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DETECTION OF ENCEPHALITOZOONOSIS AND TOXOPLASMOSIS AMONG RABBITS BY CARBON IMMUNOASSAY

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INTRODUCTION

Encephalitozoon cuniculi is an intracellular protozoon parasite of brain, kidneys and other tissues. It frequently causes chronic and latent infections in laboratory animals especially rabbits (Shaduck and Pakes,1971). Numerous serological tests have been developed for the diagnosis of encephalitozoonosis. These included the indirect fluorescent antibody tests (IFAT) (Chalupsky, et al. 1971); carbon immunoassay (CIA) (Waller, 1977 and Kellett & Bywater, 1978); immunoperoxidase test (IPT) (Gannon, 1978); skin hypersensitivity test (SHT) (Pakes, et al. ,1972); complement fixation test (CFT) (Wosu, 1975) and haemoagglutination test (HAT)(Hubner, et al. 1973).

Toxoplasmosis is a widespread disease of mammals, bird, man and laboratory animals including rats, mice, guinea pig, rabbits, dogs and cats. The most common serological test used for diagnosis of toxoplasmosis are sabin feldman dye test, indirect fluorescent atntibody test, enzyme linked immunosorbent assay, indirect haemoagglutination test and carbon immunoassay (Waller and Bergquist, 1982; Dubey and Beattie, 1988).

Carbon immunoassay is a rapid, easy and reliable serological test as compared with other tests for diagnosis of encephalitozoonosis and toxoplasmosis in rabbits

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(Waller 1977; Waller and Bergquist, 1982). Hence, the present study aims to study the seroprevalence of *E. cuniculi* and *T. gondii* in rabbits by CIA since the informations on this subject in Egypt are lacking.

MATERIALS AND METHODS

Animals: Group A: Contained 100 rabbits collected from different areas in Cairo. The blood of this group was examined for the diagnosis of *E.cuniculi* and *T. gondii* by the use of mixed antigen susepnsion of both parasites.

Group B: Contained 50 rabbits their blood samples were collected during slaughtering at Fac. Vet. Med., Cairo Univ., Giza, Egypt. This group was examined only for the diagnosis of *E. cuniculi* using Encephalitozoon antigen only. The rabbits in both groups were apparently healthy.

Serum samples: 3 ml of blood were collected from each rabbit. The serum was collected in 1 ml plastic tube and stored at -20°C until used.

Carbon Immunoassay: The carbon suspension, Encephalitozoon antigen suspension and mixed antigen of equal volume of *E.cuniculi* and *T. gondii* (contained about 2.5×10^7 *E. cuniculi* spores and the same amount of *T. gondii* tachyzoites per one ml) were kindly supplied by Dr. T. Weller (National Veterinary Institute, Uppsala, Seweden). The test was carried out on each of the 100 rabbit serum in group A as described by Waller (1977). Each serum sample was diluted 1:10 in 0.15M saline solution in 0.1 % sodium azid. Equal (10 µl) of the mixed antigen suspension and the diluted serum were mixed in a microtitre plate and left for 5 minutes to react. 10 µl of the serum antigen solution was mixed with 10 µl of carbon suspension on a microscopic slide, covered with coverslip and left for 5 minutes. The slide was examined under oil immersion objective (X 1000). The rabbit sera in group B were examined by the method described by Kellet and Bywater (1978) and Waller and Bergquist (1982). Each serum sample was diluted 1:10 as in group A. 5 µl of *E. cuniculi* antigen

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suspension was mixed with equal volume of diluted serum and carbon suspension on a microscopic slide. Covered with a coverglass and left for 5 minutes at room temperature. Examined under light microscopy using x 1000. Positive reaction on group A and B was indicated by a dark antigen margin of attached carbon particles in suspension. In case of negative reaction, they were clearly visible organisms with unstained margin against dark back ground.

RESULTS

Group A: the present study showed that examination of 100 rabbits by CIA using mixed antigen. *E. cuniculi* and *T. gondii*) revealed that 53 rabbits were positive to *Encephalitozoon cuniculi* only, 5 rabbits positive to *toxoplasma gondii* only and 15 rabbits were positive to both infection, whereas 27 rabbits showed no reaction to both parasites. It could be concluded that the incidence of *E. cuniculi* and *T. gondii* infection in rabbits were 68 % and 20 % respectively. (Fig. 1&2).

Group B: 66 % out of 50 rabbits were found to be seropositive for *E. cuniculi* by CIA using Encephalitozoon antigen suspension. (Fig. 3).

DISCUSSION

The present study showed that 66 % to 68 % of apparently healthy rabbits were seropositive to *E. cuniculi*. Reports from different countries showed higher incidence of *E. cuniculi* infection (81.8 - 100 %) using CIA, IFAT and IPT (Malherbe and Munday, 1958; Cox, et al. , 1972; chalupsky et al. 1973; Cox and Pye, 1975; Waller, 1977; Gannon, 1978 and Bywater, et al. 1980). This high prevalence of *E. cuniculi* infection among commercial breeding rabbits may be due to bad hygiene, overcrowding and urine contamination between rabbits.

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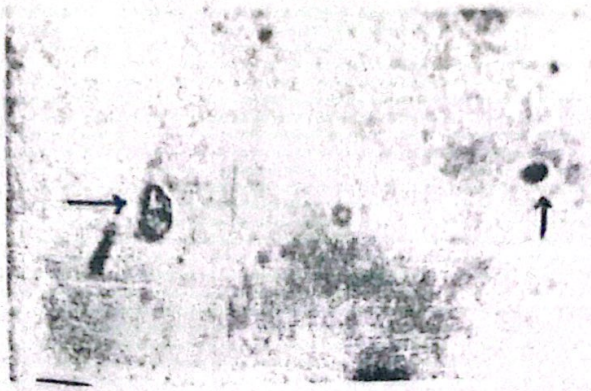

Fig. (1): Carbon immunoassay positive to *E.cuniculi* and *T. gondii* (X 1000).

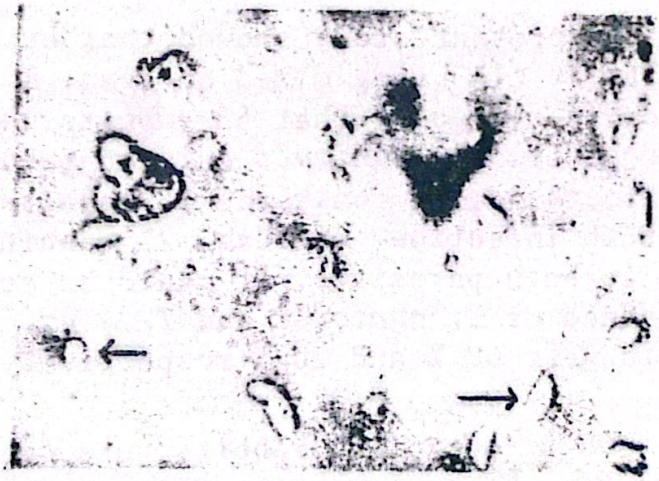

Fig. (2): Carbon immunoassay negative to *E. cuniculi* and *T. gondii* (X 1000).

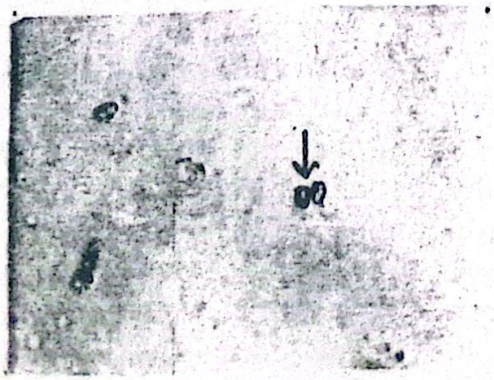

Fig. (3): Carbon immunoassay positive to *E.cuniculi* (X 1000).

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Also, transplacental transmission of the parasite Play role in increase the infection (Hunt, et al. 1972). Low incidence (5-26, 6%) were recorded by using CIA (Waller, 1977; Waller and Bergquist, 1982 and Bywater and Kellett, 1978). This low incidence may be due to good management and or the examined rabbits were recentlly infected and with very low IgG titres which can not be detected (Waller et al., 1978 and Bywater and Kellett, 1978).

Toxoplasmosis in the present study was detected in 20 % of the examined rabbits. Nearly similar incidence was recorded by Waller, 1982 (17 %) using CIA. Cross reaction was not observed in the present study between *E.cuniculi* and *T.gondii* antigen as well as in the previous reports of Wosu, et al. , 1977 and Waller and Bergquist, 1982.

SUMMARY

Examination of 100 rabbits by carbon immunoassay using mixed antigen of *Encephalitozoon cuniculi* and *toxoplasma gondii* revealed that 68 % and 20 % of rabbits were positive to these parasites respectively. Also, examination of 50 rabbits for *E.cuniculi* using *E.cuniculi* antigen suspension showed that 33 (66 %) of them were seropositive to this parasite.

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