

**Seroprevalence of *Toxoplasma gondii* infection in  
poultry kept under different housing conditions in  
Israel**

By

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(Animal/Human/Ecosystem Health) in the Department of Veterinary Tropical  
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## DECLARATION

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I hereby certify that this research is the result of my own investigation. Where use was made of the work of others, it has been duly acknowledged in the text. The results in this dissertation have not been submitted, in whole or in part, for a degree at any other tertiary institution.

**Harold Salant**

I hereby release this dissertation for examination in my capacity as supervisor.

**E. Volker Schwan**

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## SUMMARY

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SEROPREVALENCE OF *TOXOPLASMA GONDII* INFECTION IN POULTRY KEPT UNDER  
DIFFERENT HOUSING CONDITIONS IN ISRAEL

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A *Toxoplasma gondii* seroprevalence survey was conducted in poultry flocks kept under different housing systems in Israel. The seroprevalence rates were 35.4 % (46/130) in battery-raised chicken egg-layers from Jerusalem which were first raised on the ground, 12.5 % (19/152) in free-range chickens from various locations of the country and 9 % (4/45) in turkeys from the north. However, all broilers (50) and chicken breeding stock (58) kept under high biosecurity conditions in Jerusalem were found to be *T. gondii* seronegative. It is concluded that high biosecurity conditions prevent contamination with oocysts by rendering premises inaccessible to cats.

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## Chapter 1

### INTRODUCTION AND LITERATURE REVIEW

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Toxoplasmosis is caused by *Toxoplasma gondii*, a coccidian intracellular protozoan parasite. *Toxoplasma gondii* is arguably the most successful parasite worldwide. It is more than 100 years ago since *T. gondii* was initially discovered by accident in 1908 by Charles Nicolle, a scientist, who was working in North Africa and searching for a reservoir of *Leishmania* in the indigenous rodent, *Ctenodactylus gundi*, also known under the common name 'common gundi' (Nicolle & Manceaux 1908). The domestic cat and to a lesser extent wild felids act as definitive hosts while several mammal and bird species act as intermediate hosts. *Toxoplasma gondii* is capable of infecting an unusually wide range of hosts and many different host cells (Dubey & Beattie 1988) The life cycle of *T. gondii* is facultative heteroxenous.

In intermediate hosts, *T. gondii* undergoes two phases of asexual development. In the first phase, tachyzoites and endozoites multiply rapidly by repeated endodyogeny in different types of host cells (Tenter, Heckeroth & Weiss 2000). Tachyzoites of the last generation initiate the second phase of development which results in the formation of tissue cysts (Tenter *et al.* 2000). Within the tissue cysts, bradyzoites and cystozoites multiply slowly by endodyogeny (Tenter *et al.* 2000). Tissue cysts have a high affinity for neural and muscular tissues (Tenter *et al.* 2000). Tissue cysts are located predominantly in the central nervous system (CNS), the eye as well as in the skeletal and cardiac musculature, however, they may also be found in visceral organs, such as lungs, liver, and kidneys (Tenter *et al.* 2000). Tissue cysts are the final life cycle stage in the intermediate host and are immediately infectious. In some intermediate host species, they may persist for the entire life of the host (Tenter *et al.* 2000). Although the exact mechanism of this persistence is unknown, some researchers believe that tissue cysts break down periodically, with bradyzoites transforming to tachyzoites that invade host cells and again transform to bradyzoites within new tissue cysts (Dubey, Lindsay & Speer 1998; Weiss, Udem, Tanowitz & Wittner 1988). If ingested by a definitive host, the bradyzoites initiate another asexual phase of proliferation which consists of initial multiplication by

endodyogeny followed by repeated endopolygeny in epithelial cells of the small intestine (Dubey & Beattie 1988). The terminal stages of asexual multiplication initiate the sexual phase of the life cycle (Dubey & Beattie 1988). Gamogony and oocyst formation also take place in the epithelium of the small intestine (Dubey *et al.* 1998). Unsporulated oocysts are released into the intestinal lumen and passed into the environment with the faeces (Dubey *et al.* 1998). Sporogony occurs outside the host and leads to the development of infectious oocysts which contain two sporocysts, each containing four sporozoites (Dubey *et al.* 1998). There are three infectious stages in the life cycle of *T. gondii*, *i.e.* tachyzoites, bradyzoites contained in tissue cysts, and sporozoites contained in sporulated oocysts. All three stages are infectious for both intermediate and definitive hosts, via one of the following routes: (A) horizontally by oral ingestion of infectious oocysts from the environment, (B) horizontally by oral ingestion of tissue cysts contained in raw or undercooked meat or primary offal (viscera) of intermediate hosts, or (C) vertically by transplacental transmission of tachyzoites (Dubey *et al.* 1998). In addition, in several hosts tachyzoites may also be transmitted in the milk from the mother to the offspring (Tenter *et al.* 2000). Thus, *T. gondii* may be transmitted between definitive and intermediate hosts, as well as between definitive and between intermediate hosts. It is currently not known which of the various routes of transmission is more important epidemiologically (Tenter *et al.* 2000). However, simultaneous presence of both intermediate and definitive hosts is not necessary for *T. gondii* transmission cycles to be maintained. The life cycle may continue indefinitely by transmission of tissue cysts between intermediate hosts, even in the absence of cats and also by transmission of oocysts between definitive hosts, even in the absence of intermediate hosts (Tenter *et al.* 2000).

Toxoplasmosis is one of the most common parasitic zoonosis and it is estimated that a third of the world population is seropositive (Saadatnia & Golkar 2012). Humans can contract toxoplasmosis by ingesting sporulated oocysts originating from cat faeces, by consumption of tissue cysts in raw infected meat or transplacentally in primary infected women during pregnancy (Dubey & Lindsay 2004; Innes 1997). In immune competent humans, toxoplasmosis is in general clinically uneventful (Tenter



*et al.* 2000). However, severe pathological effects can result in immunocompromised individuals as well as in fetuses and neonates of woman following primary infections contracted during pregnancy (Dubey & Jones 2008).

The significance of consuming undercooked infected chicken and other poultry meat in the epidemiology of human toxoplasmosis has not been sufficiently investigated (Dubey 2010). Chickens are clinically resistant to *T. gondii* (Dubey & Beattie, 1988). Chickens infected orally with *T. gondii* oocysts remain asymptomatic or develop a mild illness (Biancifiori, Rondini, Grelloni & Frescura 1986; Dubey, Camargo, Ruff, Wilkins, Shen, Kwok & Thulliez 1993a; Miller, Frenkel & Dubey 1972). For example, in a study where chickens were infected experimentally with *T. gondii*, none of the chickens, each infected with 5 000 oocysts, were clinically affected. In addition, those chickens, each which were infected with 50 000 oocysts, remained asymptomatic with the exception of a decrease in egg production and high mortality of embryonated eggs (Biancifiori *et al.* 1986). Nevertheless, it is claimed that chickens play a significant role in the epidemiology of toxoplasmosis in the rural environment and are an efficient source of infection for cats (Dubey 2002; Dubey 2010). Sources of infection for poultry are primarily feed contaminated with sporulated oocysts from the environment and probably to a lesser extent invertebrates (earthworms, molluscs, cockroaches, flies, beetles) acting as transport hosts for oocysts (Chinchilla & Ruiz 1976; Dubey 2002; Dumètre & Dardè 2003; Frenkel 1973; Ruiz & Frenkel 1980). Sporulated oocysts are highly resistant to environmental conditions and disinfectants, and can remain infectious in moist soil or sand for up to 18 months (Tenter 2009). A previous survey in the northern part of Israel found a high infection rate in a commercially raised flock of chicken egg-layers with 42.2 % of seropositive birds harbouring viable parasites (Dubey, Salant, Sreekumar, Dahl, Vianna, Shen, Kwok, Spira, Hamburger & Lehmann 2004). In another survey conducted in the United States, viable *T. gondii* bradyzoites have been isolated from up to 100 % of chickens examined (Dubey, Webb, Sundar, Velmurugan, Bandini, Kwok & Su 2007). Of 149 infected chickens from various studies by different research groups whose individual tissues were bioassayed, 89.5 % (129 of 144) of hearts, 49.2 % (67 of 136) of brains, 44.1 % (15 of 34) of leg

muscles and 18.6 % (16 of 86) of pectoral muscles were found to be infected (Dubey 2010). However, there are now ample data from chickens and other animals indicating that *T. gondii* encysts in muscle more efficiently than in the brain (Dubey & Beattie 1988). Free-range chickens are invariably exposed to oocysts shed by cats. Cats fed naturally infected chickens tissues can shed millions of oocysts (Dubey, Graham, Blackston, Lehmann, Gennari, Ragozo, Nishi, Shen, Kwok, Hill & Thulliez 2002). In a study in which cats were fed tissues of acutely infected chickens administered *T. gondii* oocysts 10 days previously, all cats shed oocysts. (Ruiz & Frenkel 1980).

Also domestic turkeys can act as intermediate hosts but, similar to chickens, are resistant to clinical toxoplasmosis (Drobeck, Mawell, Bernstein & Dillon 1953; Dubey, *et al.* 1993a; Sedlák, Literák, Vitula & Benák 2000; Simitch, Bordjocki, Petrovitch, Tomanovitch & Savin 1961).

In many instances, especially in developing countries, chickens are killed at home or in unsupervised slaughter facilities and the viscera are left for scavengers or are improperly disposed of (Dubey, 2010). *Toxoplasma gondii* infection can be transmitted if care is not taken to wash hands thoroughly after cutting meat and during cooking of meat, however, risk assessment studies have not been undertaken (Dubey, 2010). Since Israel has the world's largest per capita consumption of turkey meat and the fourth largest per capita consumption of chicken meat (<http://www.thepoultrysite.com/>), it warrants the need to obtain data on seroprevalence in poultry flocks, particularly with regard to different housing systems.

Although *T. gondii* was not isolated from any of the 2094 chicken meat samples obtained from retail meat stores in the USA (Dubey, Hill, Jones, Hightower, Kirkland, Roberts, Marcet, Lehmann, Vianna, Miska, Sreekumar, Kwok, Shen & Gamble 2005), the authors believe that results obtained from the study do not negate that infected chickens may be a source of infection for humans primarily due to reasons related to the experimental design and conditions that the meat samples were stored before testing took place.

Published information regarding *Toxoplasma* seroprevalence in poultry kept in different housing systems is very limited. A survey conducted in northeast China reported significant differences in seroprevalence rates in cage-raised poultry (2.8 %) compared to free-range poultry (34.7 %) (Zhu, Yin, Xiao, Jiang, Andale, Lind & Chen 2008). In another survey conducted in southern China, high *Toxoplasma* antibody titers were found in 16 % of ducks, 11.4 % of free-range chickens and 4.1 % of caged chickens sampled (Yan, Yue, Yuan, He, Yin, Lin, Dubey & Zhu 2009). Serological surveys conducted in geese and ducks in Germany found that flocks kept exclusively indoors had a significantly lower number of positive birds when compared to flocks kept outdoors (Maksimov, Buschtöns, Herrmann, Conraths, Görlich, Tenter, Dubey, Nagel-Kohl, Thoms, Bötcher, Kühne & Schares 2011).

The objective of the present survey was to compare *T.gondii* seroprevalence rates of poultry flocks kept under different housing systems in Israel. The hypothesis is that the extent of exposure to sporulated oocysts depends largely on the housing system, with systems in which birds are raised above the ground and (or) overall high biosecurity providing significantly more protection from infection.

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## Chapter 2

### MATERIALS AND METHODS

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#### 2.1 Survey area

The survey was conducted on poultry flocks from the Jerusalem, North of Tel-Aviv and Samaria districts in Israel.

#### 2.2 Study animals

Blood samples from a total of 390 chickens and 45 turkeys were obtained for the survey. Chickens (*Gallus domesticus*) included in the survey belonged to several groups with respect to their housing conditions. **Group 1a** consisted of 100 2½-year-old commercially raised free-range egg-layers raised in Shilo, in the central part of Israel, approximately 45 km northeast of Tel-Aviv. **Group 1b** consisted of 52 household free-range chickens up to 1½-year-old from the mainly Arab inhabited area of East Jerusalem. **Group 2** comprised 130 battery-raised egg-laying chickens ranging between 14-127 weeks of age, from 6 different flocks in the Jerusalem area. They were housed on the ground until the age of 105 days and subsequently above the ground in cages. Chickens in all groups (1a, 1b, 2) were likely to be exposed to *T. gondii* oocysts shed by cats. **Group 3** consisted of 50 broilers kept on concrete floors until 6 weeks of age. Chickens in **Group 4** comprised 58 breeders which were over 2 years of age at sampling. Both, groups 3 and 4, were from the Jerusalem area and raised under strict biosecurity. **Group 5** comprised 45 turkeys (*Meleagris gallopavo*) used for their meat from a farm situated approximately 25 km north of Tel-Aviv. They were exposed to feral cats. Their ages were 5 months at the time of sampling.

### 2.3 Blood samples

Poultry of Groups 1a and Group 5 were destined for slaughter and subsequent human consumption. Blood samples were collected individually into serum collection tubes during the slaughtering process at Atarot 'Halal' Slaughterhouse in Jerusalem. Blood samples were centrifuged at 3000 g for 15 min and the sera stored at -20 °C until screened. Serum samples from poultry in Groups 1b, 2, 3, 4 were provided by the Israel Egg and Poultry Board, Beit Dagan and were collected by a poultry state veterinarian for routine disease screening.

### 2.4 Serological assay

Serological testing for *T. gondii* IgG antibodies was performed using the modified agglutination test (MAT) (Dubey & Desmonts 1987). Compared to other serological tests, the MAT has a high sensitivity and specificity in domestic animals (Frenkel, 1981; Dubey, Ruff, Carmargo, Shen, Wilkins, Kwok & Thulliez 1993b; Dubey 1997; Shaapan, El-Nawawi & Tawfik 2008; Casartelli-Alves, Boechat, Macedo-Couto, Ferreira, Nicolau, Neves, Millar, Vicente, Oliveira, Muniz, Bonna, Ameindoeira, Silva, Langoni, Schubach & Menezes 2014). The MAT detects only IgG antibodies as mercaptoethanol used in the test destroys IgM antibodies (Dubey 2010).

Formalinized *T. gondii* tachyzoites in phosphate-buffered saline as antigen for the MAT were provided by the Laboratoire de la Toxoplasmosse, Paris, France. Positive and negative sera from poultry were provided by the United States Department of Agriculture in Beltsville, USA. Serum samples from chickens infected with *Eimeria tenella* were provided by the Israel Egg and Poultry Board to exclude cross-reactivity of this species with *T. gondii* in the MAT. Sera from all poultry were diluted two-fold from a baseline titer of 1:5 which was regarded as positive.

## 2.5 Statistical analysis

The SPSS16 (SPSS Inc, Chicago, Illinois, USA) computer package was used for analysis of data. Data were also analyzed by using Fisher's exact test for categorical variables. Values of  $p < 0.05$  were taken as significant.

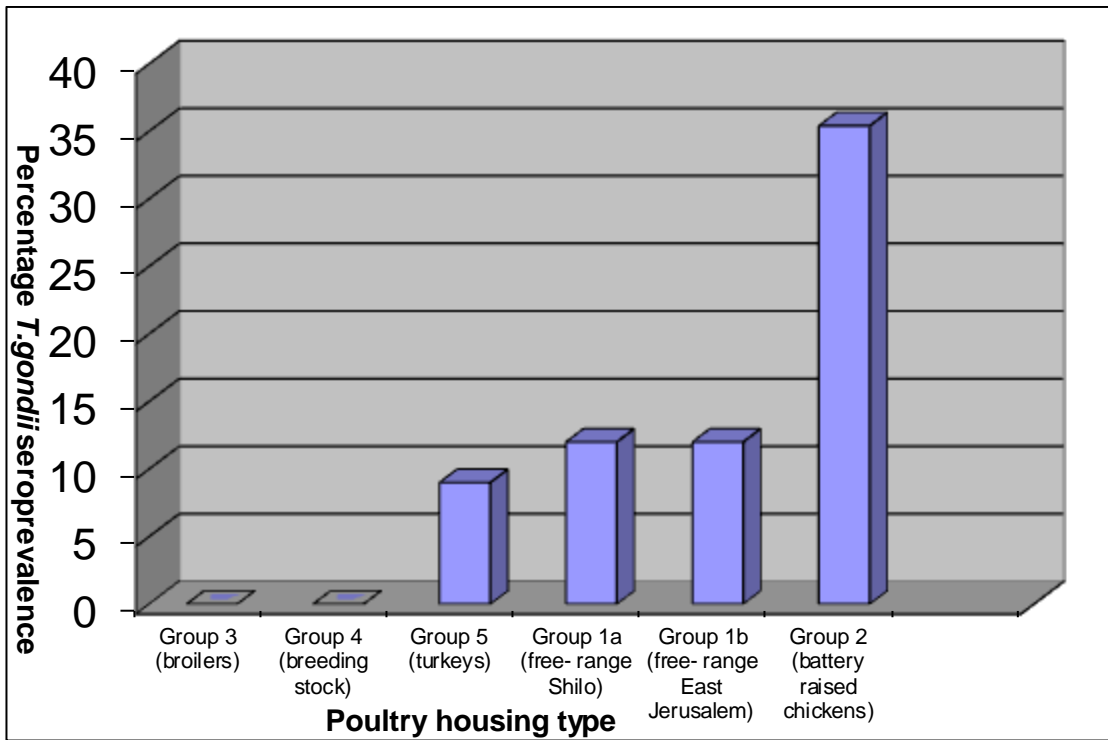
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### Chapter 3

### RESULTS

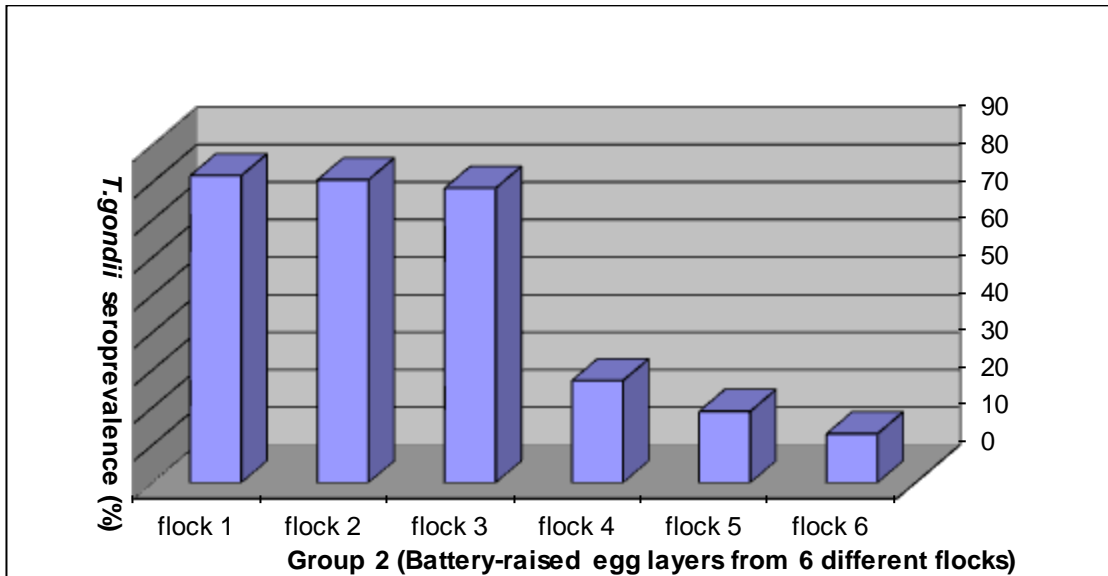
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Serum samples from a total of 390 chickens and 45 turkeys were screened for the presence of *T. gondii* antibodies. The seroprevalence in chickens in Groups 1a and 1b (free-range egg layers) was 12.5 % (19/152). Forty-six of 130 (35.4 %) battery-raised egg layers (Group 2) were found to be *T. gondii* seropositive; 0/50 (0 %) of 6-week-old broilers (Group 3) were seropositive; and 0/58 (0 %) of chickens used for breeding stock (Group 4) were seropositive. Four of 45 (8.9 %) 5-month-old commercially raised turkeys (Group 5) were seropositive. Although it had been hypothesized and, in fact was observed that the sum of groups, battery-raised chickens (Group 2), broilers (Group 3) and breeding stock (Group 4), with a total of 46 positive birds out of 238 (19.3 %), was significantly more *T. gondii* seropositive than the sum of free-range chickens (Groups 1a and 1b) and turkeys (Group 5), with a total of 23 positive birds out of 197 (11.6 %,  $p = 0.02$ ), the observation of a specifically high seroprevalence rate among battery-raised chickens (Group 2), (46/130; 35.4 %) contrary to the initial hypothesis' expectation necessitated this group to be compared to the sum of all other groups (23/305; 7.5 %). The difference was found to be of very high significance ( $p = 4.24 \times 10^{-12}$ ). Thus, this group was removed for comparison with other groups in the analysis. The sum of *T. gondii* seroprevalence amongst broilers (Group 3) and chicken breeding stock (Group 4) with a total of 0/108 (0 %) was significantly different ( $p = 2.65 \times 10^{-5}$ ) compared to the sum of seroprevalence of free range chickens (Groups 1a and 1b) and turkeys (Group 5), with a total of 23/197 (11.7 %). Similar to findings obtained by Dubey *et al.* (1993b) this survey demonstrated no cross-reaction in the MAT with sera obtained from *Eimeria tenella*-infected chickens used as controls.



**Figure 3.1:** *Toxoplasma gondii* seroprevalence in poultry kept under different housing conditions in Israel





**Figure 3.2:** *Toxoplasma gondii* seroprevalence in various flocks of different battery raised chickens in Jerusalem

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## Chapter 4

### DISCUSSION

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The survey demonstrated that poultry is widely exposed to *T. gondii* in Israel (Fig. 3.1).

The results of the survey present evidence that housing conditions which prevent oocyst-shedding cats to access premises have a significant impact on *T. gondii* seroprevalence of poultry (Fig. 3.1). Battery-raised egg layers (Group 2) had the highest cumulative seroprevalence of all 6 survey groups with 35.4 % of birds infected. Housing egg-laying chickens in batteries above the ground did not change the level of *T. gondii* seroprevalence since they were housed on the ground until the age of 105 days. Seroprevalence differed in the various flocks of Group 2 (Fig. 3.1, Fig. 3.2). A varying lack of strict biosecurity conditions in this group, which was personally observed, with rodents and cats occasionally gaining access to the housing facilities might be a possible explanation. It might also be related to differences in the age composition of these groups. Conditions of high bird concentrations in limited spaces where cats readily frequent may allow for easier *T. gondii* oocyst exposure allowing for these high seroprevalence rates to be achieved. Broilers in Israel are often raised on cement pens and on the ground under conditions of relatively high biosecurity with regard to access to cats and rodents. Broiler raising pens are thoroughly cleaned and disinfected with aldehydes after each batch of broilers is removed and before reintroduction of a new flock. This is probably also the reason why birds in Group 3 were seronegative. Also the 2-year-old breeders (Group 4) were found to be seronegative and this can only be explained by an effective housing system where biosecurity is strictly enforced and feed is not contaminated with oocysts. Zhu *et al.* (2008), Yan *et.al* (2009) and Maksimov *et al.* (2011) have reported similar findings amongst biosecurity-enforced, caged and free-range poultry in North East China and enclosed and free-range geese and ducks in Saxony, Germany. Both reports relate high *T. gondii* seroprevalence in free-range birds due to their exposure to feral cats. Unfortunately, these surveys compared only

two types of housed poultry and did not include the possibility of housing birds above the ground exclusively in their investigations.

Housing conditions as a modifying mechanism for *T. gondii* prevalence in poultry is crucial, especially in a day and age whereby animal production systems that offer outdoor access to animals have become increasingly popular in the Western world, including Israel, due to the growing general discontent of consumers with conventional bio-industrial farming practices. These open production systems offer improved animal welfare but may create new problems for animal health, resulting in increased food safety risks from parasitic infections, like toxoplasmosis. In current organic husbandry systems, the animals are kept at lower stocking densities and have outdoor access. According to the European Union regulations on organic farming, the use of antibiotics is restricted and the withdrawal times after medical treatments before delivery of products are doubled. In addition, beak trimming is prohibited and broiler chicken farmers use breeds that grow more slowly (European Council 2007). The design of new animal production systems with outdoor access requires both a thorough analysis of possible risks and optimal communication of these risks throughout the food chain and appropriate partitioning of responsibility concerning these risks. Some risks are inherent to the choice of keeping animals in a more natural environment and could be judged as an inherent responsibility of the consumer, whereas other risks may be mitigated by further refinement or adjustment of the housing or farm management system used.

In conclusion, it is important to adopt strategies to house poultry according to the ability to reduce *T. gondii* exposure from infected cats and other infected animals by using effective and affordable biosecurity measures.

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## Chapter 5

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**APPENDIX 1**

**ANIMAL ETHICS APPROVAL**



UNIVERSITEIT VAN PRETORIA  
UNIVERSITY OF PRETORIA  
YUNIBESITHI YA PRETORIA

**Animal Ethics Committee**

PROJECT TITLE	Seroprevalence of <i>Toxoplasma gondii</i> infection in poultry kept under different housing conditions in Israel
PROJECT NUMBER	V098-14
RESEARCHER/PRINCIPAL INVESTIGATOR	H Salant

STUDENT NUMBER (where applicable)	UP_79408151
DISSERTATION/THESIS SUBMITTED FOR	MSc

ANIMAL SPECIES	n/a	
NUMBER OF ANIMALS	n/a	
Approval period to use animals for research/testing purposes		October 2015-October 2016
SUPERVISOR	Dr. EV Schwan	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

<b>APPROVED</b>	Date	30 October 2015
CHAIRMAN: UP Animal Ethics Committee	Signature	

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