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Investigation of Sarcocystis Infection in Human and Cattle

A thesis

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Abstract

Sarcocystosis is important disease in Asia, especially the western regions of the continent. It is considered one of the important zoonotic diseases, the infection wide geographic spreading caused by various species. In Iraq the disease is cosmopolitan infected man and animals leading to public health disorders and economic losses in meat production. The study was made to demonstrate the Sarcocystis species from esophagus in Diyala provinces by using traditional, Serological and thermocycler PCR, As well as Sequencing, genotyping analysis of the Sarcocystis isolated from Diyala (Baqubah), Iraq.

The overall infection rate showed 65 % (65\ 100) of sarcocystis isolated from esophagus of slaughtered cattle's in Diyala provinces, the peptic digestion technique were high sensitive technique 100% according all other traditional methods. Then Trichnoscopy were 30(30%) while the squeezing less sensitive 26(26%) respectively. The male cattle have a higher infection incidence was 75%, While the females cattle rate was 53.8% from the total exanimated animals without significant difference at $P<0.05$.

The result showed that the highest infection rate were recorded in animal less than one year old 80.77% with significant differences between the age groups $P<0.01$. Followed by (3-4) year group at rate 75%. Macroscopically examination of slaughter cattle. The cyst of Sarcocystis collected creamy to white color, with different size and shapes, tack many shape lick (spindle,globular,fusiform) the sizes ranged from (2-18mm x1-5mm). As for the Microscopic examination of Sarcocystis cyst by using trichnoscopy technique, showed oval, elliptical and conical form divided into compartments were many intercostal with different measurement range (166 × 52.2) µm.

Morphology of bradyzoite by using peptic digestion, muscle blender and squeezing methods.In this methods the bradyzoites could be seen by examining muscle fluid using one drop of the sediment of the

digested, appear as banana form with a spiked end of front and rounded near end and slightly clear nucleus located near the rear end, measurements 13.2× 2.8 µm (40X).

Sarcocystis in slaughter animals was found using a traditional thermocycler PCR. Extracted and isolate of genomic DNA of Sarcocystis, depending on Gene aid, obtained from Tissue cells extraction by using peptic digestion technique according to manufacturer protocol. Primer amplified using fragment at (574 bp and 900 bp). The purity of DNA was demonstrated by Nanodrop spectrophotometer at range (1.6-1.8) ng/µL. The DNA was extracted and stored at -20^{O}C, after that the PCR determined two different regions in ribosomal RNA depending on (18s-rRNA) of specific gene were amplified for identify the cattle Sarcocystosis. The fragment at (574 bp and 900 bp) was amplified by using a specific primer. The results of genetic analysis showed that the Iraqi isolates PQ156402, PQ156403, PQ156404 have a remarkable homology (100%) with Norway (KU247922.1), China (OR553291.1, MH681972.1), Iran (KR136315.1), Turkey MF327255.1, India (KT306827.1), JapanLC171828.1, Argentina (X679468.1). The interpretation of sequencing data was recorded *Sarcocystis levinei* for the first time in Iraq. In human the detection human Sarcocystiosis by ELISA test. The total serological results were (15.22%). The blood samples were taken at different genders, in Diyala province and the infection rate was highest in male (34.48%) while the lowest in female (6.35%). according to different age the infection rate in males was higher in (25-34 years) age group (57.14%) while in females, the highest infection rate was recorded in (25-34) age group than other groups (11.54%).

Chapter One

Introduction

1. Introduction

Sarcocystosis: is a zoonotic cyst-forming, coccidian intracellular protozoan parasite of the genus Sarcocystis infected animals as well as humans; it is a member of the family Sarcocystidae within the phylum Apicomplexa diseases with a worldwide distribution (Fayer *et al*., 2015; Gareh *et al*., 2020; Dubey and Rosenthal 2023).

Sarcocystosis affects productivity and milk yield, leading to substantial economic losses and public health problems (Gareh *et al*.,2020). After 20 years, the parasite known as Miescher's tubule was identified, having originally been identified as Sarcocystis in 1843 in the striated muscle of a house mouse as white thread-like cysts without a scientific name, fayer in the year 1972 identified the parasite life cycle, as well as he described the two hosts on the life cycle of Sarcocystis (Fayer, 2004; Dubey *et al*., 2015).

The intermediate hosts for many Sarcocystis species are cattle (Vangeel *et al*., 2007). The life cycle of Sarcocystis spp. Comprises an asexual phase in intermediate hosts (herbivorous prey) and a sexual phase in final hosts (carnivorous predators). Ingesting the tissues of intermediate hosts containing sarcocysts, which harbour bradyzoites, leads to infection in definitive hosts. Within the intestine of the definitive hosts, bradyzoites are developed into gametogonia. It is considered the sexual phase, which forms the sporocysts that spread into the environment through the faeces (Dubey *et al.,* 2016; Lindsay and Dubey, 2020). Sporocysts, introduced by contaminated pasture or water, get in the intestine of the intermediate host while forming sporozoites that target the endothelium of various tissues, referred to as Intestinal Sarcocystis. The merogony or asexual phase transpires inside the blood vessels, forming the merozoites that move to muscle tissue and increase to bradyzoites (Muscular Sarcocystis) (Dubey *et*

al., 2016). Asexual stages emerge in herbivore or omnivore hosts upon ingestion of sporulated oocysts excreted in the faeces of final hosts (carnivores) (Fayer *et al*. (2015).

The organism multiplies in the blood vessels through merogony, ending when intramuscular Sarcocystis with several bradyzoites is formed. Following the definitive host's consumption of meat containing Sarcocystis, the bacteria release bradyzoites into the gut, where they begin sexual multiplication (gamogony) and ultimately release oocytes into the excrement (Fayer, 2004).

Humans may be get intestinal sarcocystosis by consuming raw or undercooked meat (hot dogs, sausages and hamburgers) with *Sarcocystis hominis* and *S. suihominis bradyzoites* (Dubey, 2015).

Sarcocystosis in cattle has been linked to impaired reproductive and productive performances (abortion, weight loss, and decreased milk production), as well as the condemnation of contaminated beef in slaughterhouses and even death (Lindsay and Dubey, 2020).

In cattle, similar diseases of Sarcocystis spp, according to the morphological characteristics such as sporocyst wall thickness, parasite location or muscle preference, and sporocyst size seen under the light microscope, have some limitations. Precise taxonomic detection needs comprehensive ultrastructural characterisation by molecular techniques and electron microscopy (Luzón *et al*., 2015).

Sarcocystis spp. may be associated with bovine eosinophilic myositis (BEM), an inflammatory response that leads to muscle fiber degeneration and results in the condemnation of contaminated meat in slaughterhouses. The macroscopic cysts induced by S. hirsute lead to economic losses during meat processing (Rubiola *et al*., 2021; Dubey and Rosenthal, 2023).

S. heydorni and *S. hominis* are pathogenic organisms that cause human disease after ingesting contaminated undercooked meat (Joseph& Igor, 2022). They provide a public health hazard, capable of inducing serious intestinal infections in immunocompromised persons and those encountering the parasite (Fayer, 2004; Lindsay and Dubey, 2020).

Cattle are home to at least three species of Sarcocystis: *Sarcocystis cruzi*, *Sarcocystis hominis* and *hirsuta*. The definitive hosts of these species are canids, felids, and primates, respectively (Dubey and Lindsay, 2006).

The prevalence of Sarcocystis in cattle is 100% in most regions of the world (Vangeel *et al*., 2007; Akhlaghi *et al*., 2016).

Diagnosing Sarcocystis in the slaughtered carcasses of cattle was accomplished through inspection at an abattoir. Pathological symptoms are not repressed to detect the infected animals (Savini, 1994). Multiple diagnostic methods, such as pepsin digestion, muscle squash and histological methods, are used for the detection of Sarcocystosis (Beyazit *et al*., 2007).

Currently, diagnosis of Sarcocystis based on molecular biology techniques is very helpful in diagnosing Sarcocystis species. These are developed methods used to detect and identify Sarcocystis species and the genetic variety of this parasite can be assessed according to different populations' hosts. Molecular markers based on ribosomal DNA, are used in Sarcocystis spp. Differentiation by sequence analysis (Sato *et al*., 2022).

1.1 Aims of the study

1. Isolation and identification of sarcocustis sp. From esophagus by conventional and molecular technique.
2. Serological detection to sarcocustiosis in human by ELISA technique.