Experimental *Neospora caninum* infection in domestic bird's embryonated eggs

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Abstract To date, there are no reports regarding comparison between different bird species in *Neospora. caninum* infection. In the present study 70 embryonated eggs from quail, partridge, broiler and egg laying chickens were divided into 7 groups equally. Six groups in each species were inoculated with different dilutions (10 , 10^2 , 10^3 , 10^4 , 10^5 , and 10^6) of tachyzoites/embryonated egg in the chorioallantoic membrane and the seventh group was considered as control. The mortality rates and clinical signs were studied. All the egg laying chickens and some of the broiler chickens and quails showed neurologic signs like. The results revealed that the mortality rate was dose dependent in broiler chicken embryonated eggs. But mortality rate was dose independent in egg laying chickens and quail. Partridge revealed 100 % mortality rate in all doses. The LD_{50} in broiler chicken embryonated was $10^{2.3}$. In conclusion, LD_{50} in the broiler chickens is the lowest between different animal models which shows that the broiler chicken embryonated egg is the best animal model for experimental inducing of neosporosis. Partridge is the most susceptible bird to *N. caninum* infection. These results reinforce that there is genetic susceptibility to *N. caninum* in chickens like mice and provide new insights to reach an inexpensive and available animal model for *N. caninum* infection.

Keywords *Neospora caninum* · Broiler chicken · Egg-laying chicken · Quail · Partridge

Abbreviations

N. caninum *Neospora caninum*
CA Chorioallantoic

Introduction

Neospora caninum is a protozoan parasite of livestock and companion animals, which can cause a serious disease in dogs and cattle (Dubey 2003). Many aspects of the life cycle of *N. caninum* are unknown. The dog and the coyote (*Canis latrans*) are the only definitive hosts known that can excrete the environmentally resistant oocysts (Gondim et al. 2004; McAllister et al. 1998). A large spectrum of domestic and wild animal considered as intermediate hosts of this parasite (sheep, goats, horses and deers). It is showed that embryonated chicken egg can be used as an animal model for experimental infection (Mansourian et al. 2009; Khodakaram-Tafti et al. 2012) and this animal model can be used for evaluating the *N. cacinum* tachyzoites (Namavari et al. 2011). It was showed that the *N. cacinum* tachyzoite virulence can be affected by the host cell (Khordadmehr et al. 2012), but these information are insufficient. Since there is lack of information about avian species as intermediate hosts of *N. caninum*, this study conducted to evaluate the infection by the parasite in two species of the chickens, partridge and quail embryonated egg.

Materials and methods

Parasites

In the present study, the *N. caninum* NC-1 isolate (Dubey et al. 1988) was used for experimental infections. According

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to the Mansourian et al. (2009) method, the parasites were cultured in Vero cell line. For preparation of the necessary inoculum doses, parasites were pelleted by centrifugation, concentration set with a Neubauer chamber, and diluted in RPMI medium.

Animals and experimental infections

The necessary inoculum doses (10 , 10^2 , 10^3 , 10^4 , 10^5 , and 10^6) were prepared and an experimental infection with *N. caninum* NC-1 isolate was conducted in quail, partridge, broiler and egg laying chickens. In each group, seventy quail, partridge, Lohman broiler and egg laying chickens randomly divided into seven equal groups. All eggs were maintained in an incubator with a controlled temperature, humidity and rotation until 8 days of incubation. At this time six groups were inoculated with different dilutions (10 , 10^2 , 10^3 , 10^4 , 10^5 , and 10^6) of tachyzoites/embryonated egg. The 7th group was considered the control and was inoculated with a sterile cell culture medium. Inoculation was performed in the chorioallantoic (CA) liquid of the embryonated eggs (Warren and Russ 1948). The embryos were observed twice daily until hatching, and any mortality was studied. Live chickens after hatch were also observed daily and any clinical signs recorded.

Statistical analysis

The LD₅₀ was calculated by the Reed and Muench method (Pizzi 1950).

Results

Mortality rate of experimental infection

As are shown in Table 1, the inoculated experiment broiler chicken groups showed mortality between 18 and 21 days of incubation, but two groups (with 10^5 and 10^6 tachyzoites) showed mortality about 4 days after inoculation. In the group with 10^6 tachyzoites, six embryos 5 days post-infection (P.I.), three, 6 days P.I., and one, on the 7th day P.I. died. In the group with 10^5 tachyzoites, one embryo 5 days P.I., three, 6 days P.I., and six, on the 7th, 8th and 9th days P.I. (two chickens per day) died. In the group with 10^4 tachyzoites, five embryos 9 days P.I., two, 10 days P.I., and one, on the 11th day P.I. died. In the group with 10^3 tachyzoites, three embryos 10 days P.I. and two, 11 days P.I. died. In the group with 10^2 tachyzoites, two embryos 11 days P.I. and one, 12 days P.I. died. In the group with 10 tachyzoites, just two embryos died 12 days P.I. In the groups with 10, 10^2 , 10^3 and 10^4 tachyzoites there were 8, 7, 5 and 2 hatchings, respectively.

The mortality rate of infected egg laying chickens is summarized in Table 2. In the group with 10, 10^2 , 10^3 , 10^4 , 10^5 , 10^6 tachyzoites, three, six, six, five, seven and nine embryos died, respectively.

The mortality rate of infected quails is summarized in Table 3. In the group with 10, 10^2 , 10^3 , 10^4 , 10^5 , 10^6 tachyzoites, six, two, seven, four, seven and ten embryos died, respectively.

Partridge revealed 100 % mortality rate in all doses.

Clinical signs after hatching

After hatching, all of the egg-laying, only one surviving Lohman broiler chickens (with 10^4 tachyzoites inoculum dose) and one quail chicken from the group with 10^4 tachyzoites showed neurologic signs. Lack of coordination, pedalling movements, hind limb paralysis, and circular walking patterns were observed in this chicken. Three broiler chickens (one chicken with 10^4 tachyzoites and two with 10^5 tachyzoites) showed arthritis in the feet joints after two weeks of inoculation. There was swelling, redness and ulceration in the surfaces of those joints.

Partridge revealed 100 % mortality rate in all doses.

LD₅₀ calculation

According to the mortality rates that were summarized in Table 1 and the calculations done using the Reed and Muench method (Pizzi 1950), LD₅₀ of broiler chickens was shown to be between 10^2 and 10^3 doses and about $10^{2.3}$ tachyzoites were administered in the CA liquid of the embryonated eggs in 0.1 ml volume per egg. In other birds, it was not possible to calculate the LD₅₀ due to the pattern of mortality rates. In egg-laying chickens and quail, the mortality rate was dose independent and in Partridge mortality rate was 100 %.

Discussion

Embryonated eggs have been used as a model for protozoan isolation, propagation, and parasite biology studies (Wunderlin et al. 1997; Que et al. 2004; Furuta et al. 2007). To date, there is no report about experimental *N. caninum* infection in different species embryonated eggs and comparison the differences between them.

In the present study, neurologic signs like ataxia, lack of coordination, pedalling movements or hind limb paralysis and circular walking patterns in just one broiler chicken from the group with 10^4 tachyzoites and one quail from the group with 10^4 tachyzoites was seen, whereas in the Furuta et al. (2007) study, only nine egg-laying chickens that hatched their shells (from groups with 10^5 and 10^6) present clinical alterations due to infection.

Table 1 The mortality rate and clinical signs of broiler chickens. ((–) negative; (+) positive)

Inoculum dose	Number of hatched broiler chicken	Mortality rate until 60 days after hatch	Neurologic signs	Arthritis
10	8	1	–	0
10 ²	7	1	–	0
10 ³	5	2	–	0
10 ⁴	2	2	1	1
10 ⁵	0	0	–	2
10 ⁶	0	0	–	0
Control	10	0	0	0

Table 2 The mortality rate and clinical signs of egg-laying chickens. ((–) negative; (+) positive)

Inoculum dose	Number of hatched egg-laying chicken	Mortality rate until 60 days after hatch	Neurologic signs	Arthritis
10	7	1	+	–
10 ²	4	0	+	–
10 ³	4	1	+	–
10 ⁴	5	2	+	–
10 ⁵	3	2	+	–
10 ⁶	1	0	+	–
Control	10	0	–	–

The mortality rate in broiler chicken was completely dependent on the inoculum dose as well which is completely different to the egg-laying chickens and quail. In the broiler chicken, groups with 10, 10², 10³ and 10⁴ tachyzoites; there were eight, seven, five and two hatchings, respectively, but there were no hatchings in groups with 10⁵ and 10⁶ tachyzoites.

The mortality rate of infected egg laying chickens was dose independent. Furuta et al. (2007) showed, with the inoculation of different doses of 10³ to 10⁴ *N. caninum* tachyzoites in egg-laying chicken embryonated eggs, showed that mortality rate was not related to inoculum dose, since all groups presented similar death patterns recorded after 7 days of infection.

Like in egg laying chickens, quail mortality rate was dose independent. The mortality rate of infected quail is summarized in Table 3. In the group with 10, 10², 10³, 10⁴, 10⁵, 10⁶ tachyzoites, six, two, seven, four, seven and ten embryos died, respectively. Therefore, both egg laying chickens and quails can not be used as a good animal models.

Partridge revealed 100 % mortality rate in all doses. But there is no other data which can be compared with our investigation. These results showed that partridge is the most susceptible bird to *N. caninum* infection and for

Table 3 The mortality rate and clinical signs of quails. ((–) negative; (+) positive)

Inoculum dose	Number of hatched quail	Mortality rate until 60 days after hatch	Neurologic signs	Arthritis
10	4	1	–	–
10 ²	8	0	–	–
10 ³	3	1	–	–
10 ⁴	6	1	1	–
10 ⁵	3	1	–	–
10 ⁶	0	–	–	–
Control	10	0	–	–

further investigation these birds should be infected with less than 10 tachyzoites per egg.

In seeking to develop a laboratory animal model for neosporosis, scientists have found that inbred mouse strains vary in their susceptibility. Most mouse strains are resistant, but BALB/c and C57BL/6 mice develop clinical neosporosis and meningoencephalitis after inoculation with the parasite (Lindsay et al. 1995; Long et al. 1998). Genetic susceptibility was also shown in bovine species (Pan et al. 2004).

In conclusion, our findings imply that genetic susceptibility should be propounded in different breeds of birds and broiler chicken embryonated egg is a completely suitable animal model for acute neosporosis. Because we can conduct the future investigations based on this LD₅₀ and it has the lowest LD₅₀ between animal models for experimental neosporosis. On the other hand, LD₅₀ in the broiler chickens is the lowest between different animal models which shows that the broiler chicken embryonated egg is. Partridge is the most susceptible bird to *N. caninum* infection. These results reinforce that there is genetic susceptibility to *N. caninum* in chickens like mice and provide new insights to reach an inexpensive and available animal model for *N. caninum* infection.

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