



First finding of antibodies to *Encephalitozoon cuniculi* in raised chickens in Egypt

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Abstract:

In the present study, the presence of antibodies against *Encephalitozoon cuniculi* in chickens of different breeds was investigated by the method of enzyme linked immunosorbent assay (ELISA). A total number of 88 serum samples were collected randomly from chickens raised in houses and commercial farms in different locations of Behera province, Egypt. The breeds sampled included Egyptian native breed (Baladi) chickens (n=35), commercial egg-laying (Hy-line) breed (n=40) and commercial broiler Sasso breed (n=13). The age of the tested birds ranged from one month up to 20 months. Antibodies against *E. cuniculi* were detected in 13/88 (14.77%) of sera examined. *E. cuniculi* antibodies were detected only in the sera of egg-laying chickens. Results of this study indicate that chickens are exposed to *E. cuniculi* infection in Egypt. These results are of epidemiological relevance and public health importance; as the presence of *E. cuniculi* in raised chickens indicates a risk of infection to humans, mainly chicken breeders. Therefore, routine screening examinations of large-scale breeding of chickens are advised considering the zoonotic potential of these parasites.

Key words: *Encephalitozoon cuniculi* ; Chicken, ELISA, Microsporidia

Introduction:

Microsporidia are a highly expanded and specialized group of unicellular intracytoplasmic protozoa that are found in wide variety of animal species. 150 genera of microsporidian parasites consisting about 1200 species have been reported until now. (Keeling and Fast, 2002). Microsporidian parasites are considered one of opportunistic pathogen especially in immunocompromised host such as

patient infected with HIV, young age and aging host as well as patient who were subjected to organ transplantation operation, travellers and contact lens wearers (Didier, 2005). They are cosmopolitan parasites in addition they produce a resistant spores that can survive in surrounding environment and still infective for fairly long time. So, such characteristic feature of these parasites aids in recognition of suspected source of infections

consequently helping in setting up a strategy of prevention and control (Kasickova et al., 2009). Several studies have been recruited to propose how the birds take apart in spreading of infective spore of microsporidia in the environment such as (Reetz, 1993 and 1999; Tocidlowski et al., 1997; Reetz et al., 2002; Snowden and Phalen, 2004; Haro et al., 2005; Lobo et al., 2006; Slodkiewicz-Kowalska et al., 2006; Graczyk et al., 2007; Kas̃ic̃kova´ et al., 2007; Bart et al., 2008 and Muller et al. 2008).

Encephalitozoon cuniculi is classified under the phylum of Microspora (Sprague et al., 1992) and is considered one of among single cell eukaryote pathogen as well. Diagnosis of *E. cuniculi* is mainly depending on the serological approach, this is because the nature of its infection which is usually symptomless (Halanova et al., 1999). Many trials have been performed to identify the existence of antibodies of *E. cuniculi* in response to its antigens such as in 1972 a study was done by Cox et al., to identify antibodies in rabbits in addition, (Stewart et al., 1979) in dog. In the same context Gannon, (1980) diagnosed the *E. cuniculi* antibodies in mice. Alongside, Stewart et al., (1981) found the parasites antibodies in man while as, Singh et al., (1982) diagnosed the disease in sheep, as well as (Waller et al., 1983) tried to detect antibodies of *E. cuniculi* in goats, pig and horses. A recent study by Abu-Akkada et al., (2015) has also confirmed the presence of *E.cuniculi* in different animal hosts in Egypt. But up till now a little is known about *E.cunciuli* in birds. Only three species of birds have been fallen under attack of *E. cuniculi* infection until now, in chronological order, chicken, cockateel (*Nymphicus hollandicus*) and pigeons

(Reetz, 1993, 1999; Kasickova et al., 2007; Bart et al., 2008) respectively.

The zoonotic relationship of microsporidian parasites between birds and human still mysterious and it was not confirmed up till now, nonetheless, some cases of ocular microsporidiosis were recorded in persons who were in close interaction with pet birds (Friedberg et al., 1990 and Yee et al., 1991). While as (Haro et al., (2005); Lobo et al. (2006) and Bart et al. (2008)) proposed that the microsporidia protozoans can be transmitted from birds to human without any borders. Furthermore, the dealing and management of exotic birds is probably a suspected source of infection (Kasickova et al., 2009).

Few data are available on the prevalence of *E. cuniculi* infection in domestic chickens and the numbers of researches have been performed to study the birds in contact with human are very limited so, we decided to investigate the presence of *E.cuniculi* in chickens (*Gallus gallus*) intended for human consumption. The basic target of the current study was to identify antibodies against *E.cuniculi* in house and commercially-raised chickens in Egypt.

Materials and methods

Collection of samples

A total of 88 serum samples were collected randomly from different locations in Behera province, Egypt. The breeds sampled included Baladi breed chickens (n=35), commercial layer breed chickens (n=40) and commercial broiler Sasso breed chickens (n=13). The age of the tested birds ranged from one month up to 20 months (Sasso breed, 1.5 months, Baladi breed; 4-6 months and egg-laying breed , 6- 20 months). Samples from Baladi chickens were collected from houses and those of commercially raised chickens were obtained from

breeder farms. Blood samples were collected and sera were separated by centrifugation and stored at -20°C until use. All sera samples were analyzed by ELISA.

***E. cuniculi* spores**

Spores of *E. cuniculi* (strain ATCC 50503) were generously provided by Dr. Esther van de Ven, QM Diagnostics (231QM), Nijmegen, Netherlands and used as an antigen in ELISA test.

Manipulation of *E. cuniculi* spores and preparation of antigen (Akerstedt, 2002)

Firstly, the spores of *E. cuniculi* were frozen and thawed for three successive times, then the spores were sonicated (30 min, 60 W) in BRANSON sonicator. Secondly, the step of measuring of the protein content that found in the supernatant was done as described by Lowry et al. (1951). Finally, the product of soluble antigen was kept at -20°C waiting for procedure.

ELISA procedures

Sera of chicken were examined by indirect ELISA as designated by Akerstedt (2002). Shortly, Coating of Polystyrene microliters plates (Immunoplate Maxisorb; Nunc, Roskilde, Denmark) with the soluble antigen at a concentration of $2\mu\text{g/ml}$. Afterward, the plates were incubated overnight at 4°C , then washed with $200\mu\text{l}$ PBS/Tween 20, and then treated with the blocking solution (3% bovine serum albumin (BSA) with 0.05 % Tween 20). A total of $50\mu\text{l}$ each of diluted tested chicken serum, negative control sera, and positive control sera (1:10) were added to each well and then incubated at 37°C for 1 h. After incubation, the plates were washed three times with $200\mu\text{l}$ PBS/0.05 % Tween 20. After washing, horseradish peroxidase (HRP) conjugated Goat anti-Chicken IgG (KOMABIOTECH) was diluted at 1:5000 in PBS-T and

added to the plates ($100\mu\text{l/well}$). Lastly, the plates were washed three times with PBS/0.05 % Tween 20, and the enzyme activity of bound peroxidase was revealed by adding $100\mu\text{l}$ of ortho-phenylenediamine substrate (OPD) (Laboratories Inc., San Diego, CA, USA) to each well. After incubation in darkness (45 min), the enzymatic color reaction was stopped by adding $100\mu\text{l}$ of 1M phosphoric acid to each well, and the optical density was read at 490 nm using a microplate reader (Corona Electrical, Japan). Cutoff value was calculated according to John (2009); $\text{Cutoff} = \text{Mean of negatives} + (3 \times \text{SD of negatives})$.

Results

88 chicken sera were totally investigated for presence of antibodies triggered by natural infection with parasites. *E. cuniculi* antibodies were detected in 14.77 % (13 out of 88) of the examined birds. Cut off value >0.508 were considered positive. Seropositive chickens were only restricted in those birds of the laying breed group (birds of the highest age). *E. cuniculi* antibodies were recorded in birds without clinical signs suggestive of encephalitozoonosis. Positive results were detected in samples from 2 localities, Abu-elamatameer and Shobrakeit at Behera province, Egypt.

Discussion

Microsporidiosis is a cosmopolitan disease that is relatively understudied in comparison to other groups of human pathogens; as a consequence, our knowledge of this group of pathogens is continually evolving (Johny and Whitman, 2008). Although the advances of diagnostic approaches and continuous improvement of molecular techniques, there are several queries about this parasite still needed to be answered,

Such as the way in which the organism is transmitted and the sources of infection as well.

The current serological assay assured the presence of antibodies of *E. cuniculi* in 14.77% (13/88) of raised chickens in Egypt. However, **Reetz et al., (2002)** described *E. bieneusi* as a first record of microsporidiosis in chicken (*Gallus gallus*). Moreover, *E. cuniculi* was incriminated in infection of only three types of birds; firstly, it was detected in chicken (**Reetz, 1993, 1999**) then later in 2007 was discovered in a cockateel (*Nymphicus hollandicus*) by (**Kas̃ic̃kova' et al., finally, Bart et al.,**) then in 2008 it was found it in pigeons.

Reetz (1993) diagnosed *E. cuniculi* in chickens using immunohistochemical methods. His results showed that *E. cuniculi* infections in chickens can cause multi-symptomatic illness but may also be clinically inapparent. Moreover, **Reetz (1999)** examined 100 chicken embryos for naturally occurring infections by immunohistochemical methods. He found that *E. cuniculi* was detected in about 40% of the embryos. His investigations demonstrated that *E. cuniculi* may be transmitted naturally to the chicken egg. Further, the results suggested that *E. cuniculi* may be a cause of death in chicken embryos. He added that in most cases, however, *E. cuniculi* infections are inapparent in embryos. Such inapparent infections may be an important way of contamination of chicken production units with *E. cuniculi*. **Bart et al., (2008)** identified microsporidial DNA in the droppings of pigeons in the Netherlands and *E. cuniculi* was the third most common microsporidial species identified. While, **Lallo et al., (2012)** demonstrated that exotic birds and pigeons play a potential role in transmission of the *Encephalitozoon*

and *Enterocytozoon* genera for humans living in urban areas, as 24.5% of birds investigated in this study were infected with these parasites.

Surprisingly, we did not detect *E. cuniculi* in Egyptian Baladi breed chickens however the method of rearing this type of chickens may predict their susceptibility to infection with *E. cuniculi* spores. Where they were reared in small area and managed by the family staff themselves in addition of using neither unreliable nor selected food stuff. The chickens range freely in the household compound and find much of their own food or spend a large proportion of their time scratching to expose hidden food (**Lambert and Radwan, 2010**) which may be contaminated with *E. cuniculi* spores. Absence of infection with *E. cuniculi* in this breed could be attributed to their resistance to infectious diseases as reported by **Abu Elezz (1994)**. On the other hand, the only breed found to be infected with *E. cuniculi* in the present study was the commercial Egg-laying type breed. Although, there is a good management and biosecurity system for chickens' rearing (**Sonaiya and Swan, 2004**) but infection with *E. cuniculi* with a relatively high percentage (14.77%) may be attributed to stress and reduced immunity caused by egg laying. Besides, battery cage confinement in which this type of breed is living does not allow birds to turn around or take part in any other natural behavior. Prolonged forced confinement increases the incidence of disease and injury.

It was found that chickens of old age (egg laying breed; > 1 year) was the age category infected while young birds (Baladi and commercial Sasso breeds) were negative to *E. cuniculi* infection. This might be returned to age-dependent exposure to the parasite and/or predisposition to

infection (Wilson et al., 2007 and Wang et al., 2018). However, Black et al., (1997) observed severe outbreaks of encephalitozoonosis with high mortality in young chicks, whereas adult birds in the same aviary appeared unaffected. Other several reports stated that avian microsporidiosis leading to high morbidity and mortality observed shortly after birds were brought into a new environment (Canning and Lom, 1986; Suter et al., 1998; Tocidlowski et al., 1997). These observations suggest that latent infections as observed with *E. cuniculi* in other animals also occur in adult birds and that the stress may reduce the degree of resistance to this infection (Mathis et al., 2005).

Since the spores of *E. cuniculi* are shed in the urine for numerous weeks (Cox and Gallichio, 1978) and they are highly resistant to environmental conditions and can survive several months in humid climates (Li et al., 2003). Therefore, the possibility of avian–avian, avian–human and human–avian transmission of microsporidia in the environment must be more carefully considered.

Graczyk et al., (2007) reported that contact with pigeon droppings for 30 minutes, for instance, when cleaning the droppings, is sufficient time to admit 3.5×10^3 infectious microsporidial spores into the human body. Moreover, bearing in mind the close relationship between chickens and humans, since, the microsporidia spore can be inhaled or get access the body through direct connection with mucosa as well as through oral route (Haro et al., 2005).

It could be concluded that the detection of *E. cuniculi* in raised chickens in the present study may have a major epidemiological impact on chicken breeders because both commercial and home-raised chickens

contribute to environmental pollution with *E. cuniculi* spores and may be potentially infectious to any person in direct contact with them, for instance to chicken fanciers or ornithologists. This should direct our attention for the need of monitoring program for tracking this parasite especially in intensive farm of egg-laying type. From different point of view chickens may be probable used as animal model for studying the biology of microsporidia and for propagation of their spores.

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الملخص العربي

الاكتشاف الأول للأجسام المضادة لطفيل إنسيفاليتوزون كونيكولي في الدجاج في

مصر

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في هذه الدراسة، تم دراسة وجود الأجسام المضادة ضد إنسيفاليتوزون كونيكولي في الدجاج من سلالات مختلفة من خلال طريقة الاليزا. تم جمع عدد ٨٨ عينة من المصل عشوائيا من الدجاج المربي في المنازل والمزارع التجارية في مواقع مختلفة من محافظة البحيرة بمصر. شملت السلالات التي تم أخذها من السلالات المصرية من البلدي (عدد ٣٥)، والسلالة التجارية (هاي-لاين) (عدد ٤٠) والسلالة التجارية ساسو (عدد ١٣). تراوح عمر الطيور المختبرة من شهر إلى ٢٠ شهرا. تم الكشف عن الأجسام المضادة ضد إنسيفاليتوزون كونيكولي في ٨٨/١٣ (١٤.٧٧%) من المصل الذي تم فحصه. تم الكشف عن الأجسام المضادة فقط في المصل من الدجاج واطع البيض. وتشير نتائج هذه الدراسة إلى أن الدجاج معرض للإصابة بالعدوى في مصر. وهذه النتائج ذات أهمية وبائية وأهمية للصحة العامة؛ كما أن وجود إنسيفاليتوزون كونيكولي في الدجاج المطروح يشير إلى خطر العدوى للإنسان، وخاصة مربي الدجاج. لذلك ينصح بعمل فحوص على نطاق واسع من الدجاج لهذه الطفيليات