The evaluation of the immune response against three *Candida albicans* vaccines by using ELISA

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**Abstract**

The *Candida albicans* infection is of minor veterinary significance, however, during the last years a considerable concern has been focused on the illness caused by these yeasts. The use of ELISA has been introduced as a new diagnostic tool to evaluate the immune response in case of various diseases including the fungal infections via measuring the level of IgG. Herein, the aim of this study was to identify the *C.albicans* microscopically and by using PCR techniques. In addition, we have used three types of vaccines (heat killed *C. albicans* vaccine with adjuvant or with culture filtrate, or mixed vaccines (HK-CA) vaccine and (CFV)) and immune response (level of IgG) of rats was evaluated by ELISA. The data showed that, the immune response of rats was significantly increased after challenge in all three types of vaccines and there was no significant differences between level of immunity emerged by heat killed *C. albicans* vaccines and culture filtrate of *C. albicans*. While, immunization by mixed vaccine (HK-CA, CFV) showed significant increase in anti-*Candida albicans* than heat killed or culture filtrate alone especially after challenge with the virulent *C. albicans* strain.

**Introduction**

Genus *Candida* is composed by yeasts that live as commensal on human and animals' microbiota. In general, they do not cause any damage to their hosts. However, due instability on chemical, physical and immunological defenses, these microorganisms can become pathogens. *Candida* species infections are rare on Veterinary Medicine. However, in the last years, a considerable raise of illness caused by these yeasts has been related on varied animal species due to the highly use of antibiotics. There are several species of *Candida* including, *C. albicans*, *C. tropicalis*, *C. pseudotropicalis*, *C. krusi* with the *C. albicans* is
the most important one responsible for infectious diseases in animals (Brito et al., 2009) such as arthritis in horses and mastitis and abortion in cattle. The vaccines that stimulate the immune response whether humoral or cellular immunity or both have been introduced as a new tool for protection against fungal pathogens, with the humoral immunity is the predominant immune response against certain pathogenic fungi such as Candida albicans (Casadevall et al., 2002). Moreover, Antibodies probably play an important part in host defense against disseminated Candidiasis (Matthews and Burnie, 1996). This suggested that humoral antibody may protect against C. albicans endocarditis, perhaps through inhibition of adhesion, a crucial early step in the pathogenesis of endocarditis (Michael et al., 1983). The importance of C. albicans vaccines and how it stimulates the immune system has been reviewed in many literatures, previously in Cardenas et a (1999) and recently in Ibrahim et al (2006).

Herein, this study was aimed mainly to identify C. albicans via microscopic examination and PCR. In addition, we have evaluated the immune response of three laboratory prepared vaccines via measuring the level of IgG by ELISA. The data obtained from this study was promising; Immunized rats have showed higher level of IgG compared with non immunized rats.

Materials and methods

1-Cultivation and determination of virulence factors of Candida albicans

Samples for C. albicans were obtained from mastitic cases (cow and buffalos). Candida albicans samples were firstly cultivated on Sabouraud’s dextrose agar medium and Rice agar medium (Refai et al, 1969) as a media for isolation and identification and then the samples stained with Lactophenol cotton blue (Collee et al, 1996). Then, samples were cultivated on specific media for determination of virulence factors, e.g. media for determination of proteinase activity (Aoki et al, 1990) and media for determination of phospholipase production. Finally, samples were cultivated on Sabouraud’s dextrose broth supplemented with bovine serum albumin 1% (Veterinary serum and Vaccine Research Institute (VSVRI) for stimulation of proteolytic enzymes to prepare C. albicans vaccine. Rabbit serum was used for demonstration of germ tube of C. albicans (Leslie and Frank, 1980) and the technique
was carried out according to Koneman et al (1992).

2- PCR evaluation of *C. albicans*

The samples collection and identification of different yeast isolates was performed according to Refai et al (1969). DNA was extracted from *C. albicans* isolates using Analytik jena kit® following the manufacturer instruction. Total chromosomal DNA isolated from *C. albicans* cells was subjected to PCR amplification with oligonucleotide primers targeting a 175 bp region as described previously by Mannarelli and Kurtzman (1998). The oligonucleotide primers (Biobasic inc.) used in the PCR reactions were:

Forward 5' TGTTGCTCTCTCGGGGGCGGC 3'
Reverse 5' AGATCATTATGCCAACATCC TAGGTTAAA 3'

PCR amplification reaction mixtures consisted of 12.5 μl Master Mix, 1 μl Forward primer, 1 μl reverse primer, 5.5 μl nuclease free water, 5 μl DNA. The PCR conditions were: Initial denaturation at 94°C for 5 min., and 35 cycles of 1 min at 94°C, 47°C for 1 min and 72 °C for 1min. Final extension at 72°C for 5 min. The PCR product was separated on agarose gel (0.9%) and stained with ethidium bromide.

Polymerase Chain Reaction for detection of the PLB1-specific region:

Total chromosomal DNA isolated from *C. albicans* cells was subjected to PCR amplification with oligonucleotide primers targeting a 751 bp region representing the 59 half of the PLB1 gene, as described previously by (Mukherjee et al., 2001). The oligonucleotide primers (Biobasic inc.) used were:

Forward 5' ATGATTTTGCATCATTTG 3'
Reverse 5' AGTATCTGGAGCTCTACC 3'

PCR amplification reaction mixtures consisted of 12.5 μl Master Mix, 1 μl Forward primer, 1 μl reverse primer, 5.5 μl nuclease free water, 5 μl DNA. The PCR conditions used was initial denaturation at 94°C for 5 min., and 35 cycles of 1 min at 94°C, 47°C for 1 min and 72 °C for 1min. Final extension at 72°C for 5 min. The PCR product was separated on agarose gel (0.9%) and stained with ethidium bromide.

3. Solutions:

In this study the KOH solution (Rebell and Taplin, 1970) and the Normal physiological saline (Leslie and Frank, 1980) were used.

3.1. Adjuvant (Montanide IMS 1113 N VG PR, Batch nr: T52531 was obtained from The Veterinary serum and Vaccine Research Institute (VSVRI).

4- Laboratory Animals:
For *C. albicans* vaccines: Twenty-Five white albino male rats weighted between 100-150g. They feed on special rat feed and divided into 5 groups (Table 1). All rats were subjected to challenge after 14 days post second vaccination.

5-Preparation of *C. albicans* cultures used for vaccines preparations: *C. albicans* were inoculated in Sabouraud's dextrose broth supplemented with bovine serum albumin for 2 days at 28°C, then centrifuged at 3000 rpm and the culture was then filtrated from yeast cell.

5.1. Heat killed *C. albicans* vaccine (HK.CA) with adjuvant: Yeast cells were washed twice in saline, counted in a hemocytometer 10⁶ ml, then inactivated by heating at 80°C for 30 minutes, and finally tested for purity and sterility for vaccine preparation (*Bromuro et al, 2002*). The yeast cells were then mixed with equal volume of adjuvant (montanide) and emulsified by repeated drawing and ejection, once emulsification took place; 2 ml of solution were given subcutaneously for each rat, while the control group received the same volume of only the adjuvant in a sterile saline (*Campbell et al, 1970*).

5-2- Culture filtrate vaccine (CFV):

The *C. albicans* culture filtrate was prepared as previously mentioned, then a part of the culture filtrate was heated at 100°C and a part inactivated with formalin (0.3%) then they all mixed together.

6- Preparation of *C. albicans* antigen for evaluation of the immune responses:

2 ml of fungus suspension were placed in a tube surrounded by ice. Then, the suspension was sonicated for 10 times 30 seconds each according to Sonicator model: Vibracell. Company: Sonics of materials INC. DANBURYCT.U.S.A. The supernatant was then taken and kept for evaluation of immune

7- Measurement of the immune response against the inoculated vaccines:

Unheparinized blood samples were collected in a sterile wisserrman tubes directly from inner canthus of eye of rats, before immunization (zero time), one week post first immunization, and 14 days post second vaccination and after challenge. Serum was then separated and kept at -20°C till examined. The immune response of rats was examined prior vaccination (zero time), after first vaccination 7 days, at 14 days after second vaccination and after challenge within 7 days.

7-1- Evaluation of the humoral immune response:
It was assessed by measuring anti- *Candida albicans* specific IgG antibodies using ELISA technique according to (Voller and Bidwell, 1986).

7-2- Evaluation of the protective efficacy of the vaccines by Challenge test:

Table (1): Experimental design and immunization of rats for *Candida albicans* vaccines as shown follow:

<table>
<thead>
<tr>
<th>Group</th>
<th>No of animals</th>
<th>Types of vaccine</th>
<th>Dose and rout</th>
<th>Number of dose</th>
<th>Challenge (dose and rout)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5 rats</td>
<td>Heat killed <em>C. albicans</em> with adjuvant</td>
<td>2 ml s/c (1 ml vaccine &amp; 1 ml adjuvant)</td>
<td>twice within one week intervals</td>
<td>I/P With 5 X 10^5.</td>
</tr>
<tr>
<td>2</td>
<td>5 rats</td>
<td>Culture filtrate</td>
<td>0.5 ml s/c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5 rats</td>
<td>Mixed vaccines</td>
<td>2 ml s/c of (Hk- CA) and .5 ml of CFV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5 rats</td>
<td>Injection with adjuvant</td>
<td>2 ml s/c (1 ml adjuvant &amp; 1 ml saline)</td>
<td>One dose</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5 rats</td>
<td>Control</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

HK= Heat Killed CFV=Culture filtrate vaccine

Results:

*Candida albicans*:

1- Isolation and identification of *C. albicans*: All isolates of *C. albicans* were positive for chlamydomspores on rice agar medium and for germ tube formation in rabbit serum as shown in photo (1 and 2).

2- Proteolytic activity (proteinase enzyme) and phospholipase activity of *C. albicans*. The isolated positive *C. albicans* samples were tested for virulence factors (production of proteinase and phospholipase enzymes) and all the samples revealed positive result.
3-Identification of *C. albicans* by PCR
In this study the PCR was used for the rapid detection of *C. albicans* yeasts. The PCR is based on a pair of universal primers. In this PCR system, the universal primer produces a 175 bp DNA fragment. As shown in figure (3).

4-Detection of phospholipase B (PLB1) gene of *C. albicans* by PCR: PCR was used for detecting phospholipase enzyme production as a rapid and more sensitive tool. As shown in the figure (4). The forward and reverse primer produces a 751 bp DNA fragment.

5- Immune response developed against the prepared vaccines
5-1- Humoral immune response developed against heat killed *Candida albicans* (HK-CA) vaccine with adjuvant
The results illustrated in table (2) indicated that The mean optical density of representing antibodies titer of anti- *C. albicans* IgG in sera of rats artificially immunized with heat killed *C. albicans* vaccine with adjuvant was significantly increased to 1.84, 1.86, and 1.87, after first vaccination, post second vaccination, and after challenge respectively compared with 1.4 and 1.38 for control and control with adjuvant respectively. Of note, there are no significant differences in immunity level between first and booster dose of vaccine (Table 2).

5-2- Humoral immune response developed against culture filtrate of *C. albicans* vaccine.
The mean optical density of anti- *C. albicans* IgG in sera of rats artificially immunized with culture filtrate was significantly increased 1.92, 1.72, and 1.91 after first vaccination, post second after challenge with the virulent *C. albicans* respectively compared with 1.4 and 1.38 for control group and control with adjuvant respectively. However, there are no significant differences in level immunity between first and booster dose of vaccine (Table 3).

5-3- Humoral immune response developed against mixed vaccine (heat killed *C. albicans* vaccine and culture filtrate):
The mean optical density values of representing antibodies titer of anti- *C. albicans* IgG in sera of rats artificially immunized with (mixed vaccines) (HK-CA) vaccine & (CFV) was significantly increased (1.99) after first vaccination reached (1.83) after booster dose of vaccine and the level immunity of anti- *C. albicans* specific IgG was significantly increased reached to (2.69) after challenge compared with control and control with adjuvant (1.4, 1.38 respectively). Also statistical
analysis showed that the level of IgG was significantly increased after challenge compared with first and booster dose of vaccination (Table 4).

5-4- ELISA mean of anti- *C. albicans* IgG in sera of rats immunized with different prepared vaccines

The statistical analysis of the data presented showed that the level of immunity was increased after challenge in all three types of vaccines with no significant differences between the level of immunity emerged by heat killed *C. albicans* vaccine and that of the culture filtrate of *C. albicans*. In contrast, rats immunized with mixed vaccine (HK-CA, CFV) showed significant increase in anti-*Candida albicans* specific IgG compared with heat killed or culture filtrate alone especially after challenge with the virulent *C. albicans* strain (Table 5).

**Photo (1):** Terminal and intercalary chlamydomspores of *C. albicans* on rice agar medium.

**Photo (2):** Germ tube formation of *C. albicans* in rabbit serum at 37°C:
Fig. (3): Amplification of 175bp by PCR for detection of C. albicans. Lane M: 1000 bp ladder, lane 1, 2, 3 and 5 showed positive samples.

Fig. (4): Amplification of 751bp by PCR for detection of phospholipase B (PLB1) gene. Lane M: 100 bp ladder, lane 2, 5 and 6 showed positive samples.
**Table (2):** ELISA mean of representing antibodies titer of anti- *C. albicans* in sera of rats immunized with heat killed vaccine with adjuvant.

<table>
<thead>
<tr>
<th>Samples</th>
<th>(zero time)</th>
<th>Control group after challenge</th>
<th>Control group with adjuvant</th>
<th>Control group with adjuvant after challenge</th>
<th>Immunized group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples1</td>
<td>1.06</td>
<td>.670</td>
<td>.690</td>
<td>1.46</td>
<td>1.74</td>
</tr>
<tr>
<td>Samples 2</td>
<td>0.79</td>
<td>1.84</td>
<td>1.14</td>
<td>1.3</td>
<td>1.93</td>
</tr>
<tr>
<td>Samples 3</td>
<td>1.07</td>
<td>1.0</td>
<td>1.2</td>
<td>1.18</td>
<td>1.88</td>
</tr>
<tr>
<td>Samples 4</td>
<td>0.62</td>
<td>1.73</td>
<td>1.24</td>
<td>1.2</td>
<td>1.69</td>
</tr>
<tr>
<td>Samples 5</td>
<td>.750</td>
<td>1.77</td>
<td>1.23</td>
<td>1.8</td>
<td>1.96</td>
</tr>
<tr>
<td>Mean and Std.Error</td>
<td>.85±.089070</td>
<td>1.4±.23768b</td>
<td>1.1±.10397b</td>
<td>1.38±.11430b</td>
<td>1.84±.05320a</td>
</tr>
</tbody>
</table>

*Mean having the same litter are not significantly different and different litters means significantly (p>0.05).

**Table (3):** ELISA mean of representing antibodies titer of anti- *Candida albicans* in sera of rats immunized with culture filtrate vaccine.

<table>
<thead>
<tr>
<th>Samples</th>
<th>(zero time)</th>
<th>Control group after challenge</th>
<th>Control group with adjuvant</th>
<th>Control group with adjuvant after challenge</th>
<th>Immunized groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples1</td>
<td>1.06</td>
<td>.670</td>
<td>.690</td>
<td>1.46</td>
<td>1.86</td>
</tr>
<tr>
<td>Samples 2</td>
<td>0.79</td>
<td>1.84</td>
<td>1.14</td>
<td>1.3</td>
<td>2</td>
</tr>
<tr>
<td>Samples 3</td>
<td>1.07</td>
<td>1.0</td>
<td>1.2</td>
<td>1.18</td>
<td>1.83</td>
</tr>
<tr>
<td>Samples 4</td>
<td>0.62</td>
<td>1.73</td>
<td>1.24</td>
<td>1.2</td>
<td>2.02</td>
</tr>
<tr>
<td>Samples 5</td>
<td>.750</td>
<td>1.77</td>
<td>1.23</td>
<td>1.8</td>
<td>1.92</td>
</tr>
<tr>
<td>Mean and Std.Error</td>
<td>0.85±.08907d</td>
<td>1.4±.23768cb</td>
<td>1.1±.10397cd</td>
<td>1.38±.11430b</td>
<td>1.92±.03736a</td>
</tr>
</tbody>
</table>

*Mean having the same litter are not significantly different (p>0.05). Different litters means significantly.
Table (4) ELISA representing antibodies titer of anti- *Candida albicans* in sera of rats immunized with (mixed vaccines) HK-CA & CFV

<table>
<thead>
<tr>
<th>Samples</th>
<th>(zero time)</th>
<th>Control group after challenge</th>
<th>Control group with adjuvant</th>
<th>Control group with adjuvant after challenge</th>
<th>Immunized group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>post first vaccination</td>
</tr>
<tr>
<td>Samples1</td>
<td>1.06</td>
<td>.670</td>
<td>.690</td>
<td>1.46</td>
<td>1.84</td>
</tr>
<tr>
<td>Samples 2</td>
<td>0.79</td>
<td>1.84</td>
<td>1.14</td>
<td>1.3</td>
<td>2.24</td>
</tr>
<tr>
<td>Samples 3</td>
<td>1.07</td>
<td>1.0</td>
<td>1.2</td>
<td>1.18</td>
<td>2.12</td>
</tr>
<tr>
<td>Samples 4</td>
<td>0.62</td>
<td>1.73</td>
<td>1.24</td>
<td>1.2</td>
<td>1.84</td>
</tr>
<tr>
<td>Samples 5</td>
<td>.750</td>
<td>1.77</td>
<td>1.23</td>
<td>1.8</td>
<td>1.94</td>
</tr>
<tr>
<td>Mean and Std. Error</td>
<td>0.85± .08907d</td>
<td>1.4± 23768c</td>
<td>1.1± 10397ed</td>
<td>1.38± 11430c</td>
<td>1.99± 07960b</td>
</tr>
</tbody>
</table>

*Mean having the same litter are not significantly different and different litters means significantly (*p*>0.05).

Table (5) ELISA mean of anti- *Candida albicans* in sera of rats immunized with different prepared vaccines in comparison with control and control with adjuvant.

<table>
<thead>
<tr>
<th>Types of group</th>
<th>zero time</th>
<th>1 w. post first vaccination</th>
<th>2 w. post 2 vaccination</th>
<th>1 weeks post challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat killed vaccine with adjuvant</td>
<td>0.85± .08907d</td>
<td>1.84± .05320b</td>
<td>1.86± .05843b</td>
<td>1.87± .06033b</td>
</tr>
<tr>
<td>Culture filtrate vaccine</td>
<td>0.85± .08907d</td>
<td>1.92± .03736b</td>
<td>1.72± .04069b</td>
<td>1.91± .19775b</td>
</tr>
<tr>
<td>Heat killed vaccine with adjuvant &amp; culture filtrate</td>
<td>0.85± .08907d</td>
<td>1.99± .07960b</td>
<td>1.83± .04823b</td>
<td>2.69± .08681a</td>
</tr>
<tr>
<td>Control group</td>
<td>0.85± .08907d</td>
<td>-</td>
<td>-</td>
<td>1.4± .23794c</td>
</tr>
<tr>
<td>Control group with adjuvant</td>
<td>0.85± .08907d</td>
<td>-</td>
<td>-</td>
<td>1.38± .11430c</td>
</tr>
</tbody>
</table>

*Mean having the same litter are not significantly different and different litters means significance (*p*>0.05).
**Discussion:**

The systemic infection of *C. albicans* is still of major concern regarding to the higher morbidity and mortality rates because of the difficulties in the treatment of this type of infection. In addition, the use of antifungal drugs as an only choice is still limited because of their toxicity and potential risk of the emergence of drug resistance (Vasquez and Sobel, 2003).

Herein, we have isolated and identified *C. albicans* on Sabouraud’s dextrose agar and rice agar medium for chlamydomspore formation (photo.1), and this agree with (Bhavan et al., 2010). In addition, the germ tube formation was detected (photo.2) which considered as presumptive identification test (Park and Lee, 2008). Moreover, the virulence of the *C. albicans* was confirmed by the production of proteinase activity, which facilitates their adhesion and tissue invasion and hence disease production (Mardegan et al., 2006). Similarly, the phospholipase activity which was considered as a determinant of *C. albicans* virulence factors was determined (Ibrahim et al., 1993). Interestingly, the stimulation of the proteinase production was enhanced by addition of bovine serum albumin similar to that previously showed by (Lerner and Goldman, 1993).

In this study the main aim was to elucidate the immune response of two prepared types of vaccines heat killed *C. albicans* vaccine (HK-CA) with adjuvant and culture filtrate vaccine (CFV). Wozniak and Fidel, (2002), reported that the Candida vaccination induces IgM and IgG3 antibodies are protective in a mouse model of vaginitis and the Protection against fungal pathogens can theoretically be elicited by vaccines that stimulate humeral or cellular immunity or both (Casadevall and Perofski 2002). In addition, (Fujibayashi et al., 2009) who prepared a polyclonal anti-*Candida albicans* antibody in chicken egg yolk (anti-*Candida albicans* IgY) by immunization with the yeast form and found that anti-*C. albicans* IgY significantly reduced the adherence of *C. albicans* to human oral epithelial cells and reduce the dissemination of Candida species. Hence, anti-*C. albicans* IgY may be considered as a prophylactic therapy. Therefore, we have assessed the immune response of two prepared vaccines via ELISA assessment of IgG. The data presented in table (2) showed that mean value of anti-* C. albicans* specific IgG in sera of rats
artificially immunized with heat killed *C. albicans* vaccine with adjuvant was significantly increased compared with control and control with adjuvant with no significant difference between first and booster dose of vaccine. These results are totally agree with that of (Abdel-Noor et al., 2006) who found that egg-laying hen immunized with a mixture of heat killed *Candida albicans* yeast cells and germ tubes produce high level of IgY (egg yolk immunoglobulin) which protected mice against a lethal dose of *Candida albicans*. Moreover, IgY is considered as a prophylactic agent or possibly an adjunct to antifungal therapy and Mice immunized with heat-inactivated whole yeast cell of *Candida albicans* developed intense specific humoral and cell-mediated immune response (Bromuro et al., 2002). Furthermore, (Zhang et al., 2009) observed that the IgG and IgA levels in murine serum were significantly increased after active immunization by S/C injection of heat inactivated *Candida albicans* spores. The data presented in table (3) showed that the levels of anti-*C. albicans* specific IgG in sera of rats artificially immunized with culture filtrate was dramatically increased in all the periods of examination (post first vaccination, 2 weeks after vaccination and after challenge with the virulent *C. albicans*) compared with control group and control with adjuvant. These results are consistent with the findings of (Schonheyder et al., 1983) who found that IgG *Candida* antibody levels were significantly higher against *C. albicans* culture filtrate. On the contrary, (Paul and Sobel 1994) demonstrated that the mice immunized subcutaneously with CCF-CFA (*C.albicans* culture filtrate antigens in complete Freund’s) produced a significant DTH reactivity in comparison with control negative mice. This is can be explained as the culture filtrate of *C. albicans* contained extra cellular proteinase and extracellular antigens which strongly stimulated the immune response. Regarding the rats immunized with mixed vaccines (HK-CA) vaccine & (CFV), the data showed that, the levels of anti- *C. albicans* IgG was notably increased to post first vaccination, at 2 weeks post second vaccination and after challenge when compared with control and control with adjuvant. Also, the level of IgG was significantly increased after challenge compared with that of first and booster dose of vaccination (Table 4). The data presented in table (5) showed that the level of immunity was increased after
challenge in all three types of vaccines compared with control with no significant differences between level of immunity emerged by heat killed \textit{C. albicans} vaccine and culture filtrate of \textit{C. albicans}. Additionally, the best vaccine gives a higher immune response against \textit{C. albicans} was the mixed vaccine (HK-CA&CFV).

\textbf{Conclusion}

\textit{C. albicans} was isolated and confirmed as a virulent strain (proteinase and phospholipase enzymes activity). Rats artificially immunized with heat killed \textit{C. albicans} vaccine with adjuvant or with culture filtrate, or mixed vaccines (HK-CA) vaccine and (CFV) showed higher anti- \textit{C. albicans} IgG in sera post first vaccination, 2 weeks post second vaccination and after challenge compared with control and control with adjuvant respectively with no significant difference in immunity level between first and booster dose of vaccine. The immune response of rats was significantly increased after challenge in all three types of vaccines and there was no significant differences between level of immunity emerged by heat killed \textit{C. albicans} vaccines and culture filtrate of \textit{C. albicans}. While, immunization by mixed vaccine (HK-CA, CFV) showed significant increase in anti-\textit{Candida albicans} than heat killed or culture filtrate alone especially after challenge with the virulent \textit{C. albicans} strain.

\textbf{References}


ملخص

الكانتيدا البيكنيز: تم عزلها من حالات التهاب ضرع من الأبقار و تصنيفها علي المنابت الخاصة بها و إجراء اختبارات الضراءة مثل (فوسفوتيزيز و بروتينيز) وأيضا باستخدام تفاعل البلمرة المتسلسل و تم تحضير اللقاحات من العناء bovine serum albumin، و لقاح الميت بالحرارة واللقاح المختلط وفي هذه التجربة تم استخدام 25 فأر تجارب مقسمة علي 5 مجموعات كل منها تخولت علي 5 فائنين.

المجموعة الأولى: بها 5 فائنين تجارب وتم حقنها بلقاح الميت بالحرارة وعند جرعة نموذجي بينهما أسبوعين.

المجموعة الثانية: بها 5 فائنين تجارب وتم حقنها بلقاح مستخلص الفطر بجرعة 2 سم تحت الجلد.

المجموعة الثالثة: بها 5 فائنين تجارب وتم حقنها باللقاح المختلط (مستخلص الفطر الميت بالحرارة) وعند جرعتين نموذجي بينهما أسبوع.

المجموعة الرابعة: بها 5 فائنين تجارب وتم حقنها بالمادة الحاملة فقط.

المجموعة الخامسة: بها 5 فائنين تجارب ولم تحدث نموذج تجربة وهي مجموعة قياسية. وتم جمع عينات الدم لفصل السيرم (مصل الدم) للمجموعات الخمسة بعد كل جرعة من اللقاح. ثم أخذت العدوى بعد 15 يوم من الجرعة الثانية من اللقاح بالعنة عالية الضراءة. وتم قياس الأجسام المضادة لمرض الكانتيدا البيكنيز باستخدام الإليزا وتبين من التحليل الأحصائي للبيانات أن كل من المجموعات الثلاث المحصنة أحدث استجابة مناعية عالية مقارنة بالمجموعات التي لم تحصين وأن مستوي المناعة ظل مرتفع حتى بعد إحداث العدوى. وأن المجموعة الثالثة المحصنة باللقاح المختلط هي الأكثر استجابة مناعية خاصة بعد إحداث العدوى.