



## Study some immune parameter of *Sarcocystis* spp. Sheep in Baghdad

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### Abstract

The purpose of this study was to measure delay hyper sensitivity in effected mice and detected Ab. type against *Sarcocystis* in sheep. In this investigation, the microscopic cysts of *Sarcocystis* were assessed in slaughtered sheep. The digestion method was used for bradyzoites observation in, esophagus and inter costal muscle samples. Serum of mice (contain antibody against *Sarcocystis*) reaction with 25 serum of randomly collection from sheep that reaction appear IgG after add drop of compounds—2-mercaptoethanol (2-ME) with Significant different between Ab (IgM and IgG) ( $P < 0.05$ )., delay type hypersensitivity (DTH) have showed increase in the thickness of right footpads of the mice's (4) and highest mean of the thickness was after 24 hours post immunization then decrease after passage of 48 hours and the after 24 hours immunized.

**Keywords:** *Sarcocystis*, virulence factors, Iraq.

### Introduction

*Sarcocystis* are intracellular protozoan parasites infecting a wide range of livestocks. Some of *Sarcocystis* genus are pathogenic for animals such as sheep and cattle which cause enormous economic losses (1). Studies in different regions of the world indicate that the prevalence of *Sarcocystis* infection in slaughtered cattle and sheep are between 70% to 100% (2 ; 3). Additionally, studies in Iran showed that the prevalence of this parasite in the animal was between 85% to 100%( 4; 5 ). For example, studies in Kerman and Ahwaz provinces indicated that 100% of animals were infected with *Sarcocystis*( 5 ;6). Different species of *Sarcocystis* have been isolated from animals worldwide. *Sarcocystis tenella* was isolated from sheep in Iran and Brazil (7; 8). In another study, *Sarcocystis moulei* was reported from reindeer (9).

Also, Nourani *et al.* isolated *Sarcocystis hominis* from cattle (10) while Kalantari *et al.* separated *S. cruzi* from cattle (11 ). Dalimi *et al.* determined *S. gigantean* and *S. arieticanis* in sheep (12).

### Immunity:-

*Sarcocystis* species are immunogenic in intermediate host , available information on cellular and humoral immune responses is only from responses directed against antigen derived from bradyzoites (13).

### Humoral Response:-

*Sarcocystis* sporocysts, developed immunoglobulin G (IgG) antibodies starting three to five weeks after

inoculation, these antibodies were detected by indirect haemagglutination (IHA), enzyme linked immunosorbent assay (ELISA) or dot ELISA, indirect fluorescent antibody (IFA) or complement fixation tests (13).

Immunoglobulin M (IgM) antibodies appear earlier than immunoglobulin G (IgG) but IgM are short lived and disappear by the time the sarcocyst matures (12).

Immunoglobulin G (IgG) antibody concentration in serum peaked during the early period of *Sarcocystis* formation, this persisted at a relatively high concentration during the chronic phase of the infection, the onset and persistence of *Sarcocystis* antibodies varied with the species of the host, species of the parasite and source of antigen, although *Sarcocystis* species share antigens within themselves, the antibody titer were higher using antigen from homologous species (15).

### Cellular Response:-

Immune cells are mobilized during *Sarcocystis* infection as might be expected of an intracellular parasite, immune cells such as lymphocytes and macrophages are mobilized and infiltrate visceral and muscular tissue (13).

This mononuclear cell infiltration begins during the third week of infection.

It may last for several months long after the parasite is no longer demonstrable in visceral tissues, also, lymphocytes from peripheral circulation show a blastogenic response when stimulated with antigen-specific *Sarcocystis* species, whether these cellular events participate in the recovery of the host from the disease has not been well established (15).

The passive transfer of resistance via cells or antibodies has not been reported, in certain animals, sarcocystosis may depress their immune status, the cellular response seen in immune animals that survive lethal challenge indicates cell-mediated immunity against *Sarcocystis*, cytotoxic antibodies or metabolites are known to destroy second generation extracellular merozoites (14).

## Materials and Methods

### Phosphate Buffer Saline (PBS) (PH=7.2):-

It was prepared by dissolving the following chemicals in 1 liter of distilled water :

Substance	Weight (g)
KH <sub>2</sub> PO <sub>4</sub>	0,2 gm
Na <sub>2</sub> HPO <sub>4</sub>	0.9 gm
NaCl	8.0
KCl	0.2

The pH of the solution was adjusted to 7.2 then sterilized by autoclave. Cooling and keep at 4 C (12).

### Experimental Design (Isolate Parasite, Ag. preparation) :-

Tissue effect with *Sarcocystis* were collected into an eppendorf tube and washed with saline thrice to remove attached bovine tissue, using fine forceps the cysts were macerated in 100 µL of saline at room temperature until a creamcoloured suspension formed, a drop of the suspension was examined under the light microscope (x 40) to confirm presence of banana-shaped cystozoites, these cystozoites were centrifuged, resuspended in PBS, recentrifuged and the supernatant discarded. The pellet of cystozoites was mixed with about 10 mL of 50% isotonic Percoll solution in a conical tube and centrifuged at 400 g for 10 min. Extraction buffer consisting of 180 µL PBS and 20 µL 10% Triton (TritonX-100, BDH laboratory, BDH Chemical Australia Pvt. Ltd., Victoria 3284, Australia) in PBS was then added to the Eppendorf, the sample was mixed well by a whirly mixer for 5 min, while alternatively placing it in ice to avoid heating. The sample was then centrifuged for 10 min at 14000 rpm in 4 °C. The resulting supernatant (antigen) was collected as aliquots into eppendorfs and stored at -20 °C.

Blood (~ 1 mL) was drawn from the heart of each mice (7-10 days) in order to select a serum sample with the highest concentration of antibodies, the serum for each animal, was stored in an eppendorf tube at -20 °C(16).

10 mice were divided randomly into two groups as follows:-

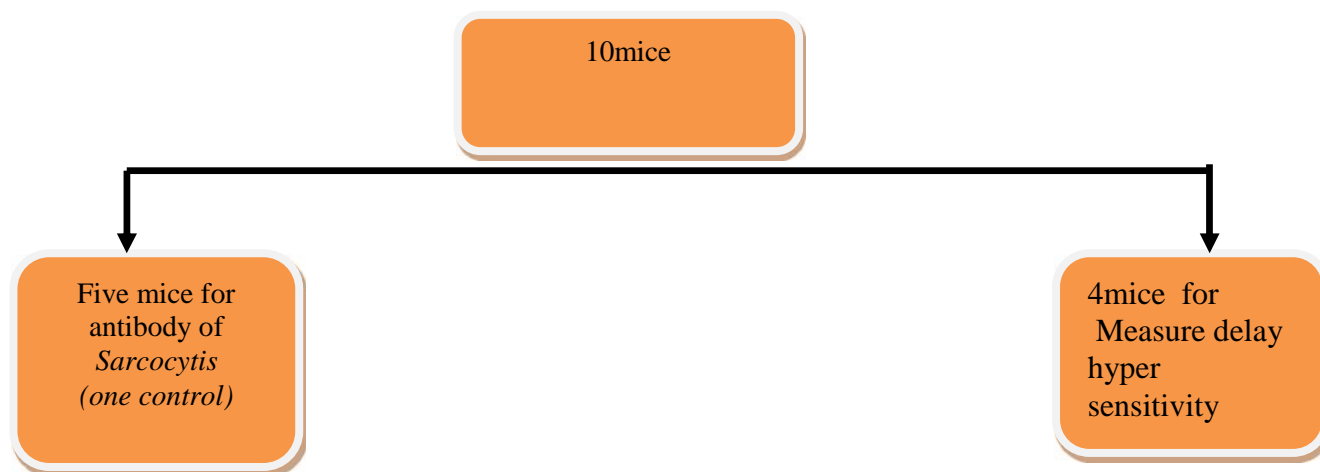
A- Experimental design :-  
A-1-antibody antigen reaction:-

Five mice were injection with 0.1 ml (interpretational with *Sarcocystis* antigen) (1 control), per mouse for anti- *Sarcocystis* antibodies after 7-10 day when appear clinical sign in mice effected. Using 25 µl serum of sheep and equal amount of serum contain Ab. ( from mice contain Ab) .

A-2-delay hyper sensitivity :-

Four mice injected with 0.1 ml in feet pad region and measure the reaction after 24 ,48 and 72 hour

### Experimental Design



### Steps of the Experiment:-

#### Methods:-

##### 1- antibody antigen reaction:-

Mice for antibody of *Sarcocystis*, after collected blood and put in centrifuge 2500 rpm for 10 minute take serum and put in deep freezing- 20 until using.

Take blood randomly from 25 sheep's than put in centrifuge 2500rpm for 10 minute take serum and put in deep freezing -20 until using.

Serum of mice put in water bath 56 C for 30 minute to discard the agglutination of complement after that , put drop( 25  $\mu$ l) from of mice serum ( contain antibody anti *Sarcocystis* ) to drop 25  $\mu$ l from serum of sheep's than wait 2-5 mint to occur antibody antigen reaction.

Then put in this reaction drop (25  $\mu$ l)of mercaptoethanol for detection the type of antibody IgG or IgM by the agglutination appear detect IgG while IgM Agglutination disappear mean the mercaptoethanol work in J chain and destroy it(17).

##### 2- delay hyper sensitivity:-

This test was done according to(14) as below:-

About 0.1 ml of a souluble antigen of *Sarcocystis* was injected intradermaly in right hind footpad of 4 mice while lift hind footpad injected by 0.1 ml of sterile PBS (PH 7.2)

Four mice injected with 0.1 ml in feet pad region and measure the reaction after 24 48 and 72 hour measure by Calipar digital.

FOR all immunized and thickness of skin was measured by vernier caliper in 0 days , 24 , 48 and 72 hours post injection.

### Results and Discussion

#### Antibody antigen reaction:-

Serum of mice (contain antibody against *Sarcocystis*) reaction with 25 serum of randomly collection from sheep that reaction appear as figure (17). table (4:21).

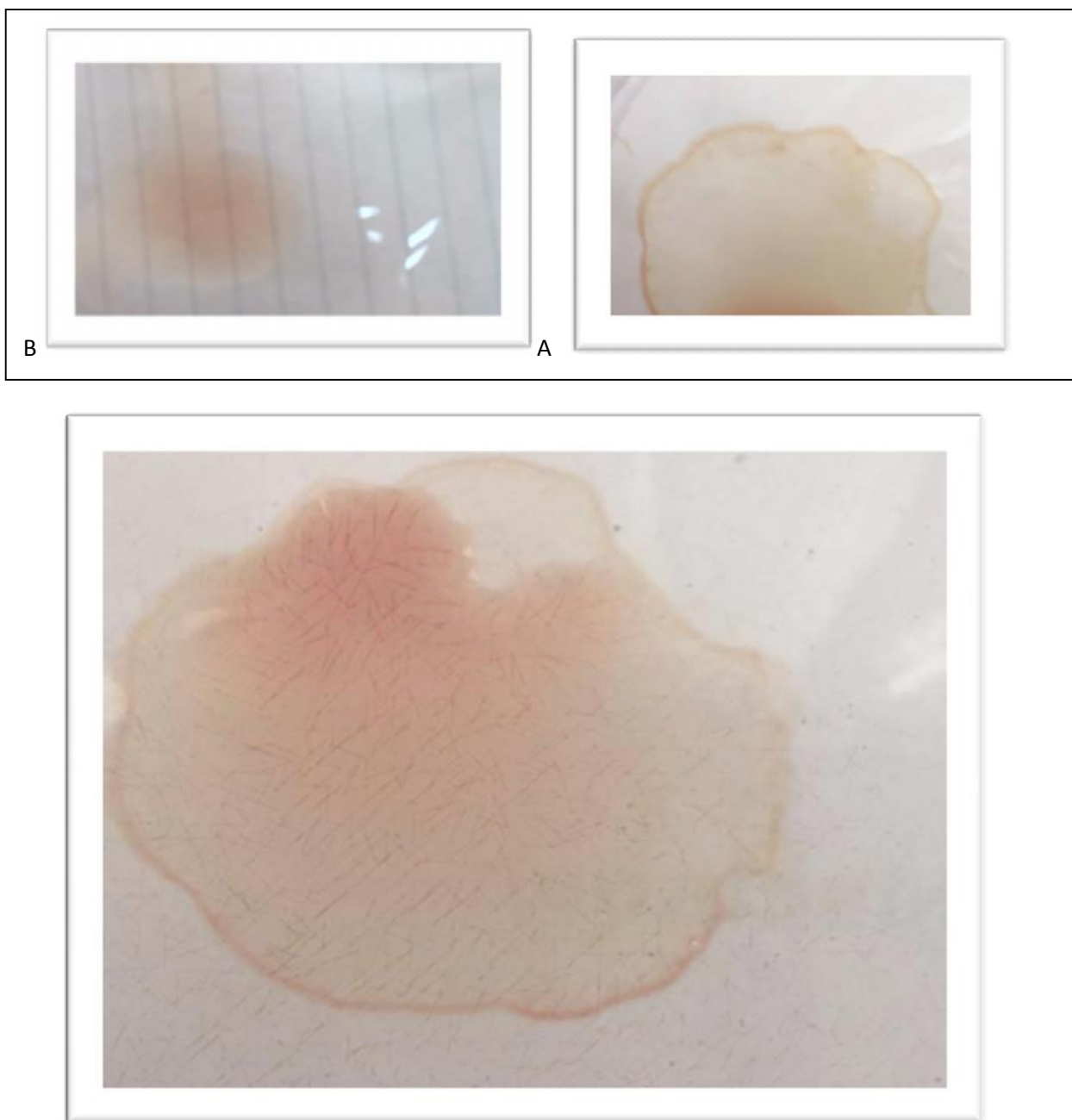


Fig .1 After add drop from mercaptoeathanol , agglutination still antibody is IgG).

Table (1 ) show the number of Ab. And Ag. Reaction

Randomly sheep serum	IgM	IgG
25	-	25

X<sup>2</sup>=0.05: Significant different between Ab (IgM and IgG).

**Delay type hypersensitivity (DTH)skin test:-**

The result of delay type hypersensitivity (DTH) have showed increase in the thickness of right footpads of

the mice's (4) and highest mean of the thickness was after 24 hours post immunization then decrease after passage of 48 hours and the after 24 hours immunized .

### Immune parameter:-

On the other hand, some serological assays including enzyme linked immunosorbent assay (ELISA) and indirect fluorescent antibody test (IFAT), based on bradyzoites derived from *Sarcocystis* have been assessed for serological diagnosis of sarcocystosis in sheep as well as some other animals, the bradyzoites of different *Sarcocystis* spp. have very antigenic similarities and therefore they have considerable cross reactions with other *Sarcocystis* spp.

Host immune response to the infection might have being responsible for the fall in prevalence in the older sheep, broken and degenerating *Sarcocystis* surrounded by host inflammatory reactions in muscle tissue of infected pigs and goats have been reported (14).

It has been reported that *Sarcocystis* within the muscle fibres had usually no associated host reaction but some toxic substances may be released, causing a strong immune response, when they were ruptured (15).

The rupture of the *Sarcocystis*, which may occur spontaneously or be caused by a host-immune reaction, is more likely to occur in old cysts found in older animals and may cause progressive reduction in cyst number over time (12).

For the development of specific antigens for serological tests and studying immune response in the patients and animal.

Using mercabitoethanol to detect type of antibody after reaction with Ag. Of *Sarcocystis* with Ab., mercabitoethanol action on J chain that chain found in IgM type, and that lead after add to the reaction disappear any agglutination occur between Ag. And Ab.

The DTH responses usually eliminate the intracellular pathogens but in some individuals the pathogen is not eliminated in spite of DTH responses, consequently more macrophages accumulate around the site of microbial presence, that adhere to each other, the active macrophages in turn secrete a number of cytokines and biological active substance that cause inflammation and destruction of microbial, the T helper cell secrete many cytokines such as (MIF, INF, GM-CSF, TNF, LIF, IL8 AND IL2) in turn activate the nearby lymphocytes and macrophages (17).

The DHT response has been used as an indicator of cell mediated immune status and is dependent upon

both T helper 1(Th1) driven responses as well as cell recruitment and chemotaxis to local site, as a result, the DHT functional response may be influenced by disruption either Th1 – driven, antigen – dependent T cell development or mobilization sensitized T cell to local site (18).

Delayed type hypersensitivity reaction (DTH) consists of a sequential cascade of steps depending on different types of T cells, as well as mast cells, endothelial cells and macrophages (19).

When small quantities of antigen are injected dermally, a hallmark response is elicited which includes induration, swelling and monocytic infiltration into the site of the lesion within 24 to 72 hours (20).

It has been shown that CD4+ TH1 lymphocytes ("inflammatory type") play a central role in DTH reaction (21).

This reaction has been shown to be absolutely dependent on the presence of memory T cells, both the CD4+ and CD8+ fractions of cells have been shown to modulate response, contemporary debate regarding the reaction is focused on the role of the Th1 and Th2 cells originally discovered by (22).

It has been postulated that the Th1 cell is the "inducer" of a DTH response since it secretes interferon gamma (IFN), a potent stimulator of macrophages and induces a cell mediated immune response black (1999), while the Th2 cells secrete cytokines such as IL-4, IL-5, and IL-6, which activate B cells and induce humoral immunity, Induction of the Th1 or Th2 phenotype is due to the antigen presenting cells secreting IL-12 which induces Th1 cells or secreting IL-10 which induces Th2 cells( 21).. Mosmann and others originally proposed that the Th1 cell that secretes IFN is the only T cell capable of inducing a DTH reaction (15).

The kinetics of the DTH responses are slightly different, with Th2 DTH lasting only 48 hours as opposed to 72 hours for Th1 DTH, but this is a relatively minor difference (23).

The low immune response, elicited by whole killed *Sarcocystis* antigens, may be associated with activation of CD4+ T cells and CD8+ T cells, which depended on the antigens exposure on APCs and the density of peptide MHC- complex on APCs (24).



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