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# Serologic survey for toxoplasmosis in domestic birds from the Czech Republic

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Short title: Domestic birds and *T. gondii* antibodies

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**Summary**

This study was carried out on domestic fowls in the Czech Republic in the years 2003 and 2004. A total of 1,108 samples obtained from 538 water birds (geese and ducks) and 570 gallinaceous birds (chickens and turkeys) were tested for specific antibodies against *T. gondii* using the Indirect fluorescence antibody test (IFAT). In geese, 43% prevalence (77 positive out of 178 examined) was found with titres of 40, 320 and 2,560 in 40, 29 and 8 geese, respectively. In ducks, 14% prevalence (52 out of 360) was found with titres of 40 and 320 in 46 and 6 ducks, respectively. In gallinaceous birds, only one of 293 broiler chickens was positive with a titre of 40; sera of 217 hens and 60 turkeys were negative. We found extremely higher antibody prevalence in geese and ducks compared to gallinaceous birds. In Europe, it is the first seroprevalence study in domestic birds by IFAT.

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## Introduction

The parasite *Toxoplasma gondii* causes infections that are widely prevalent in human and warm-blooded animals worldwide (Dubey and Beattie, 1988). Humans can be infected mainly by ingesting *T. gondii* tissue cysts from undercooked meat or *T. gondii* oocysts from the environment contaminated with cat feces. Domestic birds are a good indicator of environmental contamination with parasite oocysts because of their feeding behaviours. Moreover, birds can serve as a potential source of infection for humans. In chickens, *T. gondii* were found in skeletal muscles, heart, brain, ovary, oviduct, kidney, spleen, liver, lung, pancreas, gizzard, proventriculus, intestine, and retina and even in eggs (Jacobs and Melton, 1966; Kaneto et al., 1997). Tissue cysts of *T. gondii* were found in breast and leg muscles, heart, brain, liver and stomach of experimentally infected domestic ducks (Bártová et al., 2004).

For testing of *T. gondii* antibodies in domestic birds, different methods were used in several countries. Indirect fluorescence antibody test (IFAT) was used to test chickens from India (Sreekumar et al., 2001) and Costa Rica (Abrahams-Sandi and Vargas-Brenes, 2005). Modified agglutination test (MAT) was used to test chickens from several countries, e.g. India (Sreekumar et al., 2003), Israel (Dubey et al. 2004b), Mexico (Dubey et al. 2004a) and Chile (Dubey et al., 2006) and to test chickens and ducks from Egypt (Dubey et al., 2003).

In the Czech Republic, the last serologic study in domestic birds was made by the Sabin-Feldman reaction during the years 1981-1990 (Literák and Hejlíček, 1993). This work is aiming to estimate recent seroprevalence in domestic birds by the IFAT. This method was used in the present prevalence study in several groups of domestic and wildlife animals in the Czech Republic, in order to compare results obtained with the same method.

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## Materials and Methods

**Blood samples.** During the years 2003 and 2004, blood samples of 1108 domestic birds from the Czech Republic were examined for the presence of *T. gondii* antibodies. The birds were slaughtered at 14 slaughterhouses and blood was taken by veterinary inspectors during the time of avian influenza surveillance. Birds came from 57 different large-scale farms and one slaughter house of 13 districts (Figure 1) to obtain representative sampling from the whole Czech Republic, taking into account the size and the importance of farms. The blood samples were sent to the State Veterinary Institute in Prague. The blood was centrifuged and sera were stored at –18 °C until assayed. Sera of 178 geese, 360 ducks and 570 gallinaceous birds (293 broilers, 217 hens and 60 turkeys) were tested by the IFAT.

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**Indirect fluorescence antibody test.** Antibodies to *T. gondii* were detected in the sera by the IFAT using a commercially available antigen Sevatest Toxoplasma Antigen IFR (Sevac, Prague, the Czech Republic), anti-chicken IgG conjugate (Sigma Aldrich) and anti-duck IgG conjugate (KPL, USA). The sera were diluted in a two-fold series starting at 1:40 as a basic dilution; a titre ≥ 40 was considered positive. The other dilutions used were 1:320 and 1:2560. Sera of naturally infected domestic geese, Coscoroba swan (*Coscoroba coscoroba*) and Paradise shelduck (*Tadorna variegata*) and sera from experimentally infected chickens, turkeys and ducks, positive in both the Latex agglutination test (LAT) and the IFAT, served as the positive control (Table 1). Sera negative in both the LAT and the IFAT served as the negative controls.

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## Results



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In water birds (geese and ducks) a prevalence of 24 % (129 positive of 538) was found. Antibodies to *T. gondii* were detected in 43 % (77/178) of geese with titres 40, 320 and 2560 in 40, 29 and eight geese, respectively. Geese came from four different farms with seroprevalences ranging from 19 % to 64 %. In ducks, 14 % (52/360) prevalence was found with titres of 40 and 320 in 46 and 6 ducks, respectively. Ducks came from nine different farms with seroprevalences ranging from 3 % to 29 % (Table 2). In gallinaceous birds, only one of 293 broiler chickens originating from 29 farms was positive with a basic titre of 40; sera of 217 hens coming from 11 farms and 60 turkeys located on 6 farms, were negative (Table 3). Data did not allow statistical evaluation because of the differences between districts (different number of bird sampled, different number and size of farms examined).

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**Discussion**

The seroprevalence of *T. gondii* in domestic birds varies within countries due to the methods used for testing, the number of examined animals, type and hygiene of animal breeding, etc. In our study, the gallinaceous birds came from large-scale farms with restriction of cat access. This can explain the very low seroprevalence found in this group of birds compared to data obtained in other countries. Antibodies against *T. gondii* were detected by the MAT in 40 % of chickens from Egypt (Dubey et al., 2003) and in 47 % of free-range chickens from Israel (Dubey et al., 2004b). In Costa Rica (Abrahams-Sandi and Vargas-Brenes, 2005) and India (Sreekumar et al., 2001), a prevalence of 41 % and 22 % were found applying IFAT for chicken sera. There are no data available from the European countries, so this is the first seroprevalence study in domestic birds by the IFAT in Europe. In contrast to 40 % prevalence

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in chickens and 16 % in ducks from Egypt (Dubey et al., 2003), we found higher prevalence in ducks compared to chickens.

Our results are similar to those obtained in the Czech Republic in the past, even though a different method was used. Literák and Hejlíček (1993) made a long-term serological study (1981-1990) in domestic birds coming from South Bohemian district by the SFR. They found a higher prevalence in geese (16 %) compared to ducks (2 %). In chickens a prvevalence of 5 % was noticed in birds from a small backyard, whereas only one positive chicken was obtained from large-scale commercial farms.

The higher prevalence of antibodies in water birds (geese and ducks) is a good indicator of environmental contamination with oocysts in the sampled areas. Different prevalence was found in geese and ducks. For example, prevalence in geese was 55% (22/40) and 0% (0/39) in ducks coming from the same farm (D) from the OL district. Different prevalence in geese and ducks can be explained by differences in age of the tested animals, the type of breeding and their feeding habits. In the case of ducks, broilers (young birds) produced in large halls were slaughtered and tested. Since geese are not frequently slaughtered, the blood for the surveillance was obtained from breeding stock. These geese were adult with free access to natural water sources (ponds, lakes) and thus with a greater chance to be infected.

Based on the obtained data ducks and geese can represent a higher risk of infection for consumers, because of a higher prevalence found in this group of birds.

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**Table 1.** *Characteristic of positive and negative controls included in IFAT.*

| Controls | Characteristic of controls | n | LAT results | IFAT results |
|---|---|---|---|---|
| Chicken (negative) | Chicken serum, SPF breed, 2 weeks old | 2 | - | - |
| | Chicken serum before *T. gondii* experimental infection (Sedlak et al 2000) | 5 | - | - |
| Chicken (positive) | Chicken serum 28 d.p.i. with *T. gondii* oocystes (Sedlak et al., 2000) | 5 | + | + |
| Turkey ( negative) | Turkey serum before *T. gondii* experimental infection (Sedlak et al., 2000) | 4 | - | - |
| Turkey (positive) | Turkey serum 21 d.p.i. with *T. gondii* oocystes (Sedlak et al., 2000) | 4 | + | + |
| Duck (negative) | Duck serum before *T. gondii*experimental infection (Bartova et al., 2004) | 4 | - | - |
| Duck (positive) | Duck serum 28 d.p.i. with *T. gondii* oocystes (Bartova et al., 2004) | 4 | + | + |
| Geese (negative) | Geese serum | 4 | - | - |
| Geese (positive) | Sera of naturally infected domestic geese, Coscoroba swan (*Coscoroba coscoroba*) and Paradise shelduck (*Tadorna variegata*) | 4, 1, 1 | + | + |

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**Table 2.** *Results of T. gondii seroprevalence study in ducks and geese*

| Species | District | Farm | No. of serum samples | No. of positive samples (%) | Titres | | |
|---|---|---|---|---|---|---|---|
| | | | | | 40 | 320 | 2560 |
| **Goose** | South Bohemian | A | 73 | 14 (19%) | 14 | 2 | – |
| | | B | 39 | 25 (64%) | 25 | 23 | 5 |
| | | C | 26 | 16 (62%) | 16 | 2 | 1 |
| | Olomouc | D | 40 | 22 (55%) | 22 | 10 | 2 |
| *Total* | | | *178* | *77 (43%)* | *77* | *37* | *8* |
| **Duck** | South Bohemian | E | 40 | 3 (8%) | 3 | – | – |
| | | F | 39 | 8 (21%) | 8 | – | – |
| | | G | 40 | 1 (3%) | 1 | – | – |
| | | H | 40 | 0 | 0 | – | – |
| | | I | 33 | 5 (15%) | 5 | 2 | – |
| | | J* | 39 | 8 (21%) | 8 | – | – |
| | Hradec Králové | K | 48 | 15 (31%) | 15 | 2 | – |
| | Pardubice | L | 42 | 12 (29%) | 12 | 2 | – |
| | Olomouc | D | 39 | 0 | 0 | – | – |
| *Total* | | | *360* | *52 (14%)* | *52* | *6* | – |

* J = slaughter house

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**Table 3.** *Results of T. gondii seroprevalence study in gallinaceous birds*

| Species | District | No. of farms | No. of serum samples | No. of positive samples (%) |
|---|---|---|---|---|
| **Broiler** | South Bohemian | 3 | 30 | – |
| | Plzeň | 2 | 20 | – |
| | Ústí nad Labem | 3 | 30 | 1* |
| | Hradec Králové | 3 | 30 | – |
| | Central Bohemian | 5 | 50 | – |
| | Pardubice | 2 | 21 | – |
| | Vysočina | 2 | 21 | – |
| | South Moravian | 7 | 71 | – |
| | Moravian-Silesian | 1 | 10 | – |
| | Zlín | 1 | 10 | – |
| *Total* | | *29* | *263* | *1 (0.4%)* |
| **Hen** | South Bohemian | 1 | 11 | – |
| | Ústí nad Labem | 1 | 30 | – |
| | Hradec Králové | 1 | 50 | – |
| | Central Bohemian | 2 | 50 | – |
| | Pardubice | 4 | 56 | – |
| | South Moravian | 2 | 20 | – |
| *Total* | | *11* | *217* | – |
| **Turkey** | Plzeň | 2 | 20 | – |
| | Karlovy Vary | 1 | 10 | – |
| | Liberec | 1 | 10 | – |
| | Vysočina | 1 | 10 | – |
| | Moravian-Silesian | 1 | 10 | – |
| *Total* | | *6* | *60* | – |

* positive sample with titre 40

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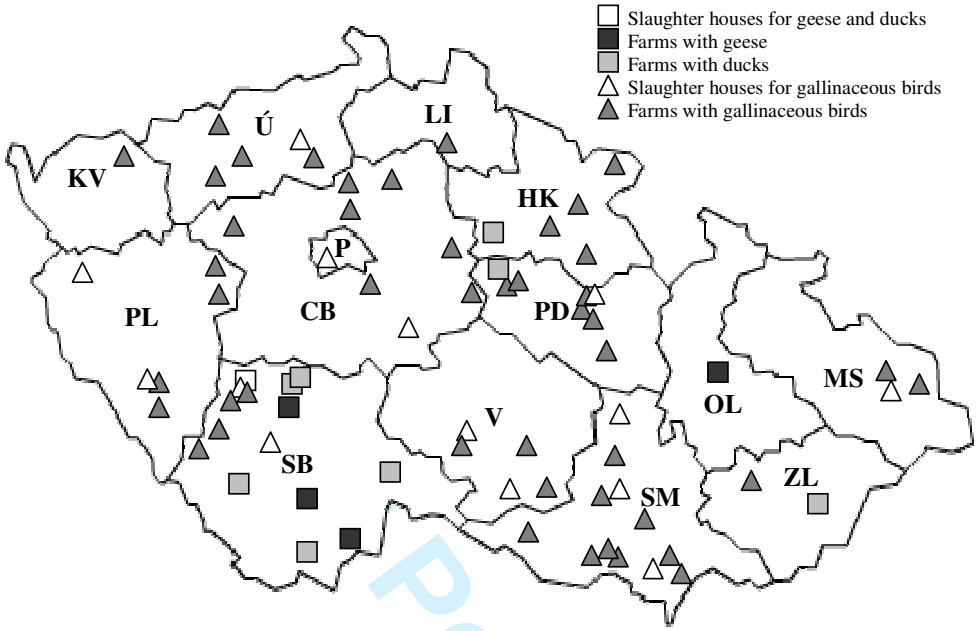
**Figure 1.** *Map of the Czech Republic showing the sampled areas.*




Districts: SB – South Bohemian; PL – Plzeň; KV – Karlovy Vary; Ú – Ústí nad Labem; LI – Liberec; CB – Central Bohemian; P – Prague; HK – Hradec Králové; PD – Pardubice; V – Vysočina; SM – South Moravian; OL – Olomouc; MS – Moravian-Silesian; ZL – Zlín.

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