), 13-21(2012).
6. Anugua, S., Petersson-Wolfe, C.S., Combs, Jr, G.F. and Petersson, K.H. Effect of vitamin E on the immune system of ewes during late pregnancy and lactation. *Small Rumin. Res.;* **111,**83-89(2013). https://doi.org/10.1016/j.smallrumres.2012.10.010.
7. Undiandeye, U.J., et al., Immunostimulatory Effect of Levamisole on the Immune Response of Goats to Peste des petits ruminants (PPR) Vaccination. *World Journal of Vaccines*, **4**, 88-95 (2014).
8. Alhidary, I.A., Shini, S., Al Jassim, R., Abudabos, A.M. and Gaughan, J.B. Effects of selenium and vitamin E on performance, physiological response, and selenium balance in heat-stressed sheep. *J. Anim. Sci.*, **93**,576-588 (2015). doi: 10.2527/jas.2014-8419. PMID: 26020746.
9. El-Shahedy, M. and Abdel Fatah, S. Immunostimulant activity of levamisole to polyvalent FMD vaccine in buffaloes. *Suez Canal Vet. Med. J.*, **20**(2), 449-61(2015).
10. Dabbir, B.K.R. and Nanjundaiah, K. Enhancement of immunity of sheep pox vaccinations with levamisole and bioplex. *J. Bio. Innov.,* **9**, 550–556(2020).
11. Doğan, E. Effects of Levamisole Application on Immunity System in Anthrax-Vaccinated Cattle Kocatepe. *Vet. J.*, **15**(3),348-354(2022).
12. Khan, M.A., Khan, S.B., Ahmad, S., Ahmad, I., Ikramul, H., Prince, K., Ullah, A., Shoaib, M., Zaman, S., Aqib, A.I., Rashid, G., Ali, M., Khan, I.U., Khan, I., Ullah, N. and Shahid, M. Effect of Vitamin E Supplementation on Humoral Immunity Following the Administration of Enterotoxaemia Vaccine in Goats. *Pakistan Journal of Zoology*,**51**(6),2377-2379(2019). DOI:http://dx.doi.org/10.17582/journal.pjz/2019.51.6.sc3
13. Darwish, A.A., El-Sify, A.M. and Allam, T.A. Clinicopathological study on some vaccines in Barki ewes. *AJVS*., **76**(2), 142770, pp. 104-114 (2023). doi: 10.5455/ajvs.142770.;
14. Darwish, A.A., El-dakroury MF. Clinicopathological evaluation of some immunostimulants' effects in Barki lambs. *Iraqi Journal of Veterinary Sciences*, **37**(3), 765-773(2023). DOI:10.33899/ijvs.2023.136587.2595
15. Office International Des Epizootic (OIE) Terrestrial Manual (2018): Manual of standards for diagnostic tests of vaccines: chapter .9. 2 (pages 1178-1184).
16. Feldman, B.F., Zinkl, J.C. and Jain, N.C. 2000. "Schalm's Veterinary Hematology", 5th (ed.), Lippincott Williams & Wilkins, Philadelphia, London.
17. Wilkinson, P.C. 1981. "Techniques in Clinical Immunology", 2nd (ed.), Black well scientific publications, London.
18. Reed, L.J. and H. Muench. A simple method of estimating fifty percent and points. *Am. J. Hyg.*, **27**, 493-497(1938).
19. Sndecor, G. W. and Cochran, W. G. 1980. Statistical Methods. Iowa State Univ. Press. USA.
20. Kostinov, M.P., Akhmatova, N.K., Khromova, E.A. and Kostinova, A.M. Cytokine Profile in Human Peripheral Blood Mononuclear Leukocytes Exposed to Immunoadjuvant and Adjuvant-Free Vaccines Against Influenza. *Front. Immunol.***, 11**,351-1361(2020). doi: 10.3389/fimmu.2020.01351
21. Elsayed, E.M.F., El Shahidy, M. and EL-Senosy, R. 'Field Evaluation of Sheep Pox Vaccine in Sheep and Goats in Egypt'. *Suez Canal Veterinary Medical Journal. SCVMJ*, **28**(2), Articleno. 1132, pp.353-366(2023). doi: 10.21608/scvmj.2023.210396.1132.
22. Oreiby, A.; Seada, A.S.; Abou Elazab, M.F.; Abdo, W.; Kassab, M.; Hegazy, Y. 1132 Khalifa, H.O.; Matsumoto, T. Emergency Vaccination as a Control Strategy against Sheeppox Outbreak in a Highly Susceptible Population. *Animals*, **12**, 2084 (2022). https:// doi.org/10.3390/ani12162084
23. Ayres, J.A., Barraviera, B., Calvi, S.A., Carvalho, N.R. and Peracoli, M.T. Antibody and cytokine serum levels in patients subjected to anti-rabies prophylaxis with serum vaccination. *J. Venom. Anim. Toxins incl. Trop. Dis.*, 12(3), 435-455(2006).
24. El-Din, W., Ibrahim E., Daoud, H. and Ali, S. Humeral and cellular immune response of Egyptian trivalent foot and mouth disease oil vaccine in sheep. *Res. Opin. Anim. Vet. Sci.*, **4**(4), 178- 185(2014).
25. Simon, W.L., Salk, H.M., Ovsyannikova, I.G., Kennedy, R.B. and Poland, G.A. Cytokine production associated with smallpox vaccine responses. *Immunotherapy*. **6**(10),1097-1112 (2014). doi: 10.2217/imt.14.72.
26. Christian, L.M., Porter, K., Karlsson, E. and Schultz-Cherry, S. Proinflammatory cytokine responses correspond with subjective side effects after influenza virus vaccination. *Vaccine*, **33**(29), 3360-3366 (2015). doi: 10.1016/j.vaccine.2015.05.008

27. Mawa, P.A., Webb, E.L., Filali-Mouhim, A., Nkurunungi, G., Sekaly, R.P., Lule, S.A., Prentice, S., Nash, S., Dockrell, H.M., Elliott, A.M. and Cose, S. Maternal BCG Scar Is Associated with Increased Infant Proinflammatory Immune Responses. *Vaccine***,** **35**(2), 273- 282(2017). http://dx.doi.org/10.1016/j.vaccine.2016.11.079.

28. Jarikre, T.A., Taiwo, J.O., Emikpe, B.O. and Akpavie, S.O. Protective effect of intranasal peste des petits ruminants virus and bacterin vaccinations: Clinical, hematological, serological, and serum oxidative stress changes in challenged goats. *Vet.World*, **12**(7), 945-950 (2019).

29. Bergamaschi, C., Terpos, E., Rosati, M., Angel, M., Bear, J., Stellas, D., Karaliota, S., Apostolakou, F., Bagratuni, T., Patseas, D., Gumeni, S., Trougakos, I.P., Dimopoulos, M.A., Felber, B.K. and Pavlakis, G.N. Systemic IL-15, IFN-γ, and IP-10/CXCL10 signature associated with effective immune response to SARS-CoV-2 in BNT162b2 mRNA vaccine recipients. *Cell Rep.*, **36**(6), 109504- 109521(2021). doi: 10.1016/j.celrep.2021.109504.

30. Andersen, S.A., Petersen, H.H., Ersbøll, A.K., Falk-Rønne, J. and Jacobsen, S. Vaccination elicits a prominent acute phase response in horses. *Vet. J.*,**191**(2),199-202(2012). doi:10.1016/j.tvjl.2011.01.019.

31. Arthington, J.D., Cooke, R.F., Maddock, T.D., Araujo, D.B., Moriel, P., Dilorenzo, N. and Lamb, G.C. Effects of vaccination on the acute-phase protein response and measures of performance in growing beef calves. *J. Anim. Sci.*, **91**(4), 1831-1837(2013). doi: 10.2527/jas.2012-5724.

32. Gundasheva, D. and Tsachev, I. Laboratory field studies on acute phase response of horses after vaccination against equine influenza virus and equine herpes virus. *Trakia J. Sci.*, **1**, 88-92 (2015). doi:10.15547/tjs.2015.01.01.

33. Shawky, S., Thabet, N., Orabi, S. and Nayel, M. A Comparative Study on the Hemato-Biochemical and Immunological Effects of the Hexavalent FMD Vaccine Alone or in Combination with Trivalent FMD Vaccine in Cattle. *J. Biosci. Med.*, **4**, 6-26 (2016). DOI: 10.4236/jbm.2016.41003.

34. Phillips, M., Cataneo, R., Chaturvedi, A., Danaher, P.J., Devadiga, A., Legendre, D.A., Nail, K.L., Schmitt, P. and Wai, J. Effect of influenza vaccination on oxidative stress products in breath. *J. Breath Res.*, **4**, 1-8 (2010). doi:10.1088/1752-7155/4/2/026001.

35. Kumar, A., Gupta, V.K., Mandil, R., Verma, A.K., Rahal, A. and Yadav, S.K. Mapping of oxidative stress in immune response induced by polymer gel based Brucella melitensis vaccine in mice. *Ind. J. Anim. Sci.*, **88**(7), 771–778 (2018).

36. Yadav, S, Kumar, A., Yadav, S.K., Singh, V.K. and Rahal, A. Interplay of oxidative stress and antioxidants in mice mastitis model. *National Seminar on Rttepsr Nduat Ayodhya*, **3**, 247-225(2020).

37. Dursun, A.D., Saricam, E., Sariyildiz, G.T., Iscanli, M.D., Cantekin, Ö.F. The Evaluation of Oxidative Stress in the Young Adults with COVID-19 mRNA Vaccines Induced Acute Pericarditis- Myopericarditis. *Int. J. Gen. Med.*, **15**, 161-167(2022). doi: 10.2147/IJGM.S347977.

38. Paulson, R.F., Ruan, B., Hao, S. and Chen, Y. 2020. Stress Erythropoiesis is a Key Inflammatory Response. *Cells.* **9**(3), 634-648 (2020). doi: 10.3390/cells9030634.

39. Arfuso, F., Giudice, E., Di Pietro, S., Piccione, G. and Giannetto, C. Modulation of Serum Protein Electrophoretic Pattern and Leukocyte Population in Horses Vaccinated against West Nile Virus. *Animals*, **11**, 477-287(2021). https:// doi.org/10.3390/ani11020477

40. Prentice, S., Webb, E.L., Dockrel, H.M., Kaleebu, P., Elliott, A.M. and Cose, S. Investigating the non-specific effects of BCG vaccination on the innate immune system in Ugandan neonates: study protocol for a randomized controlled trial. *Trials*, **16**, 149-160(2015). doi: 10.1186/s13063- 015-0682-5.

41. Biswajit, D., Suvakanta, D., Chandra, C.R. and Jashabir, C. An overview of levamisole hydrochloride with immuno-stimulant activity. *Am. J. Pharm. Health Res.*, **2**(4),1-9 (2014).

42. Mohamed, E.H., Baiomy, A.A., Ibrahim, Z.S. and Soliman, M.M. Modulatory effects of levamisole and garlic oil on the immune response of Wistar rats: Biochemical, immunohistochemical, molecular and immunological study. *Mol. Med. Rep.*, **14**(3), 2755-63(2016). DOI: 10.3892/mmr.2016.5551.

43. Krehbiel, C.R., Terrill, S.J., Mills, R.L., Gifford, C.A., Step, D.L. and Richards, C.J. Burciaga Robles, L.O. 2012. Inflammation and nutrient metabolism in ruminants. Presentation at the 23rd Symposium of Florida Dairy Extension. University of Florida, USA.

44. Mehrzad, J., Maleki, M., Raji, A. and Razmi, G. An ultrastructural investigation of the blood neutrophils in camel (Camelus dromedarius). *Comp. Clin. Path*., **23**(4), 885-892 (2014).

45. de Almeida, J.M.و Stefani, LM., Loyola, W., Krabbe, E.L.,Tamehiro, C.Y. and Quinteiro Filho, W.M. Effect of vitamin E on the immune system of SPF birds vaccinated against infectious bronchitis virus. *emina: i ncia A r ria 1923 ondrina*, **35**(4), 1923-1933 (2014). DOI: 10.5433/1679-0359.2014v35n4p1923.

46. Mohammed, S.A. and Ali, A.A. Effect of selenium nanoparticles against protoscoleces of Echinococcus granulosus in vitro and hydatid cysts in mice. *Iraqi J. Sci. Vet.* **36**(I), 95-202(2022). DOI: 10.33899/ijvs.2022.135838.2535

47. Gamil, N.M., Mohamed, S.M., Abou-Bakr, D.A. and Elsaid, B.S. Effect of vitamin E on the progression of renal ischemia perfusion injury in female rats. *Al-Azhar Med. J.*, **46**(4), 781-792(2017). DOI: 10.12816/0045166.

48. Kumar, A., Mehta, J.S., Purohit, G.N., Kumar, A., Narula, H.K. Effects of non-enzymatic antioxidants on serum total proteins and its fractions in Magra rams in arid region of Rajasthan. *J. Pharm. Innov.*, **8**(6), 542-547(2019). https://www.thepharmajournal.com/archives/2019/vol8issue6/PartJ/8-6-28-918.pdf.

49. Sigoloa, S., Khazaeib, R., Seidavib, A., Ayasanc, T., Galloa, A., Prandinia, A. Effects of supra-nutritional levels of vitamin E and vitamin C on growth performance and blood parameters of Japanese quails. *Ital. J. Anim. Sci.*, **18**(1), 140–146(2019). https://doi.org/10.1080/1828051X.2018.1500496

التأثير المناعى لليفاميزول، البى سى جى، و فيتامين هـ + سيلينوم علي الاستجابة المناعية للنعاج للقاح جدرى الاغنام

أسماء عبدالله درويش[1]، منى عبدالله محمود[1]، جهاد رشاد دنيا[1]، أميرة عبد النبى السعيد[2] وأية الله ابراهيم بسيونى [2]

[1] قسم صحة الحيوان و الدواجن، مركز بحوث الصحراء، القاهرة، مصر.

[2] قسم الجدرى، مركز بحوث المصل و اللقاح البيطرية، مركز البحوث الزراعية، القاهرة، مصر.

الملخص

تعد عدوى جدري الأغنام مصدر قلق اجتماعي واقتصادي لمربي الأغنام، فهي تشكل تهديدًا كبيرًا لإنتاج الأغنام الناجح على مستوى العالم. يعد التحصين هو الإجراء الوقائي الأكثر مباشرة وفعالية ضد الأمراض المعدية. في هذا العمل قمنا بدراسة التأثير المناعي لبعض المنشطات المناعية على الاستجابة المناعية الخلطية والفطرية للنعاج البرقي للقاح جدري الأغنام. تم تطعيم ستين نعجة برقي بلقاح الجدري المضعف الحياة ثم تم تقسيمها إلى: مجموعة الجدري: تم حقنها بلقاح الجدرى فقط، مجموعة BCG: لقاح + 0.1 مل لقاح BCG، مجموعة E+Se: لقاح + 0.5 مل E+Se / 10 كغم وزن الجسم ، مجموعة الليفاميزول: لقاح + 1مل من ليفابان® 10%/50 كجم من وزن الجسم. تم جمع عينات الدم منهم، وتم تقدير المعاييرالمناعية الخلطية (NI) و الفطريةو المرضية السريرية وتحليلها إحصائيا. حصلت مجموعة E+Se على أفضل NI بين المجموعات المدروسة مع الحد الأدنى من التغيرات المناعية الفطرية وأظهرت اختبارات الدم ووظائف الكبد والكلى و الالبيومين والقدرة الكلية لمقاومة التاكسد معدلات طبيعية مع تحسن فى مستويات البروتين الكلى والجلوبيولين ومؤشر البلعمة للعدلات. في المقابل، كان لدى مجموعة الليفاميزول قيم NI قريبة من مجموعة Pox وكانت مجموعة BCG لديها NI أقل من مجموعة Pox. كانت التغيرات المناعية الفطرية والمتغيرات الكيميائية الحيوية اللاحقة أكثر وضوحًا في مجموعة الليفاميزول وBCG مقارنة بمجموعة الجدري وفي مجموعة BCG مقارنة بمجموعة الليفاميزول. الاستنتاج: حقن E+Se قبل لقاح الجدري المضعف للحياة أدى إلى تحسن ملحوظ في الاستجابة المناعية الخلطية للنعاج للقاح وتقليل الآثار الجانبية المرتبطة بعملية التحصين.

الكلمات الدالة: تحصين جدرى الضأن، مؤشر التحييد، المتغيرات الباثولوجية الاكلينيكية، الليفاميزول، بى سى جى، هـ+سيلينوم.