Analysis of *Toxoplasma gondii* clonal type-specific antibody reactions in experimentally infected turkeys and chickens by peptide microarray

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**Background**

- Toxoplasmosis occurs in humans and animals worldwide.
- *T. gondii* may cause severe clinical disease. There are indications of correlations between clinical manifestations and the clonal type of *T. gondii* humans are infected with.
- *Toxoplasma gondii* has a clonal population structure. In North America and Europe three clonal types (I, II, III) dominate with clonal type II being the most prevalent one.
- There is limited information about the ability of turkeys and chickens to develop clonal type specific antibody response.

**Aims of this study**

- Development of a serotyping test based on peptide microarray using reference sera from experimentally infected turkeys and chickens.
- Analyse the ability of turkeys and chickens to develop a *T. gondii* clonal type-specific antibody response (IgY).

**Material and Methods**

**Peptides**

- 101 peptides representing sequences of *T. gondii* clonal type specific antigenic sites were analysed in peptide microarray.
- The peptide panel consisted of peptides presenting single clonal type specific polymorphisms (I [n = 27], II [n = 29] and III [n = 21]) as well as common polymorphisms for two clonal types simultaneously (II/III [n = 6], III/I/III [n = 12], II/III/III [n = 6]).

**Reference sera**

- Reference sera: 120 sera from experimentally infected chickens and turkeys inoculated with different doses of *T. gondii* tachyzoites (104, 103 and 102) collected from isolates representative for types I (RH), II (ME49) or III (NED) and uninfected controls. The sera were collected at 0, 2, 5, 7 and 9 weeks post infection (wpi).

**Results**

- All experimentally infected turkeys and chickens seroconverted in the TgSAG1-ELISA (Maksimov et al., 2011; Schaeres et al., 2016) (Fig. 1).
- After screening the peptides with reference sera from chickens and turkeys, 30 and 37 peptides were identified that showed type specific reactions with sera collected 2, 5, 7 and 9 wpi as determined by Receiver Operating Characteristics (ROC) analysis. These peptides originated from eight *T. gondii* antigens (ROP1, GRA1, GRA3, GRA5, GRA6, GRA7, SAG2A, SAG3). In addition, turkey sera recognized peptides derived from the SAG1 *T. gondii* antigen.
- With selected peptides it was possible to determine until 7 wpi the *T. gondii* clonal type, by which groups of chickens and turkeys had been infected (Fig. 2). Differences in recognized peptide patterns were observed between individual animals as well as between different time points post infection.
- Most of the animals recognized peptide patterns, by which at least the infection with one out of three *T. gondii* clonal types could be excluded. At 9 wpi, most of the experimentally infected chickens (78% [14/18]) and turkeys (78% [14/18]) did no longer react with the selected peptides (Fig. 2 B, C and D).

**Summary**

- Experimentally infected chickens and turkeys are able to develop a clonal type specific IgY antibody response.
- Serotyping of the infection in individual chickens or turkeys was only possible when the whole peptide panel was applied.
- Serotyping using the selected peptides was only possible in a certain time period post infection.

**References**

1. Maksimov et al., 2011, Veterinary Parasitology 182 (2011) 140–149