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Experimental toxoplasmosis in red-legged partridges (*Alectoris rufa*) fed *Toxoplasma gondii* oocysts

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Abstract

Thirty red-legged partridges (*Alectoris rufa*), 5-month-old, were orally inoculated with oocysts of the OV-51/95 strain of *Toxoplasma gondii*. Birds were distributed into five groups and received, respectively, 10 (group A, 4 birds), 50 (group B, 14 birds), 10^2 (group C, 4 birds), 10^3 (group D, 4 birds) and 10^4 (group E, 4 birds) oocysts. One partridge from group B and one from group E died suddenly of acute toxoplasmosis at 7 day after inoculation (DAI) with demonstrable *T. gondii* in several tissues. The rest of birds remained clinically normal until killed at 44, 58, 65, 72, 79 or 100 DAI. Brain, heart, liver and skeletal muscle from these partridges were bioassayed individually in mice; *T. gondii* was demonstrated in all these tissues, except in heart of three birds inoculated, respectively, with 10, 50 and 10^2 oocysts. Lesions were not seen in histologic sections of tissues from surviving partridges. These results suggest that red-legged partridges are resistant to clinical toxoplasmosis.

Keywords: Red-legged partridges; *Alectoris rufa*; *Toxoplasma gondii*; Toxoplasmosis; Oocyst

1. Introduction

Toxoplasma gondii is a protozoan that has been reported in many species of warm-blooded animals, including avian species. Felids are the only hosts that can spread oocysts in the environment (Dubey and Beattie, 1988). Birds can act as intermediate hosts of *T. gondii*. Natural toxoplasmosis has been described in domestic and wild birds, but clinical cases and outbreaks are not frequent, because infection often occurs subclinically or without specific symptoms in birds (Dubey, 2002). In wild gallinaceous birds, spontaneous fatal toxoplasmosis has been described in partridges (*Perdix perdix*) by Pokorný (1955), in wild turkeys (*Meleagridis gallopavo*) by Howerth and Rodenroth (1985) and Quist et al. (1995), and in Erckel's francolin (*Francolinus erckelii*) by Work et al. (2002). However, there are several reports of isolation of viable *T. gondii* from tissues of partridges, pheasants (*Phasianus colchicus*) and turkeys (Literák et al., 1992; Lindsay et al., 1994) captured in the wild without clinical signs. Experimental *T. gondii* infec-

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tions have been reported in several wild galliformes (Simitch et al., 1965; Dubey et al., 1993a,b, 1994a,b, 1995; Sedlák et al., 2000).

In Spain, the red-legged partridge (*Alectoris rufa*) is the most frequently raised game bird, and its meat constitutes the base of a large number of regional gastronomic dishes. Actually, there are no references on experimental infection in red-legged partridge with *T. gondii* or natural toxoplasmosis in this bird species.

The objective of the present report was to study the susceptibility of red-legged partridges to oral infection with different doses of *T. gondii* oocysts.

2. Materials and methods

2.1. Experimental infection of partridges with *T. gondii*

Thirty red-legged partridges (23 females and 7 males), 5-month-old, weighing 244–478 g, reared in battery, were used in the present experiment. Partridges were hatched from eggs set at the same time, and had no antibodies to *T. gondii* by the modified agglutination test (MAT) as described by Dubey and Desmonts (1987). The MAT was performed using a commercial test kit (''Antigène ToxoAD'' from bioMerièux, Marcy I'Etoile, France).

Partridges were distributed into four groups (A, C–E) of 4 birds each, and one group of 14 birds (group B). Birds were orally inoculated with oocysts of the OV-51/95 ovine strain of *T. gondii*; this strain was isolated from an ovine abortion, and it is of low virulence to mice, but produces many tissue cysts in their brains.

Oocysts were obtained from the faeces of a cat that was fed *T. gondii* tissue cysts of the OV-51/95 strain (Dubey et al., 1972). After collecting (Dubey, 1995), oocysts were sporulated in 2% sulfuric acid at room temperature, and then stored for a week at 4 °C until use in the experiment. Before inoculation, the oocyst suspension was neutralized with 3.3% sodium hydroxide (Dubey and Beattie, 1988).

Inocula were obtained by visual counting of sporulated oocyst in a Neubauer–Levy chamber and adjusting suspensions by dilution in physiological saline so that the desired dose was obtained. A volume of 0.5 ml of oocyst suspension was deposited directly into the crop of partridges by means of a canula; birds were each inoculated with 10 oocysts (group A), 50 (group B), 10^2 (group C), 10^3 (group D) and 10^4 oocysts (group E).

Inoculated birds were maintained in separate sterilized wire cages, and their bedding and excreta were collected for 10 days after inoculation (DAI) and incinerated to kill oocysts that might have passed unexcysted in faeces (Dubey and Frenkel, 1973). Partridges had access to poultry ration and water ad libitum without any anticoccidial drugs throughout the experimental phase.

2.2. Clinical examination

Red-legged partridges were observed at least twice daily throughout the experiment for clinical symptoms and mortality.

2.3. Necropsy and histological examination

Partridges that died or were sacrified during the experiment were necropsied. Among surviving birds, one from each group was killed at 44, 58, 65 (only birds from groups A, B, C and D) and 72 DAI. From 79 DAI to the end of the experiment, two red-legged partridges from group B were killed at weekly intervals. The last partridges were killed 100 DAI.

For histopathologic examination, portions of the cerebrum, cerebellum, eye, heart, pectoral and semitendinosus muscles, tongue, trachea, lung, esophagus, proventriculus, gizzard, small and large intestines, liver, spleen, kidneys, pancreas, gonads and bursa of Fabricius were taken immediately and fixed in 10% neutral buffered formalin. Paraffin-embedded sections were cut at 5 μm thickness, stained with hematoxylin and eosin (H–E), and examined microscopically. Ten non-serial sections from each tissue sample were examined.

2.4. Bioassay

Tissue samples from all partridges were bioassayed in mice for *T. gondii* (Dubey and Beattie, 1988). The bioassay was carried out the same day of death or sacrifice.

Before bioassay, *T. gondii* infection in partridges was confirmed by preliminary direct microscopic