Protective effect of cytosine-phosphate-guanosine oligodeoxynucleotide against experimental mastitis induced by *Cryptococcus neoformans* infection in goats

Nidhal R. Mahdi[1], Shaimaa N. Yassein[2] and Jenan M. Khalaf[3]

[1,2] Department of Microbiology, [3] Unite of Zoonosis, College of Veterinary Medicine, Baghdad University, Iraq.

E-mail: dr.nidhalraoof@yahoo.com

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Summary

The present study investigated the effect of synthetic non-methylated oligonucleotides containing Cytosine-phosphate-guanosine dinucleotides (Cytosine-phosphate-guanosine oligodeoxynucleotide**)** on caprine mastitis with Heat Killed *Cryptococcus neoformans* Ag. 20 healthy local breed does were used with weight ranging of 25-30 Kg and free of mastitis by examination via California Mastitis Test and Somatic Cell Count. The does were allotted into four equal groups, the first group (G1) was treated intramammary with 100µg/kg of Cytosine-phosphate-guanosine oligodeoxy nucleotide on fifth day postpartum in the right mammary gland while the left mammary gland served as control and were infused with sterile phosphate buffered saline. On day 8 postpartum repeat dosages of Cytosine-phosphate-guanosine oligodeoxynucleotide and phosphate buffered saline were infused respectively. On day 9 pp the right mammary gland was infused with 2ml of $2x10^8$ cell/ml of Heat Killed *Cryptococcus neoformans* Ag. The second group (G2) was infused at day 9 postpartum with 2ml of $2x10^8$ cell/ml of Heat Killed *Cryptococcus neoformans* Ag in the right mammary gland only. The third group (G3) was left until the challenge test done after one week of immunization in the G1 and G2, by inoculation of 2ml of $5x10^6$ viable *C. neoformans* in the right mammary gland. The fourth group (G4) was kept as a control receiving 2ml of sterile PBS. Blood samples were collected at 0, 5, 10, 20, 30 and 40 days of the study, to determine the antibody titer by passive haemagglutination assay, while the cell mediated immunity was evaluated by detecting the goat Interferon Gamma by ELISA test and Phagocytic index. Also the cell mediated immunity was determined by delayed type hypersensitivity test after 21 days of immunization. The results showed a significant variation ($P \leq 0.05$) between vaccinated groups (G1 and G2) and the control. However, there was a significant increase ($P \leq 0.05$) of skin thickness shown after 48 hrs in the G1compared to G2. High level of Interferon Gamma concentration was noticed in the G1 as compared with other groups. Moreover, cell mediated immunity developed effectively in the G1 which was noted by a significant increase ($P \leq 0.05$) of phagocytic activity of polymorphonuclear cells. The high level of antibody titer was observed in the G1 as compared with other groups. In conclusion: These results suggest that vaccination with Cytosine-phosphate-guanosine oligodeoxynucleotide plus Heat Killed *Cryptococcus neoformans* Ag intramammary lead to a good protection of caprine mammary glands against *C. neoformans* mastitis.

Keywords: Caprine mastitis, Heat Killed *Cryptococcus neoformans* antigen, Cytosine-phosphate-guanosine oligodeoxynucleotide.

Introduction

It was found that purified deoxynucleotides (DNA) from *Mycobacterium bovis* bacille Calmette–Guérin (BCG) possessed immune stimulatory effects, including the activation of natural killer (NK) cells and production of type-1 and type-2 IFN *in vitro* and the promotion of tumour regression *in vivo* (1). Other findings confirmed that purified bacterial DNA induced B cell proliferation and immunoglobulin secretion, while vertebrate DNA did not (2). This is due to the bacterial genome as compared to vertebrate DNA contains a higher frequency of unmethylated deoxycytidyl-deoxyguanosine (CpG) dinucleo -tides while the methylation of 80% of the CpG was found in vertebrates (3). Small oligodeoxynucleotides (ODN) with unmethyl -ated CpG dinucleotides (CpG-ODN) are able to exactly mimic the immunostimulatory activity of bacterial DNA (bDNA) and are distinguished by the Toll-like receptor 9 (TLR9) (4). DNA from protozoa was reported in previous studies to stimulate B cell proliferation and macrophage cytokine synthesis in a TLR9-dependent manner,

although they are eukaryotic microorganisms. These findings raise a possibility that DNA from *Cryptococcus neoformans* may be involved in the activation of host immune responses, as in the case of protozoa. In correspond with this hypothesis, DNA from some fungi, such as *Schizosaccharomyces pompe* and *Paracoccidioides brasiliensis*, is also reported to stimulate a battery of immune responses (5). Synthetic CpG-ODNs have been shown to have potentials as adjuvants for vaccines (6). Although CpG-ODNs are a good adjuvant, they can have an even greater adjuvant activity if formulated or coadministered with other compounds, such as particulates, mineral salts, saponins, liposomes, cationic peptides, polysaccharides and bacterial toxins and the synthetic polymers, polyphosphazenes (7). CpG-ODN appears to be a promising class of adjuvants for a wide variety of vaccine candidates such as hepatitis viral surface antigen, inactivated influenza virus and viral peptides (8) because these compounds have shown safety profiles similar to conventional vaccines, So it could be used in clinical trials as adjuvants for multiple immunotherapies and for treating infectious diseases, cancers and allergies (9).

The therapeutic applications of CpG-ODN are investigated for adjuvant activity in immunotherapy,anti-infection (10 and 11), anti-asthmatic and allergy (12) and for anti-tumor (13). The present study aims to characterize the immunostimulatory effect of CPG-ODN on the goat`s mammary gland defence which may help to develop new mastitis prophylaxis and treatment.

Materials and Methods

Twenty healthy local breed does aged 2-4 years, weight range (25-30 Kg) free of mastitic pathogens by examination via California Mastitis Test (CMT) and Somatic Cell Count (SCC) and clinically free from any other infectious disease were used. Goats and mammary glands were monitored during 5days before starting the experiment.

The antigens were prepared from a virulent *C. neoformans* strain that was isolated from caprine mastitis and cultured in BHI broth for 3 days at 37˚C, then 2 ml of this broth cultured directly on SDA for 5 days at 33˚C then harvesting was done by 10 ml of phosphate buffered saline (PBS) (pH=7.2) and a suspension was accumulated in test tubes then centrifuged to 4000 rpm for 20 min. and washed 3 times in PBS. The Cryptococcal cells were counted by using a haemocytometer chamber according to (14). Heat Killed *C. neoformans* Ag (HKCn Ag): The sediment was resuspended by PBS, then put in a screw cap bottle in a water bath and heated at 60˚C for 1 hrs. The lack of viability of Cryptococcal cells was confirmed by cultivation of suspension on Sabouraud Dextrose Agar (SDA) and absence of growth considered positive indicated of killing (15).

Soluble Sonicated *C. neoformans* Ag (SS Cn Ag): It was prepared after harvesting virulent *C. neoformans*, centrifuged and the sediment was resuspended by PBS, then put in a screw cap bottle in a cooled sonicator oscillator and surrounded by enough amount of ice to prevent the temperature elevation during the sonication, put down the probe inside the sample so it can reach the bottom of the bottle without touching it. The actual work of sonicator was 60 min. and in range 1min work, 1min. rest with 50 voltage. During work, there was a replacement of ice that surrounded the sample, and aloopful was taken from sample and examined by staining with India ink to make sure that the sonication process was occurred, then a loopful of sample was inoculated on SDA to make sure that no growth occure to 24-48 hrs. (16). The sonicated suspension was centrifuged by cold centrifuge with 16000rpm for 30min. and the supernatant was taken and the sediment discarded, the supernatant passed through a 0.22 µm millipore filter and protein was estimated 16gm/ml by using Biurate Kit (Randox Lab.) and kept it under 4˚C until used in skin test (17).

The lactating does were divided into four groups, each the first group (G1) was treated with 100µg/kg of CPG-ODN on day 5 postpartum (pp) in the right mammary gland while the left mammary gland served as control and was infused with sterile PBS. On day 8pp repeat dosage of CPG-ODN and PBS were infused respectively. On day 9 pp the right mammary gland was infused with 2ml of $2x10^8$ cell/ml of HKCn Ag. The second group (G2) was infused at 9 pp with 2ml of $2x10^8$ cell/ml of HKCn Ag in the right mammary

gland only. The third group (G3) was left until the challenge test that done after one week of the immunization in the G1 and G2. by inoculation of 2ml of $5x10^6$ viable *C. neoformans* in the right mammary gland. The fourth group (G4) served as control receiving 2ml of sterile PBS according to (18).

Blood samples were collected at 0, 5, 10, 20, 30 and 40 days of study. These samples were drawn aseptically from the external jugular vein by vein puncture into vacutainer tube containing Lithium- Heparin10 IU/L for studying the Phagocytic index, while other samples were allowed to coagulate in refrigerator for 24 hrs. then serum was collected by centrifugation of blood samples and stored at-18˚C for detection and evaluation of antibody titer and IFN-γ. The twenty goats in experimental study were examined for rectal temperature, heart rate, respiratory rate, general attitude and specific udder examination according to (19) at study period.

Evaluation of Immune response by: Cell Mediated Immune Response by Delayed Type Hypersensitivity reaction (DTH) was assessed by skin thickness according to (20) by using Soluble Sonicated *C. neoformans* Ag protein intradermaly of the upper lateral part of udder on the right side. This test was carried out after 21 days of immunization. (The 0 day was considered the day of HKCn Ag administra -tion in both immunized groups). Detection of Goat Interferon Gamma by ELISA Kit (according to the manufactures directions by CUSABIO, also Phagocytosis index according to (21).

Evaluation of humeral immunity by Passive Haemagglutination Test according to (22). The challenge test was performed to the right udder of each goat in the immunized and non-immunized groups after one week of immunization by inoculation intramammary 2 ml of viable *C. neoformans* containing 5 x 10^6 cells/ ml while the left udder control of all groups. After more than 30 days post challenge, all does that exposed to challenge dose were killed and postmortum examination was done. The collected data were subjected to statistical analysis, ANOVA test was used to find out the significant differences. The statistical significance was accepted as $P \leq 0.05$. Values were reported as mean and standard error (Mean±SE).

Results and Discussion

The present study showed that G1, at 24 hrs. that the mean values of skin thickness against soluble sonicated *C.neoformans* Ag (2.40 ±0.15) mm in goats were higher than those in the G2 which immunized with HKCn Ag alone (1.30±0.57) mm. At 48 hrs, the value of skin thickness in the G1 was more elevated when compared with the G2 (Table, 1).

Table, 1: Skin thickness (mm) in immunized and control groups (Mean ± SE).

Skin thickness (mm).			
Groups	After 24hrs. (Mean ± SE)	After 48hrs. (Mean ± SE)	After 72hrs. (Mean ± SE)
G1	2.40±0.15 Ab	3.26±0.23 Aa	2.60±0.15 Ab
G2	1.30±0.57 Bb	1.70±0.11 Ba	1.16±0.33 Bb
G3 (control –ve)	0.13±0.33 B	0.00±0.00 C	0.00±0.00 C

The different capital letters in column refer to significant differences (P≤0.05) among groups and different small letters in row refer to significant differences (P≤0.05) among periods.

The skin test was performed to evaluate the cellular immune response in immunized and non-immunized groups of goats. The positive reaction of the skin test was showed in both immunized groups and the peak of thickness was at 48 hrs. when compared with control group that showed no skin reaction at injection site. This test revealed that the use of CPG-ODN as immunostimulatory agent resulted in stimulation of cellular immune response more than the second group that immunized with HKCn Ag alone. This may be ascribed to that the oligo-DNA (ODN) containing unmethylated CpG motif activate dendritic cells (DC) to induce pro -inflammatory cytokines and chemokines which results in the development of a pattern of Th1-like immune activation as mentioned by (23) and to promote an MHC II-restricted, antigen-specific CD4+T-cell response. This idea is in consistency with (24) who demonstrated the role of CpG-containing oligodeoxy- nucleotides to stimulate production of proinflammatory cytokines and chemokines from murine conventional and plasmacytoid dendritic cells. Also this opinion coincids with (25) who discovered that The oligo-DNA (ODN) containing this motif

activates murine dendritic cells (DC) to produce IL-12 and expression of co-stimulatory molecules such as CD40, which results in the development of a pattern of Th1-like immune activation as pointed by (26 and 27).

The results of DTH responses in the G1 and G2 indicated that HKCn Ag with or without CPG-ODN elicited cell mediated immune response and it is induced by CD4+ and CD8+T cells which produced IFN-γ that played an essential role in the initiation of DTH reaction and this confirmed through the observation of induration of skin at the site of inoculation of antigens and increase skin thickness due to accumulation of immune cells particularly macrophages and DTH cells and other inflammatory cells, this evidence is in agreement with (28 and 29) who demonstrated that CPG-ODN act as adjuvant through indirect effects on other immune cells such as macrophages, nmonocytes, and T cells, CpG enhance antigen presentation and induce the production of high levels of Th1 cytokines, resulting in the production of potent antigen-specific Th1-type immune responses as described by (30).

On the other hand, the present study showed that the immunized animals with Heat killed Ag expressed low value of DTH reaction as compared with those values of the G1. This may be due to influence of the heat on protein Ags. However, immunization with HKCn Ag induced both $CD4^+$ and $CD8^+$T cells whichare involved in the anticryptococcal DTH response as mentioned (31). The lowest value of DTH reaction in the G2 may be attributed to the ability of HKCn Ag to induce T_{DH} cell population without T_{AMP} cells and this evidence is in agreement with (32) who used HKCn Ag and chitosan in immunization of mice. Beside the Depletion of $CD8^+$ T cells resulted in reduced delayed-type hypersensitivity (DTH) responses without affecting antigen recognition or lymphocyte proliferation in vitro, suggesting that $CD8^+$ T cells enhanced host defenses against *C. neoformans* as mentioned by (33).

The results showed in (Table, 2) indicates that immunized animals with CPG-ODN plus HKCn Ag group expressed high levels in the mean concentration of IFN- γ (2.54±1.44) and in the group which immunized with HKCn Ag was (1.76±1.12) compared with control negative group (0.48±0.43). The G1 showed highly significant differences value of IFN- γ means concentration on (P≤0. 05) compared with the control group.

Table, 2: The differences in theIFN-γ concentrations means in immunized and non-immunized groups that were measured by ELISA method, (Mean ± SE).

Group	(Mean ± SE)					
	0 day	After 5 day	After 10 day	After 20 day	After 30 day	After 40 day
G1	0.73 ±1.2 (N.S)	1.22 ±1.1 Ac	2.07 ±1.56 Ab	2.54 ±1.44 Aa	1.98 ±1.1 Ab	1.62 ±0.98 Ab
G2	0.61 ±1.32 (N.S)	0.79 ±0.24 B	1.22 ±0.78 Bb	1.76 ±1.12 Ba	1.13 ±0.56 Bb	0.88 ±0.45 Bc
G3 (control –ve)	0.51 ±0.22 (N.S)	0.57 ±0.1 B	0.59 ±0.09 a	0.48 ±0.43 c	0.49 ±0.36 C	0.54 ±0.32 B

The different capital letters in column refer to significant differences (P≤0.05) among groups and different small letters in row refer to significant differences (P≤0.05) among period. (N.S)= non significant.

The results of IFN- γ examination were concomitant with those of DTH responses, based on these data, this study suggested that the CPG-ODN plus HKCn Ag stimulated immune cells such as macrophages, dendritic cells, natural killer cells and T- cells to produce IFN- γ which play an important role in attraction and activation of macrophages which were the principle cells in the induction of DTH response and cell mediated immune responses.The present finding revealed that the high level of IFN- γ mean concentration in the G1 may return to the action of CPG-ODN as an adjuvant and this observation is in consistence with (34) who found that a stronger induction of IFN-γ and IL-12 may be beneficial to enhancing Th1 type immune responses when the CpG-ODN was formulated to a vaccine as an adjuvant, so, The immune responses elicited by CpG-ODN lead to the consideration of using CpG as an adjuvant. Other researchers (35) were discovered that in vivo injections of CpG-ODN induced systemic or local Th1-biased immune responses, including the synthesis of IL-12 and IFN-γ.

The contribution of both $CD4^+$ and $CD8^+$ T cells to produce IFN-γ was induced by CpG-ODN treatment of different animal models,

although their contribution varied from one model to another, and discovered the using of heat-killed leishmania antigen and CpG oligodeoxynucleotides induces long-term memory $CD4^+$ and $CD8^+$ T cell responses and protection against leishmania (36). The major source of IFN-γ production was $CD8^+$ T cells rather than $CD4^+$ T cells in lung on day 7 after infection with *C. neoformans*, although the overall proportion of intracellular IFN-γ+ $CD8^+$T cells was not very high (2·6%) (1). In contrast, on day 14 $CD4^+$ T cells became the major IFN-γ-producing cells instead of $CD8^+$ T cells. This observation of the G1 is similar to the data that reported by (37) who studied the effects of CpG ODN adjuvant on the immune responses elicited by a quadrovalent mastitis vaccine in dairy cows and they conducted to peripheral blood mononuclear cells (PBMC) from cows immunized with CpG-ODNs as the adjuvant had a significantly increased expression of IFN-γ (11 v.s. 4 folds) and these results indicated that inclusion of CpG ODNs as the adjuvant in an inactivated mastitis vaccine can enhance Th1 type immune responses, which might be beneficial to the elimination of bacteria by phagocytes. Our evidence showed that the G2 which immunized with HKCn Ag alone have less value of IFN-γ concentration when compared with the 1st group and this result was associated with the result of DTH reaction in the same group and this may be due to unability HKCn Ag to induce T_{amp} cells which were responsible for augmented IFN-γ production as mentioned by (15). Moreover, (38) explained that the DTH reaction was correlated with protective immunity when combination occurred with mononuclear cells infiltration and elevated IFN-γ levels in DTH reaction sites. The high IFN-γ levels without DTH response do not provide protective immunity to mice infected with *C. neoformans* (39).

Phagocytosis indices were increased significantly in immunized groups (G1 was 46.4±0.7 and the G2 was 29.2±2.1) and in the infected group (G3 was 23.9±1.8) as compared with the control group which gave (12.4±0.2). The result showed a high significant variation at (P≤0.05) between G1 and G2, also significant variation at (P≤0.05) between immunized groups and G4 (control group) was present (Table, 3).

Table, 3: The phagocytic indices in immunized, infected and control groups (Mean ± SE).

Groups of goats	G1 (Mean±SE)	G2 (Mean±SE)	G3 (Mean±SE)	G4 (Mean±SE)
Phagocytic indices	**46.4±0.7 A**	**29.2±2.1 B**	**23.9±1.8 C**	**12.4±0.2 D**

The different letters= significant differences (P≤0.05).

The result of the current study was expected to the observation in the G1 due to stimulation and activation of phagocytic cells (monocyte and neutrophils) through the immunostimulatory effect of CPG-ODN adjuvant which enhanced opsonising antibodies like IgG2a isotype because the phagocytic rate of these cells increased in the presence of opsonising antibodies against specific pathogens, this observation is in line with (40). However, the phagocytosis represents the most important defense mechanisms against mammary gland pathogens. *In vivo*, *C neoformans* has a polysaccharide capsule that impairs phagocytosis but, as a result of binding specific opsonising antibodies against this capsule, phagocytosis will reactivated and become effective (41). There was no similar research on phagocytosis with HKCn Ag in goats, but in sheep (42) reported that the live attenuated *S. aureus* vaccine was more helpful in phagocytosis than killed vaccine through stimulating production of IgG1 and IgG2. Also, the present study revealed that the phagocytosis value of the infected group was higher than data observed in the control group, this result was in agree with (43) who recorded that the significant increase of phagocytic activity occurred after injection of *Mycoplasms agalactia* in goats. During diapedesis of PMN into the mammary gland, several functionally important receptors were up-regulated, allowing for a more efficient phagocytosis and killing of invading pathogens. While PMN are phagocytosing and destroying the invading pathogens, they inadvertently release chemical mediators which induce swelling of secretory epithelium cytoplasm, sloughing of secretory cells, and decreased secretory activity (44). In addition to macrophages, neutrophils were responsible

for bacterial phagocytosis and can activate the immune system by release of cytokines and pro-inflammatory mediators and facilitate the innate immune response, including neutrophils migration and bactericidal functions (45).

However, mammary macrophage numbers tend to be lower during inflammation and they possess fewer Fc receptors, possibly their rate of phagocytosis decreased when compared to neutrophils, then, phagocytic activity rapidly decreases with continued exposure to inhibitory factors such as milk fat globules and casein in mammary secretions (46). On the other hand, it was found that a 3-fold difference exists among cows in the ability of milk to support phagocytosis and a 2-fold difference in the ability of PMN to Phagocytose (47). Antibody titers of immunized and control groups by Passive Haemagglutination test: The present study revealed that the goats administered with CPG-ODN then HKCn Ag (G1) showed high values of Ab titers (298.±112.88) against SSCn Ag as compared with those values of the G2 that immunized with HKCn Ag only (12.00±4.00) and those values of the control group (3.33±0.60) at 40 days of immunization (Table, 4).

Table, 4: Antibody titers in serum of immunized and control groups (Mean ± SE).

Group	(Mean ± SE)					
	0 day	After 5 day	After 10 day	After 20 day	After 30 day	After 40 day
G1	53.33 ±10.6 C	85.33 ±21.3 Ac	213.3 ±42.66 Ab	341.3 ±85.33 Aa	341.3 ±85.3 Aa	298.6 ±112.8 Aa
G2	6.66 ±1.33 B	10.66 ±2.66 B	21.33 ±5.33 B	26.66 ±14.11 B	37.33 ±5.33 B	12.00 ±4.00 B
G3	3.33 ±0.66 B	3.33 ±0.66 B	3.33 ±0.60 B	3.33 ±0.66 B	2.66 ±0.66 B	3.33 ±0.66 B

The different capital letters in column refer to significant differences (P≤0.05) among groups and different small letters in row refer to significant differences (P≤0.05) among period.

There was suggests that both types of immunization's methods stimulated humeral immune response but with different values. There was a significant increase in antibody titers at (P≤0.05) that observed in the G1 as compared with the G2 and G3, respectively, also the G2 showed a significant difference at (P≤0.05) as compared with the G3 during the study period at 5, 10, 20, 30 and 40 days. These results indicated that the G1demonstrated best along the experiment which showed the highest titer of serum antibody as shown in (Table, 4).

The highest levels of Abs titers in serum of goats that administered CPG-ODN then HKCn Ag may be ascribed to the action of CPG-ODN that have novel adjuvants to promote opsonising antibodies such as those of the IgG2a isotype , this observation coincides with by (48), also CpG-DNA induces proliferation of almost all (>95%) B cells and increases immunoglobulin (Ig) secretion. This B cell activation by CpG-DNA is T cell independent and antigen non-specific. However, (49) reported that B cell activation by low concentrations of CpG-DNA has strong synergy with signals delivered through the B cell antigen receptor for both B cell proliferation and immunoglobulin secretion. The present study demonstrated that the immunized animals which expressed high values of CMI response, also revealed high levels of Abs, So this result may indicate an interaction between CMI and humeral immunity.

The animals in the non-immunized group showed different signs of udder inflammation during the first three days after inoculation which characterized by pain, warming, and increasing in the size of the infected halves when compared with uninfected left udder halves (Fig. 1), this observation revealed to clinical mastitis formation, but after that these signs disappeared bit by bit and the udder size began to decline until reached to smallest size at the end of experiment with more firmness in consistency when compared with non-infected left udder halves (Fig. 2). In contrast, the G1 (immunized with CPG-ODN then HKCn Ag) showed no alteration in udder halves morphology which mean that the clinical mastitis didn't develop in challenged halves of immunized animals (Fig. 3), this may be due to an increase in levels of immunoglobulins that possess the ability of opsonising *C. neoformans* for phagocytosis by neutrophils accompanied with that CpG-DNA induced more rapid migration of PMNs from blood to mammary tissue at the initial stage of

infection. This is corresponded with (48 and 50) who highlighted on the Protective effect of CpG-DNA against mastitis induced by Staphylococcus aureus and Escherichia coli infection in a rat model.Although there have been no reports on the effect of HKCn Ag in prevent caprine mastitis.

Figure, 1: The right infected half of goat showed an increase in the size when compared with non-infected left udder halves (control group after 3 days of challenge).

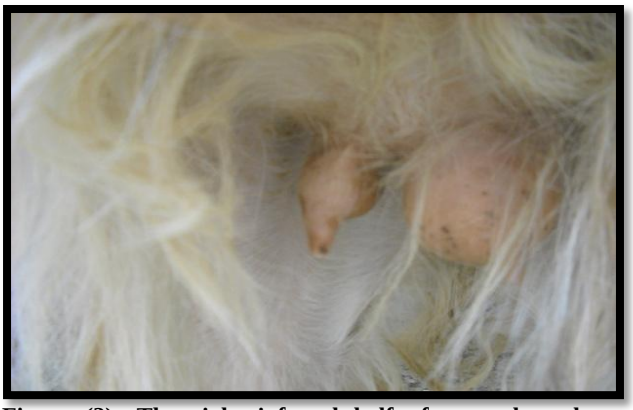
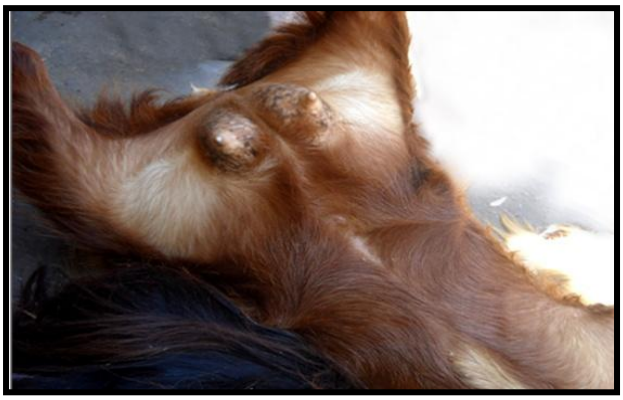
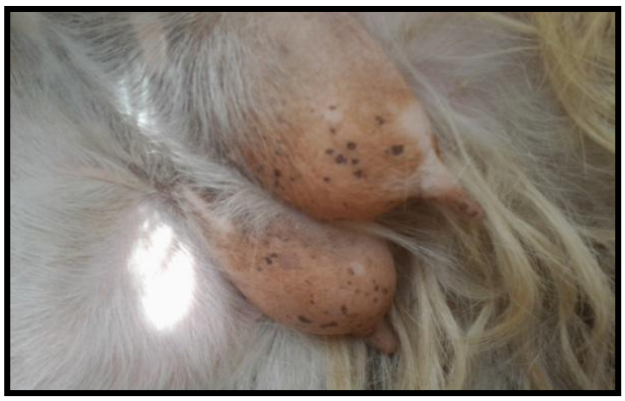


Figure (2): The right infected half of goat showed a decrease in the size when compared with non- in-fected left udder halves (control group after challenge at the 35 days of experiment).



Figure, 3: No change in the udder halves of goat morphology of the 1st group that with CPG-ODN then HKCn Ag along the study.

The 2nd group revealed a slight increase in the size of injected halves at the begining of study but less than that in infected group (Fig. 4). While systemic examination (Temperature, heart rate and respiratory rate) showed no significant changes in all groups.



Figure, 4: The slight increase in the size of injected halves in goat of the 2nd group at the beginning of study but less than infected group.

C. neoformans reisolation from immunized and non immunized udder halves: The *C. neoformans* couldn't be isolated from any internal organs of all groups, while this yeast was reisolated with heavy growth from the mastitic mammary secretions of all infected udder halves in the G3 at 5th to more than 30th day post inoculation, and in the present study. This yeast was not isolated neither from the opposite non-infected udder halves nor from udder of immunized animals with CPG-ODN then HKCn Ag. Whereas the G2 showed fungal growth in cultivation of their milk with low percent. The researchers (51) pointed that the clearance of Cryptococcus infection requires the development of a Th1-type CMI and activation of leukocytes. Neutrophils and macrophages are the two phagocytic cells in the natural host defense that were most likely to be responsible for clearing the cryptococcal cells from the tissues. Additionally, the cytokine profile at the site of infection determines the rate of clearance, So, the host who has higher IFN-γ at the site of infection had a faster rate of clearance compared to those with lower levels (52). The results of present study suggested that the CPG-ODN improved mammary gland defense and thereby, had a beneficial effect against mastitis caused by *C. neoformans* infection in goats. This observation was in line with (17) who explained the effect of CPG-ODN on the mammary gland defense during mastitis

induced by *E.coli* infection in goats. This study concluded that CPG-ODN induced Th1-cell to express cell mediated immunity (CMI) and activated B cells to produce humeral immunity and CPG-ODN possesses the ability in the induction of strong protective immunity, proper recruitment and function of effector cells (lymphocytes and macrophages), and, ultimately, effective cryptococcal clearance from the infected mammary glands of goat.

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التأثير الواقي للسايتوسين فوسفات كوانوسين ضد الاصابة التجريبية بإلتهاب الضرع المتسبب عن الإصابة بالمكورات الخبيئة في الماعز

نضال رؤوف مهدي[1] وشيماء نبهان ياسين[2] وجنان محمود خلف[3]

[3]وحدة الأمراض المشتركة، [1.2] فرع الأحياء المجهرية، كلية الطب البيطري، جامعة بغداد، العراق.

E-mail: dr.nidhalraoof@yahoo.com

الخلاصة

أظهرت هذه الدراسة تأثير استعمال المحفز المناعي السايتوسين فوسفات كوانوسين ضد الإصابة بإلتهاب الضرع في الماعز مع مستضد خميرة المكورات الخبيئة المقتول بالحرارة حيث استعملت 20 رأسا من الماعز المحلي تراوحت اعمارها بين 2-4 سنوات ومتوسط وزن 25-30 كغم وقد تم فحص جميع الحيوانات باختبار كاليفورنيا واختبارعد الخلايا الجسمية للتحري عن خلوها من التهاب الضرع. وقسمت الحيوانات على 4 مجاميع متساوية. المجموعة الاولى: حقنت ب 100 مايكروغرام/كغم من وزن الجسم بالمحفز المناعي السايتوسين فوسفات كوانوسين بعد 5 ايام من الولادة وكررت الجرعة عند اليوم الـ 8 من الولادة وفي اليوم الـ 9 حُقنّت 2 مل من 2×10^8 من المستضد المقتول في الشطر الأيمن من الضرع أما الشطر الأيسر فقد ترك كسيطرة وحقن بدارئ الفوسفات الملحي الفسلجي. المجموعة الثانية: حقنت ب 2 مل من 2×10^8 من المستضد المقتول فقط وذلك بعد 9 أيام من الولادة في الشطر الأيمن فقط. المجموعة الثالثة: تركت لحين اجراء اختبار التحدي الذي اجري بعد اسبوع من تمنيع الحيوانات في المجموعتين الاولى والثانية وذلك بحقن 2 مل من *C.neoformans* وبتركيز وبتركيز 2×10^6 في الشطر الايمن من الضرع. المجموعة الرابعة:مجموعة السيطرة فقد حقنت ب2 مل دارئ المحلول الملحي الفسلجي. جمعت عينات الدم لفحص المناعة الخلوية المتمثلة بقياس تركيز الانترفيرون كاما وفحص البلعمة ولغرض قياس مستوى الاجسام المضادة بواسطة فحص التلازن الدموي غير المباشر ابتداء من اليوم 0 و5 و10 و20 و30 و40 من الدراسة. أظهرت النتائج أن التمنيع بواسطة المستضد المقتول مع او بدون المحفز المناعي السايتوسين فوسفات كوانوسين ادى الى تحفيز استجابة مناعية خلوية وخلطية جيدة اذ ان جميع الحيوانات الممنعة أظهرت نتائج ايجابية في الفحص الجلدي باستعمال ألمستضدات الطافية الناتجة من عملية تكسيرخميرة *C.neoformans* كما اوضحت نتائج الدراسة ان حيوانات المجموعة الاولى اظهرت مستوى عال من تركيزالانترفيرون كاما مقارنة بالمجاميع الاخرى اضافة الى تأثر المناعة الخلوية في قابلية الالتهام للخلايا متعددة الانوية وخلايا وحيدة النواة بزيادة معنوية وخاصة في المجموعة الأولى، اما فيما يخص المناعة الخلطية فان الحيوانات التي جرى تمنيعها مسبقا كما في المجموعة الأولى اظهرت معاييرعالية من الاجسام المضادة مقارنة بالمجاميع الاخرى. ومن هذا كله يتضح أن التلقيح باستخدام المحفز المناعي السايتوسين فوسفات كوانوسين قبل المستضد المقتول داخل الحلمة قد ولد حماية للغدد اللبنية للماعز ضد التهاب الضرع بخميرة *C.neoformans*.

الكلمات المفتاحية: التهاب الضرع في الماعز، المستضد المقتول بالحرارة لخميرة المكورات الخبيئة، سايتوسين فوسفات كوانوسين.