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Characteristics of non-cerebral coenurosis in tropical goats

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ABSTRACT

The epidemiological, clinical, and biochemical profile of non-cerebral coenurosis in goats and the morphological characteristics of the responsible metacestodes (cysts) were examined in a cross-sectional survey of slaughtered goats in abattoirs of the United Arab Emirates (U.A.E.) originating from Abu Dhabi and various tropical countries. The age, country of origin, and location of each cyst in the body of goats were recorded. Blood samples collected from infected and matching healthy goats were subjected to biochemical analysis. Data on the morphological characteristics of the cysts as well as the clusters, scoleces, and rostellar hooks in one cyst from each affected carcass were collected. The data collected were subjected to statistical analysis. A total of 2,284 slaughtered goats were examined and 40 goats were diagnosed as infected with non-cerebral coenurus cysts. The prevalence of non-cerebral coenurosis was 1.75% and the degree of parasite aggregation (k) was 0.003, which is indicative of overdispersion ($k < 1$). The only abnormalities observed in the infected goats were palpation of large single cysts in thigh muscles and higher serum aspartate aminotransferase (AST) value. A total of 76 non-cerebral coenurus cysts from 14 different body locations were collected. No cysts were found in the brain or spinal cord. Cysts located in psoas muscles had on average significantly bigger volumes and higher numbers of scoleces and clusters compared to cysts located in other body parts (P-value = 0.000). Significant differences in the morphometric measurements of the rostellar hooks were observed between cysts found in goats from different countries of origin (P-value < 0.05) perhaps due to initial steps of allopatric speciation by geographic isolation. A significant positive correlation was found between number of scoleces and volume of cysts ($b = 6.37 > 5$; R-Sq = 89.4%; P-value = 0.000) and between number of clusters and number of scoleces ($b = 25.13 > 1$; R-Sq = 79.8%; P-value = 0.000) indicative of following a positive allometric growth as well as between number of clusters and volume of cysts ($b = 0.25 < 0.5$; R-Sq = 69.4%; P-value = 0.000) indicative of following a negative allometric growth. The biological significance of the observed allometries is not known, but perhaps for evolutionary reasons the parasite is investing its resources more on the growth of scoleces, less on the growth of cyst volume, and even less on the number of clusters.

1. Introduction

The term "non-cerebral coenurosis" refers to the occurrence of coenurus cysts in body locations of the host other than the brain and the spinal cord. Non-cerebral coenurosis was first described in sheep (Benkovskij, 1899) and then in goats (Gaiger, 1907). The parasite responsible for non-cerebral coenurosis was initially named *Multiceps gaigeri* (Hall, 1916) in goats, and *M. Skrjabini* (Popov, 1937) in sheep (Schuster et al., 2010). However, the later literature considered *M. gaigeri* as the same species with *T. multiceps*, while *M. skrjabini* was rather treated as an unknown entity (Verster, 1969; Soulsby, 1982; Loos-Frank, 2000; Smith and Sherman, 2009).

Recently, the occurrence of non-cerebral coenurosis in sheep has been confirmed (Christodoulopoulos et al., 2013); while the geographical distribution of the disease in both goats and sheep covers a wide range of tropical countries in Asia, Middle East and Africa (Sharma et al., 1995; Sharma and Chauhan, 2006; Oryan et al., 2010; Schuster et al., 2010; Christodoulopoulos et al., 2013). Furthermore, investigation of two mitochondrial genes (CO1 and ND1) supported the opinion that *M. gaigeri* belongs to the same species of

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T. multiceps and only an intraspecific variation was noted between them (Oryan et al., 2010; Varcasia et al., 2012).

The lack of systematic information on non-cerebral coenurosis in goats was the rationale for undertaking a cross-sectional abattoir survey of non-cerebral coenurus cysts in goats in Abu Dhabi (United Arab Emirates). The objective of the present study was to investigate the epidemiological, clinical, and biochemical profile of non-cerebral coenurosis in goats and the morphological characteristics of the responsible metacestodes (cysts).

2. Material and methods

2.1. Goats

Non-cerebral coenurus cysts were collected from slaughtered goats in three abattoirs namely Bawadi, Falaj-Hazaa, and Yahar in the city of Al Ain of the Abu Dhabi Emirate of the United Arab Emirates (U.A.E.) during January 2011-August 2013. The majority of goats had been imported from various neighbouring countries for slaughtering.

2.2. Ante-mortem clinical examination of goats

All goats were given a full clinical examination before slaughter, including measurements of temperature, heart, and respiratory rate. In addition, the major groups of muscles were palpated in order to detect possible swelling or pain.

2.3. Cyst and data collection

The carcass of slaughtered goats was inspected by visual examination as well as by palpation. The suspected cyst was removed by dissecting the surrounding tissue. If a coenurus cyst was suspected in the carcass, the brain and the spinal cord were also examined visually for the presence of coenurus cysts by dissecting the head and splitting the carcass in half respectively using the saw of the slaughterhouse.

Cysts suspected as coenurus were removed, placed in a labelled plastic bag, and transferred to the laboratory in a portable fridge at 4–8 °C within an hour. In addition, the following data were recorded for goats with suspected coenurus cysts: age, country of origin, and location of each cyst in the body.

2.4. Blood samples

Following the usual ante-mortem clinical inspection of the goats, a 5 ml and a 10 ml blood sample were collected by jugular venipuncture in test tubes with and without heparin respectively. Subsequently, the animals proceeded to slaughtering followed by a detailed post mortem examination by the meat inspector of the slaughterhouse and also by one of the authors (GC or AK). Blood samples from goats found with non-cerebral coenurosis were matched with blood samples from healthy goats of the same batch of animals, same breed, same gender, and approximately same age. The blood samples were subjected to biochemical analysis.

2.5. Biochemical analysis

Blood serum albumin (Doumas et al., 1971) and total proteins (Weichselbaum, 1946) were determined using colorimetric methods. Serum aspartate aminotransferase (AST) (Bergmeyer et al., 1986), gamma-glutamyl transpeptidase (GGT) and creatine kinase (CK) (Szasz et al., 1976) were determined using enzymatic kinetic methods. All measurements were assayed at 37 °C by means of the same spectrophotometer (Shimatzu UV-1601, Tokyo, Japan). In order to exclude the possibility of selenium deficiency which may affect CK, selenium determination was carried out in whole blood by a fluorometric method in a spectrofluorometer (Hitachi Model F-2000) (Christodoulopoulos et al., 2003). The laboratory normal reference values for serum albumin, total proteins, AST, GGT, and CK were 24–44 g/l, 64–78 g/l, 58–350 IU/l, 5–89 IU/l and 20–194 IU/l, respectively. The threshold selenium concentration in whole blood below which goats were considered selenium deficient was 0.07 mg/dl (McComb et al., 2010).

2.6. Examination of cysts

The cysts collected during carcass inspection were examined to confirm their identity and measure their morphological characteristics. A cyst was initially identified as a coenurus cyst when it contained a bladder filled with a watery fluid and having a thin and transparent wall with numerous scoleces attached to its inner surface.

The morphological characteristics of each cyst were measured by establishing its volume by placing it in a measuring cylinder filled with tap water and by laying it on a flat surface and counting the number of scoleces and their arrangement in clusters. As cluster was considered any group of scoleces attached in the cyst membrane in proximity and surrounded by a distinguished area of membrane that was free of scoleces; random, single scoleces surrounded by a distinguished area of membrane free of scoleces were ignored.

For the final confirmation of the identification, a piece of the larval membrane containing a cluster of scoleces was placed on a slide along with some drops of normal saline. A cover slip was pressed tightly on the slide to provoke the evagination of the scoleces and the scoleces of the cluster were examined under a light microscope. The identification of the coenurus larvae was based on the recognition of the rostellar hooks along with the four surrounding suckers in the evaginated scoleces (Soulsby, 1968; Loos-Frank, 2000 Loos-Frank, 2000).

2.7. Examination of rostellar hooks

The rostellar hooks were further examined in one cyst from each affected carcass. For this purpose, five to eight scoleces of each cyst, randomly selected, were cut and placed face down on slides (the top was placed downwards, while the extremity previously attached to the larvae membrane was placed upwards). Some drops of Berlèse solution (cleaning and mounting medium; TCS biosciences, United Kingdom) were added and a cover slip was placed and pressed on each scolex rigorously. The slides were left to dry for 1–2 h and subsequently were observed under a light microscope.

Some of the rostella were posing in the scoleces in such a way that permitted the counting of the total number of small and large hooks (Fig. 1). Only these rostella were used for counting the number of hooks and measuring the dimensions of the rostellar hooks. In case that no rostellum was properly posing, the process was repeated with new scoleces until one rostellum at least from each cyst allowed the proper counting of the hooks.

The measurement of the dimensions of the hooks was accomplished by photographing the rostellum with a digital camera coupled to a light microscope. Measurement of the dimensions was performed using a computerized image analysis system (ImageJ by Softonic®). The accuracy of the measuring method had been previously tested by measuring a scale of known length (10 μm) fixed next to a scolex. Only hooks lying completely 'en face', were measured. Seven morphometric measurements were performed on each scolex: Sum of large and small hooks per scolex, length of large hook, length of handle of large hook, length of blade of large hook,