P63

Toxoplasma gondii strain and dose effect on body weight, serum antibodies response and systemic distribution in intraperitoneally infected domestic turkeys

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Domestic turkeys represent a very important food source for humans and they also serve as an important intermediate host of the zoonotic parasite *Toxoplasma gondii*. In the present study, 24 four-week old, female domestic turkeys were separated into four treatment groups. Each of the groups was intraperitoneally infected with either a virulent or avirulent strain at one of two doses ($1X10^5$ or $1X10^8$). In addition, 10 birds were injected with sterile PBS only and served as a negative control group. The study aimed to investigate the parasite tissue tropism, seroconversion time line, weight gain and feed conversion in relation to the parasite dose and strain. Birds were monitored twice daily throughout the experiment. Clinical signs, weight, and mortality were recorded. Serum samples were collected every week starting the day before the infection until day 42 of the experiment and then every other week until 95days post infection. Sera were tested with the modified agglutination test to detect *T. gondii* antibodies. At termination of the study, multiple tissue samples were collected and tested for *T. gondii* by qPCR and light microscopy using H&E staining. Clinical signs and weight gain of the birds were related to the dose and strain of *T. gondii* used to infect the birds. Birds infected with $1X10^8$ tachyzoites of either strain showed the most severe clinical signs and the highest and earliest antibody conversion. The group infected with the higher dose of the virulent strain showed lower weight gain compared to other groups. Brain tissues were the most commonly infected tissue as determined by qPCR. qPCR will be compared to histopathology and results will be discussed. These results demonstrate that *T. gondii* is an important disease of domestic turkeys, especially birds that are raised outdoors in organic or backyard propagation facilities.

P64

Effect of meat processing on viability of Toxoplasma gondii: Towards replacement of mouse bioassay by in vitro testing

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Felines are the definitive hosts of *T. gondii* and primary infection results in fecal shedding of infectious oocysts. Infected intermediate hosts will develop tissue cysts, which are infective to both cats and intermediate hosts. Meat containing viable tissue cysts is considered one of the main sources of human infection. In contrast to fresh meat, raw meat products usually undergo processing, including salting and mixing in additives such as acetate and lactate, which affects the viability of *T. gondii*. However, the experiments currently described in literature, are not always performed in line with the processing methods applied in industry. Therefore we aimed to study the effect of salting and additives according to the recipes used by commercial producers. Mouse or cat bioassay is the gold standard to demonstrate the presence of viable *T. gondii*. However, it is costly, time consuming and for ethical reasons not preferred for large-scale studies. Therefore, our second aim was to develop an alternative for mouse bioassay that can be used to determine the effect of processing on the viability of *T. gondii* tissue cysts. We focused on a tissue culture method to determine the parasite's ability to multiply, and a PMA-based assay to selectively detect DNA from live cells. Results with the PMA-based method were inconsistent and did not sufficiently discriminate between live and dead parasites. The tissue culture method showed promising results, but further optimization is needed before it can replace or reduce the number of mouse bioassays needed. Small scale experiments with minced meat incubated for 20h with low concentrations of salt, lactate and acetate showed a large but incomplete reduction of the number of infected mice. In future, in vitro methods are needed to allow more extensive testing of product-specific

processing methods, thereby providing a better indication of the risk of *T. gondii* infection for consumers.

P65

Bumped kinase inhibitor BKI-1748: Studies on in vitro efficacy, safety and in vivo effects in pregnant mice infected with the highly virulent N. caninum isolate Nc-Spain7

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Neospora caninum is one of the major causes of abortion in cattle and thus exerts considerable economic impact. Bumped kinase inhibitors (BKIs), which target calcium dependent protein kinase 1 (CDPK1), represent a promising class of compounds that are active against numerous apicomplexans species. *In vitro*, BKI-1748 added concomitantly to infection of human foreskin fibroblast (HFF) monolayers with *N. caninum* tachyzoites impaired parasite proliferation with an IC50 of 68 nM. When BKI-1748 (2.5 µM) was added to already infected HFF monolayers, treatment efficacy was much lower, but resulted in the formation of large multinucleated schizont-like structures that contain newly formed zoites, which were unable to separate and form tachyzoites. These multinucleated complexes remained largely viable for several weeks. As *N. caninum* tachyzoites are vertically transmitted, it is essential that BKI-treatment does not interfere with embryonic development and pregnancy outcome. BKI-1748 was therefore assessed for potential interference in embryo development using a Zebrafish (*Danio rerio*) model. Analysis for lethality/malformations demonstrated no embryonic growth interference within a time frame of 96 hours with BKI-1748 treatments at concentrations up to 10 µM, while embryo development impairment was evident at 20 µM and above. In pregnant mice treated by oral gavage with either 50, 20 or 5 mg/kg BKI-1748 emulsified in corn oil during days 9-13 of pregnancy, the highest concentration resulted in severe loss of offspring, while 20 and 5 mg/kg did not impair neither fertility nor pup survival. Based on these results, we are currently performing a BKI-1748 treatment study in pregnant mice that are infected with 10^5 *N. caninum* Nc-Spain7 tachyzoites at day 7 of pregnancy, and are then treated with 20 or 5 mg/kg/day for 5 days, starting 2 days post infection. We will present data on clinical signs, fertility, litter size, neonatal and postnatal mortality.

P66

Treatment with bumped kinase inhibitor 1294 alters Neospora caninum protein expression, which lead to a diversified humoral immune response in N. caninum infected mice

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Bumped kinase inhibitors such as BKI-1294 target apicomplexan calcium-dependent protein kinase 1 (CDPK1), a protein kinase that is involved in host cell invasion and egress. CDPK1 is encoded by apicoplast DNA, and thus an interesting drug target. In vitro, the drug inhibits host cell invasion, and induces the formation of multinucleated complexes (MNCs) by interfering in the process of completing cytokinesis. However, the drug does not act parasiticidal. MNCs are composed of several parasite nuclei and newly formed zoites with an inner membrane complex but lacking a SAG1-positive outer membrane. They form a schizont-like organism that remains trapped within the host cell, but intact tachyzoites with an outer SAG1-positive membrane are formed readily and resume proliferation once the drug is removed. In spite of the lack of parasiticidal activity, BKI-1294 dramatically reduced cerebral infection and vertical transmission in mice. IFA and RT-PCR showed that these MNCs exhibit a dysregulated antigen expression pattern compared to non-treated tachyzoites. Thus, we hypothesize that BKI-1294 treatment induces the expression of other antigenic parasite proteins that could potentiate the immune response. We developed a procedure for enrichment of MNCs and performed comparative