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# A STUDY ON THE IMMUNE RESPONSE OF CATTLE NATURALLY INFESTED WITH BLOOD PARASITES AND VACCINATED WITH SHEEP POX VACCINE PRIVATE

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## SUMMARY

The immune response of naturally infested cattle with Theileria annulata, and vaccinated with sheep pox virus vaccine was studied in comparison with the immunity of vaccinated healthy animals. Fourteen bulls were proved to be susceptible to sheep pox virus vaccination via screening of their sera using serum neutralization test. Six of these animals were confirmed to be infested with theileriosis via examination of Giemsa stained blood smears and immunofluorescent investigation of another blood films. Another six non-infested animals beside the infested one with theileriosis were vaccinated at the same time with sheep pox virus vaccine and two healthy animals were kept as control. Heparinized blood samples were collected for evaluation of the cell mediated immune response using lymphocyte transformation assay and the sera were separated for evaluation of the humoral immunity using serum neutralization, double antibody sandwich ELISA and dot immunoblot ELISA techniques. A challenge test using the virulent lumpy skin disease virus was carried out at 90 day post vaccination for evaluating the immune status of all animals. Results revealed a significant decrease in lymphocytic cells in animals infested with theileriosis and the level of lymphocytic cells varied according to the severity of infection. Also, the antibody titres and the challenge with virulent virus showed highly depressed of antibody levels in acute infested animals, partial suppression in chronic infested animals with theileriosis and positive reaction to challenge in severely infected animals and controls. It could be concluded that the degree of theileriosis infestation in cattle should be considered during the vaccination campaign against lumpy skin disease to avoid the failure of vaccination.

## INTRODUCTION

Cattle are infected with lumpy skin disease (LSD) which is caused by a strain of capripox virus and is antigenically related to sheep and goat pox viruses (Davies, 1991). In African countries and Egypt, the cattle could be immunized against LSD using sheep pox virus vaccine (SPVV) (Woods, 1988 and Michael et al., 1994). The efficacy of any vaccine depends generally on the environmental conditions and stress factors affecting the vaccinated animals, one of the main factors is the infestation with blood parasites carried by ticks especially theileria (Morrison, 1989). Theileriosis is considered as one of the immunosuppressive diseases affecting cattle and its role in vaccination failure were recorded with some viral vaccines (Wafula and Wamwayi, 1989; Mouaz et al., 1997; Elian and Hegazy, 1999 and Iman et al., 2003).

The aim of this study is to investigate the role played by theileriosis in cattle vaccinated with SPVV and evaluate the cellular and humoral immune status of these animals for their protection against LSD under field conditions.

## MATERIAL AND METHODS

### 1. Animals:

A total of fourteen cross-breed Friesian bulls were divided into three groups as follows:

**Group (I):**

Six bulls were proved to be free from neutralizing antibodies specific for SPV and LSDV as well as they were free from blood parasites.

**Group (II):**

Six animals were infested naturally with theileriosis, two of them were found in the acute stage and the other four animals showed chronic infestation of theileriosis. Animals of groups (I) and (II) were vaccinated intradermally (ID) in the tail fold each with one ml of SPVV containing the field dose (3 log10 $TCID_{50}$/ml).

**Group (III):**

Two bulls were kept as non-vaccinated and non-infested control. All animals were kept in insect proof stable at the Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.

### 2. Viruses:

A vaccinal strain of Kenyan SPV was adapted on Vero cell cultures and had a titre of 5.5 log10 $TCID_{50}$/ml (Michael et al., 1994). It was used for animal vaccination and various serological techniques. A virulent LSDV (Ismailia strain) was adapted on bovine fetal lung cells and had a titre of 4.8 log10 $TCID_{50}$/ml and used for challenge test (Michael et al., 1994).

### 3. Blood and serum samples:

Blood smears were used for detection of Theileria annulata stages. Heparinized blood samples were

taken for evaluation of the cellular immunity and sera were collected for investigation of the humoral immune response.

**4. Staining the blood smears and immunofluorescent assay (IFA):**

Giemsa stained blood smears were prepared according to Schalm et al. (1975). IFA was done using anti-bovine IgG fluorescein conjugate and reference hyperimmune serum for Theileria annulata according to published procedures (Burridge, 1971).

**5. Lymphocyte transformation assay (LTA):**

It was carried out using heparinized blood samples for lymphocyte blastogenesis assay at 1, 3, 5, 7, 10, 14 and 21 day post vaccination (DPV) according to Rai et al. (1985).

**6. Serum neutralization test (SNT):**

It was used for estimation of the neutralizing antibodies titres at 0, 7, 14, 21, 28, 45, 60 and 90 DPV according to Sharma et al. (1987). The titre was expressed as the reciprocal of each serum dilution is that end dilution.

**7. Double antibody sandwich ELISA (DASE):**

It was used for accurate estimation of antibodies titres according to Paweska et al. (2003). The test based on sandwich formation in which the plates are coated with sheep anti-SPV hyperimmune serum and then reacted with SPV antigen.

**8. Dot immunoblot ELISA (DIE):**

It was done on high titre positive sera according to Tiwari et al. (1996). In this technique, a nitrocellulose membrane was prepared to be coated with SPV antigen and the examined sera were evaluated using anti-bovine IgG HRPO conjugate and 4-chloro-1-naphthol substrate solution.

**9. Challenge test:**

It was applied on all animals at 90 DPV using the virulent LSDV according to OIE (1989). Each animal was inoculated subcutaneously (S/C) in the lateral aspect of the neck with 0.2ml of the virulent LSDV containing 100 $TCID_{50}$/ml.

## RESULTS AND DISCUSSION

Giemsa stained blood smears and immunofluorescence investigation of blood films of animals infested with theileriosis (Group II) revealed the presence of different morphological erythrocytic Theileria annulata stages and schizonts. Also, the specific apple green fluorescence was observed in the examined blood films and confirmed the clinical symptoms of theileriosis in the infected animals (Photo 1 and 2). These results are similar to those obtained by Burridge (1971) and Conrad et al. (1985).

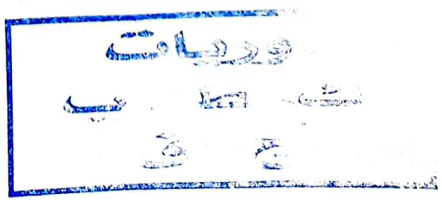

Table (1): Cell mediated immune response using lymphocyte transformation assay

| DPV | Group (I) OD values | Group (II) OD values | | Group (III) OD values |
|---|---|---|---|---|
| | | AIA | CIA | |
| 1 | 0.375 | 0.145 | 0.350 | 0.300 |
| 3 | 0.500 | 0.148 | 0.420 | 0.300 |
| 5 | 0.700 | 0.175 | 0.475 | 0.300 |
| 7 | 1.500 | 0.290 | 0.920 | 0.300 |
| 10 | 1.200 | 0.200 | 0.900 | 0.300 |
| 14 | 0.750 | 0.160 | 0.510 | 0.300 |
| 21 | 0.750 | 0.160 | 0.500 | 0.300 |

DPV: Days Post Vaccination.
OD: Optical Density.
Group (I): Vaccinated healthy animal.
Group (II): Vaccinated and infected animals.
Group (III): Non-vaccinated non-infected controls.
AIA: Acute infected animals.
CIA: Chronic infected animals.

Table (2): Humoral immune response using serum neutralization test and ELISA

| DPV | Group (I) Antibody titres | | | Group (II) Antibody titres | | | | | | Group (III)/ Antibody titres | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | AIA | | | CIA | | | | | |
| | DIE | DIE | DIE | SNT | DASE | DIE | SNT | DASE | DIE | SNT | DASE | DIE |
| 0 | 4 | 80 | -ve | 2 | 20 | ND | 4 | 80 | ND | 4 | 80 | ND |
| 7 | 8 | 400 | -ve | 2 | 80 | ND | 4 | 300 | ND | 4 | 80 | ND |
| 14 | 32 | 1400 | +ve | 4 | 180 | ND | 16 | 1200 | ND | 4 | 80 | ND |
| 21 | 128 | 5000 | +ve | 16 | 200 | ND | 64 | 3500 | +ve | 4 | 80 | ND |
| 28 | 128 | 5000 | +ve | 16 | 180 | ND | 64 | 3500 | +ve | 4 | 80 | ND |
| 45 | 64 | 4000 | +ve | 8 | 100 | ND | 64 | 3000 | +ve | 4 | 80 | ND |
| 60 | 64 | 4000 | +ve | 8 | 80 | ND | 64 | 3000 | +ve | 4 | 80 | ND |
| 90 | 64 | 4000 | +ve | 8 | 80 | ND | 64 | 3000 | +ve | 4 | 80 | ND |

DPV: Days Post Vaccination.
Group (I): Vaccinated healthy animal.
Group (II): Vaccinated and infected animals.
Group (III): Non-vaccinated non-infected controls.
AIA: Acute infected animals.
CIA: Chronic infected animals.
ND: Not Done.

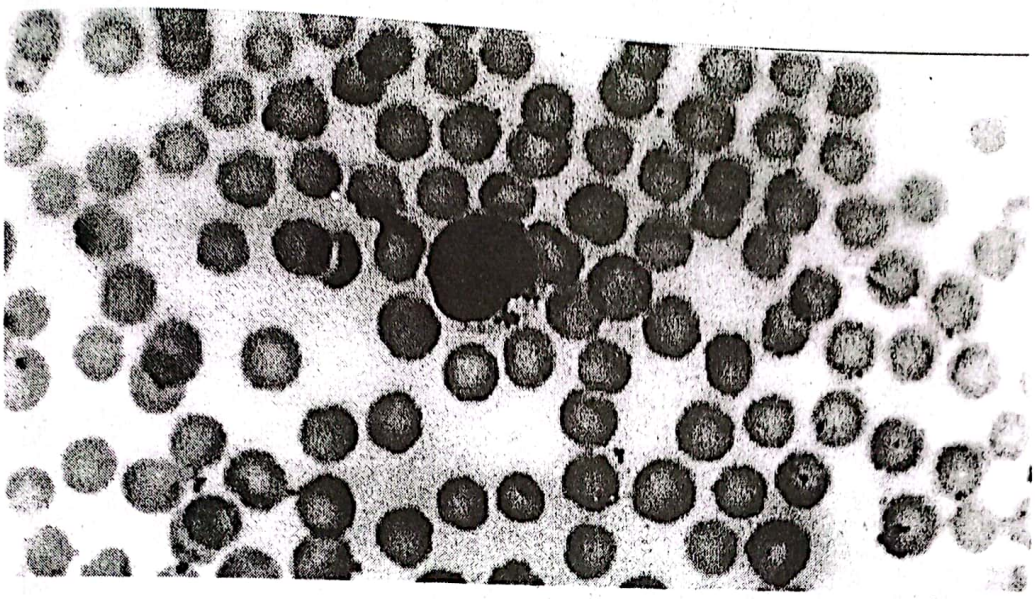

Photo (1): Giemsa stained blood films showing different piroplasmic forms of Theileria annulata in the erythrocytes of infested animals

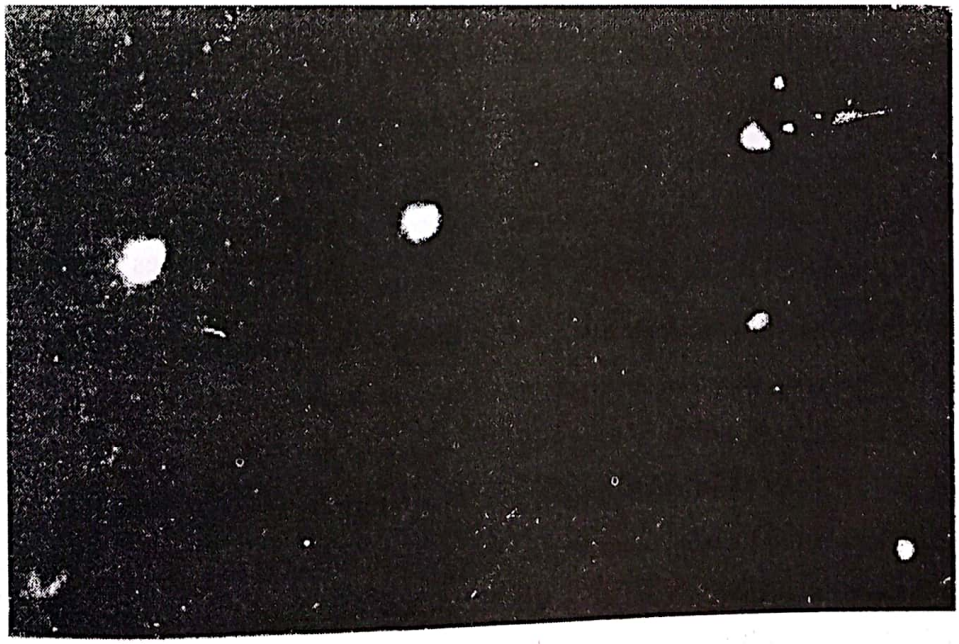

Photo (2): Apple green fluorescence reaction of Theileria annulata schizont antigen in the erythrocytes of infested animals

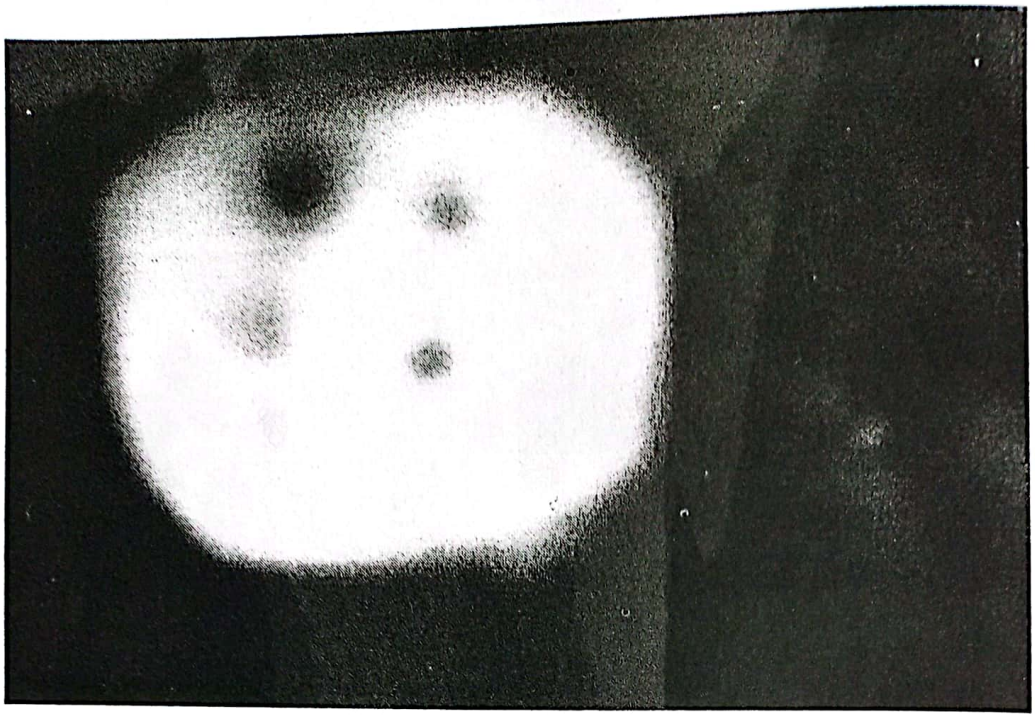

Photo (3): Blue dots of positive sera of vaccinated and chronic infested animals with theileriosis

Results of cell mediated immune response by LTA revealed that the level of lymphocyte cells were varied completely between animals in group (I) and group (II), also between acute and chronic infected animals in group (II). The obtained results recorded in table (1) showed severe depression of lymphocytic cells in acute infested animals with theileriosis and slight decreasing of lymphocyte cells in chronic infected animals of group (II) in comparison with animals in group (I). These results are in agreement with findings of Carson and Phillips (1981) who stated that Theileria annulata may even inhibit lymphocytes and macrophages that are ordinary engorged in anti-microbial activity. Also, Benach et al. (1982) and Ellissalde et al. (1983) reported that (T) and (B) cell responses to non-specific mitogens were reduced during acute phase of babesia infections. Similar results were obtained by Campbell et al. (1995) who stated that Theileria annulata usually causes severe lymphocyte proliferative alterations which initially develop in the draining lymph nodes where release of schizontal bodies from producing lymphocytes may lead to rupture of these cells. Also, they added that Theileria annulata may also affect T lymphocytes in away that shares in the failure of the host to mount an effective immune response.

Results of humoral immunity using SNT, DASE and DIE were recorded in table (2) which showed that the antibodies titres were affected obviously due to theileriosis and severe depression and partial suppression of antibody titres were noticed in

acute and chronic infested animals respectively. DIE showed specific blue dots with samples of high titres in group (I) and chronic infected animals in group (II) (Photo 3). These results are in harmony with that obtained by Wanger et al. (1975), Scott et al. (1977), Rurangiraw et al. (1980) and Mouaz et al., 1997, who found that the reduction in the rinderpest immune curve was proportional to the degree of infestation with acute theileriosis and the suppression was complete during the acute phase of infection. In addition, James et al. (1981) mentioned that non-specific suppression of total IgM and IgG has observed during Babesia bovis infestions, maximal suppression occurred at peak parasitaemia which that interfered with immune mechanism of the host. Similar results were obtained by Sharp and Langley (1983) and Soulsby (1987) who recorded that theileriosis induce immunodepression between 12-24 days following infection with theileria corresponding to the period of peak clinical reaction and stated that the immune suppression during the acute phase of theileria infestation is nearly complete whereas during the chronic phase is much less marked. Moreover, the antibodies titres by SNT and ELISA were previously ascertained by Elian and Hegazy (1999) and Iman et al. (2003) who found that the antibodies titres post vaccination with Rift valley fever and rota and corona viruses were sharply decreased in acute experimentally infected cattle and the antibody titer was partially decreased in chronic artificially infested animals with theileriosis. This immunosuppression was due to lymphoproliferative alterations by Theileria annulata leading to decreasing of the antibody titres.

Results of challenge test with virulent LSDV revealed a complete protection of all vaccinated animals with SPVV except the acute infested animals with theileriosis. The positive reactions in challenge acute infested animals with theileriosis and controls were due to lowering the antibody titres (8) at challenge time (90 DPV). These antibody titres were considered not protective against the virulent virus in comparison with antibody titres of protected animals (64). This result was confirmed by that obtained by Saber et al. (1993) and Michael et al. (1997) who recorded that the protective antibody titre of vaccinated cattle with SPVV against LSD was 64 or 1.5 log10 $TCID_{50}$.

In conclusion, the results of the present work proved that the infested cattle with blood parasites especially Theileria annulata impact the process of vaccination if the infection performed at the peak level of antibody response (Acute infection) and make sharp decrease in the level of antibodies. Also, this study revealed that the degree of infection affect the antibodies curve and the acute stage of theileriosis had the more suppressive effect than the chronic infestation, so the blood parasites is a factor may interfere with vaccination campaigns causing failure of vaccination.

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