The emergence of infectious diseases has become an important threat to biodiversity. In recent years, ranaviral infections have caused high mortality in amphibians and have been linked to amphibian population declines in Spain (Price et al. 2014). Mass mortality of amphibians due to ranaviral infections have been reported in the Americas, Europe, and Asia (Green et al. 2002; Fox et al. 2006; Une et al. 2009; Geng et al. 2011). Amphibian ranaviruses were first found in Europe in late 1980s (Cunningham et al. 1996) in the southeast of England, where local declines of common frog (Rana temporaria) populations have been observed (Teacher et al. 2010). In Central Europe, Ranavirus infections have been reported mostly in anurans (Duffus et al. 2015). The first mass mortality event in the wild in continental Europe was in Croatia (Fijan et al 1991). Other events were posteriorly reported in the Netherlands for water frogs (Pelophylax spp.) and the common newt (Lissotriton vulgaris) (Kik et al. 2010). In Denmark (Ariel et al. 2009) and Switzerland (Stöhr et al. 2013a) ranaviral disease and associated mass mortality events in Pelophylax kl. esculentus (edible frog) occurred. Additionally there was an outbreak in France in 2012 in the Park Mercantour (Alpes-Maritimes) affecting Rana temporaria (Angot 2014; Miao et al. 2016). In Spain, Ranavirus infection was reported in the common midwife toad (Alytes obstetricans) and in the alpine newt (Ichthyosaura alpestris) in their native ranges (Balseiro et al. 2010). More recently, these two species, together with the common toads (Bufo spinosus), were reported being affected by this disease in the Picos de Europa National Park (Spanish Cantabric Chain) in 2008 (Balseiro et al. 2010).

During the year 2014, an introduced population of alpine newt (Ichthyosaura alpestris) was found in the northeastern part of the Iberian Peninsula (Montesquiu Castle Park, Catalonia) (42.137505°N, 2.227895°E, 830 m elev.). Sampling by Fibla et al (2015) captured 103 adult newts, estimating a population size of about 360 individual. The animals were apparently healthy and were deposited at the facilities of the Catalonian Reptile and Amphibian Rehabilitation Center (CRARC). In order to check the health status of these introduced newts, individuals were sampled immediately before they were housed in new and disinfected tanks. Blood smears were taken from a selection of ten randomized newts. One drop of blood collected from the ventral coccygeal vein was used to perform blood smears. Also from within this selected group, sterile dry swabs were used to obtain samples from the back and ventral skin of four individuals. The four swabs were tested for Batrachochytrium dendrobatidis, B. salamandrivorans and Ranavirus spp. using established PCR techniques. Batrachochytrium dendrobatidis and B. salamandrivorans standards were used in the PCRs and were obtained from An Martel (Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium). Screening of the samples for B. dendrobatidis was carried out using a real-time PCR (see Boyle et al. 2004). DNA preparation
was carried out with the Roche MagNA Pure 96 system using the MagNa Pure 96 DNA and Viral NA small volume kits according to the manufacturer’s instructions. The detection of *B. salamandrivorans* was carried out separately using real-time PCR as described by Blooi et al. (2013). The *Ranavirus* spp. detection from the swabs was carried out using a conventional PCR targeting the major capsid protein (MCP) gene as described by Mao et al. (1997) and Marschang et al. (1999). The primers used were the Mao et al. (1997) MCP 4 and 5, but MCP 5 was modified as indicated in Marschang et al. 1999.

We found that 4 of 4 newts screened for pathogens were positive for *Ranavirus* spp. infections and negative for both fungi. Two of the four positive newts were sacrificed by use of general anesthesia (percutaneous pentobarbital; Baier 2005) in order to collect tissue samples (skin and internal organs such as liver, intestine and kidneys). The tissues were prepared for histological examination and both the slides and the blood smears were analyzed in the laboratory at CRARC, in order to detect any cellular changes related with inflammation, infection or parasitism.

None of the tissues analyzed histologically showed any signs of disease, and no inclusion bodies were detected either in tissues or blood smears. The newts positive for *Ranavirus* spp. were specifically analyzed to detect inclusion bodies in their erythrocytes or leucocytes. All newts were free of any clinical signs of ranaviral disease (ulcerated skin, shedding of skin, hemorrhagic skin or abnormal behavior) even under histological analysis. The newts that were positive for *Ranavirus* were considered subclinically infected and consequently were not diseased. Related to this is the fact that different species of american amphibians have been described with subclinical infections and some of these species may serve as long term reservoirs for ranaviruses ( Brenes 2013; Brunner et al. 2015; Miller et al. 2015).

No mortality or morbidity has been noted in the remaining captured newts, which have been housed in an isolated aquarium since capture. In order to evaluate the sanitary risk of this situation, we collected skin swabs from two adults and two larvae specimens of the natterjack toad (*Epidalea calamita*) and the common midwife toad (* Alytes obstetricians*) living in the same pond from which the newts were removed. Samples from all of these animals were negative for all of the pathogens that were checked (*Ranavirus* spp. and *Batrachochytrium* spp.). However, the sample size is small, and the skin swabs might not be particularly sensitive (false negatives) for detecting *Ranavirus* infections, so more samples are needed in order to better understand the evolution of this disease in the infected area.

In conclusion, we report the first data on the occurrence of a *Ranavirus* in the northeastern region of Spain in a population of introduced newts. The introduction of pathogens or pathogen pollution is an important aspect of the illegal trade of exotic pets. This has been shown to play a role in the distribution of ranaviruses in various hosts both in the wild and in captive animals before (Picco and Collins 2008; Schloegel et al. 2009; Stöhr et al. 2013b; Kolby et al. 2014). Genetic studies (see Fibla et al. 2015) show that *Ranavirus*-positive newts had the same geographical origin as the newts affected by the outbreak in their natural area of distribution (Price et al. 2014). Thus, our study highlights the chance of a pathogen introduction caused by the introduction of alpine newts in a new area. As a consequence, the illegal trade and introductions could increase the spreading of this pathogen.

In our case, it is impossible to confirm if the newts found in Catalonia where caught, presumably illegally, in their native range, and then released in another place. Both commercially raised and wild caught newts are potential sources of diseases when they are released in nature. Implementation of quarantine procedures, including pathogen detection methods in introduced amphibians (with or without symptoms) captured in the wild, as well as surveillance guidelines with qualified diagnostic support, are strongly recommended (Gray et al. 2015). It is recommended that microscopical evaluation of biopsies and blood smears be supported by PCR techniques in order to detect silent carriers and unapparent infections. Despite the negative results in the native species of amphibians, the confirmed presence of asymptomatic carriers is enough to make special surveillance of the native amphibians inhabiting the affected area over a long period of time (months and even years). More research is needed in these animals to provide more information about what species of *Ranavirus*, detection of the pathogen in the inner organs and which degree of pathogenicity is expected in the native species living in this area.

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Literature cited


Stöhr, A.C., Blahak, S., Heckers, K.O., Wiechert, J., Behncke, H., Mathes, K., Günther, P., Zwart, P., Ball I., Rüschoff, B.,

