Harmonisation of regulations in the European Union and the European Economic Area, as of January 1, 2012, has led to an increase in the number of rescue dogs imported to Norway from Eastern European countries, in particular Romania. Today the only requirements for dogs entering Norway are rabies vaccination and prophylactic *Echinococcus multilocularis* treatment. The aim of this study was to investigate the antibody levels to rabies virus in vaccinated rescue dogs and to examine if the dogs had sufficient antibody response according to the recommended titre ≥0.5 IU/ml by the World Organisation for Animal Health (OIE). A significant proportion (53%, 95% CI (41% to 65%)) of imported rescue dogs from Eastern Europe were found to have inadequate titres after rabies vaccination. Moreover, 41 per cent of the dogs had antibody levels below or equal to 0.2 IU/ml, and among these, 14 dogs had titres ≤0.1 IU/ml, which is considered negative in the fluorescent antibody virus neutralisation assay. This study indicates that the present regulation increases the risk of introducing rabies from member states where rabies is still prevalent to countries considered free from rabies.

**Introduction**

The transport of companion animals across borders provides a real threat for the spread and introduction of various infectious pathogens, including rabies virus. The European Union (EU) implemented a harmonised pet movement policy for non-commercial movement of dogs, cats and ferrets under EU regulation 998/2003 of the European Community (EU 2003). As member of the European Economic Area, Norway also follows this regulation. It states that (i) all animals should be identified by tattoo and/or microchip, (ii) be accompanied by a passport issued by a veterinarian authorised by the competent authority certifying valid anti-rabies vaccination, and (iii) a 21 day waiting period in case of primary vaccination. Until January 1, 2012, countries considered free of rabies were granted a temporary derogation from the policy, allowing them to implement specific regulations regarding the transport of pets across their borders. Until end of 2011, five countries (the UK, Ireland, Malta, Sweden and Norway) required an individual serological test for rabies neutralising antibodies before entry into the country (Fooks and others 2011). Today, identification by microchip, a passport certifying valid anti-rabies vaccination as well as prophylactic *Echinococcus multilocularis* treatment are the only entry requirement for dogs entering Norway. This change of movement policy has led to an increase in the number of rescue dogs imported from Eastern European countries for re-homing in Norway through advertisement on the internet. According to data recorded by the customs authority at Oslo Gardermoen airport, the non-commercial movement of dogs from the EU has increased from about 5000 in 2011 to approximately 7500 in 2012 (personal communication; Ole-Herman Tronerud, Norwegian Food Safety Authority, January 2015). Since serious infectious diseases such as echinococcosis and rabies are endemic in Eastern Europe, a report on the health hazards linked to import of rescue dogs to Norway was requested by the Norwegian Food Safety Authority (Norwegian Veterinary Institute 2013). The current paper reports the results of an investigation of the antibody level to rabies virus in vaccinated rescue dogs imported to Norway. The aim was to examine if the internationally accepted threshold antibody titre of ≥0.5 IU/ml was reached in these dogs.

**Material and Methods**

The criteria for inclusion in the study were that the dog (i) was considered a stray animal, that is, not under the direct control by a person, in its country of origin and (ii) had arrived from Romania, Hungary, the Balkans or the Baltic countries during 2012. Dog owners were encouraged to visit a veterinary clinic for blood sampling, analysis costs being covered by the Norwegian Food Safety Authority. A total of 75 blood samples were submitted to the Norwegian Veterinary Institute from veterinary clinics throughout the country and sent to the National Veterinary Institute in Sweden for analysis. The antibody responses were determined by the OIE approved fluorescent antibody virus neutralisation (FAVN) test (Cliquet and others 1998). A control group of 1766 owned dogs from Sweden, that had antibody titre analysis carried out at the same laboratory, was selected from a previous study. The dogs in this control group had received one injection of rabies vaccine and were sampled four months to six months after vaccination (Berndtsson and others 2011). An antibody titre ≥0.5 IU/ml is the internationally accepted threshold after rabies vaccination of dogs (OIE Terrestrial manual 2015). Titres ≤0.1 IU/ml are considered negative in the FAVN assay.
The blood samples from the rescue dogs were accompanied by a submission form containing information on age, breed and sex. In addition, passport details such as date of vaccination (reported for 56 of 75 dogs) and vaccine label (reported for 38 of 75 dogs) was requested. A number of different vaccines, both monovalent and polyvalent products, were used such as Rabies og Eurican DHPII-LR (Merial, France), Nobivac Rabies og Nobivac DHPII +LR (Merck, the Netherlands), Biocan R (Bioveta, Czech Republic), Hexadog (M.C.I. Merial, Morocco), Vaugard Rabies (Pfizer, USA). Proportions and exact CIs were calculated using R V2.12.0 with EpiR package, and group comparisons were done using Fisher’s exact test.

Results

The screening of specific antibody titres to rabies virus in imported rescue dogs demonstrated that only 35 of the 75 dogs (proportion 47%, 95% exact CI (55% to 59%)) showed a satisfactory antibody level ≥0.5 IU/ml. In addition, 51 dogs (41% (30% to 53%)) had titres ≤0.2 IU/ml and among these, 14 dogs (19% (11% to 29%)) had titres ≤0.1 IU/ml, which is considered negative in the FAVN assay (Fig 1). Among the 56 dogs with a reported vaccination date, 50 per cent had antibody titre <0.5 IU/ml.

Sixty-three of the 75 dogs came from Romania, 8 came from Hungary, and 4 dogs the country of origin was not reported. All dogs imported from Hungary had antibody titres ≥0.5 IU/ml.

The 1766 dogs used as control group were tested four months to six months after vaccination. To compare the level of antibodies detected in vaccinated rescue dogs and conventionally owned dogs the data were restricted from the rescue dogs to the control group rescue dogs in this study were vaccinated against rabies at least 21 days before arrival in Norway. However, more than half of the dogs did not achieve the satisfactory antibody level recommended by WHO and OIE. Most alarming was the finding that 19 per cent of the dogs had serum titres ≤0.1 IU/ml raising doubt about whether they had been vaccinated at all.

The two main objectives of antibody testing are to check if the animal (i) has been vaccinated according to recommendations and (ii) has developed an adequate humoral immune response. Previous studies in conventional pet dogs show that the antibody response is influenced by vaccine product used, number of vaccine doses in the primary immunisation schedule, interval between vaccination and blood sampling, age, size and breed of the dog (Cliquet and others 2003, Mansfield and others 2004, Kennedy and others 2007, Jakel and others 2008). However, taking these aspects into consideration, more than 85 per cent of vaccinated dogs achieve an adequate immune response after one dosage of rabies vaccine (Fooks and others 2002, Council of Europe 2008, Van de Zande and others 2009, Berndtsson and others 2011). Several studies report that the interval between vaccination and testing will affect the proportion of dogs with titres above 0.5 IU/ml since peak antibody values are seen four weeks to six weeks postvaccination (Cliquet and others 2003, Mansfield and others 2004, Jakel and others 2008). Therefore analysis was restricted to only those dogs that had been vaccinated four months to six months before sampling to standardise with the control group (Berndtsson and others 2011), and the difference between imported rescue dogs and owned Swedish dogs was statistically significant. Only 45.5 per cent of the rescue dogs showed a sufficient antibody response four months to six months postvaccination compared with 87.5 per cent of the conventionally owned dogs. There is no systematic comparison of the rabies antibody titre and protection from challenge between immunosuppressed and healthy dogs (Morters and others 2014). Many rescue dogs have poor body condition, as well as deficiencies and underlying infections which might have a negative impact on the immune response (Davlin and Vonvillé 2012). Still, mass vaccination campaigns in free-roaming dogs are very successful (Cleaveland and others 2006, Thiptara and others 2011, Morters and others 2014), and one study in Peru demonstrated that 97 per cent of the free-roaming dogs had antibody titre ≥0.5 IU/ml 12 months postvaccination (Chomel and others 1988). The rabies vaccines used in the present study are all inactivated and approved for the European market. If stored or administered according to the manufacturers’ instructions, they should be expected to provide a satisfactory response in the majority of dogs examined in the present study. Hence, one might question if dogs with no detectable antibody responses have been properly vaccinated before rehousing and adoption to Norway.

The impact of these findings on human and animal health is complex to assess. In veterinary medicine, rabies vaccination is considered mainly a preventative measure, to be applied before dogs are exposed to rabies. The antibody titre of vaccinated animals before they move from a rabies-free area to a rabies-endemic area, indicates their level of immunity, and is therefore directly related to their future risk of infection (Aubert 1992). A three-week delay after vaccination usually ensures

**FIG 1:** Distribution of antibody titre to rabies virus in 75 imported rescue dogs with a certified valid anti-rabies vaccination. Titre ≥0.5 IU/ml is the internationally accepted threshold level after vaccination and antibody titre ≤0.1 IU/ml is considered negative (fluorescent antibody virus neutralisation test)

**FIG 2:** Antibody titre to rabies virus in 57 vaccinated dogs, shown as a function of time after vaccination. Titre ≥0.5 IU/ml is represented as 0.5 IU/ml
sufficient high levels of protective antibodies, and is therefore adequate for these types of movement. However, this is no longer the case when dogs are moved from rabies-endemic areas into rabies-free areas, particularly for free-roaming dogs which may unknowingly have been exposed to rabies virus before vaccination. The effect of vaccination on dogs already incubating rabies is debated, and seems to depend on type of challenge (dose, route, natural or experimental) and time between challenge and vaccination (Hanlon and others 2002, Manickama and others 2008, Wilson and others 2010). Antibody titres alone are not able to reveal if animals are infected or not (Hanlon and others 2002, Manickama and others 2008), unless it is known that the animal has been observed over a time period longer than the maximum incubation period for rabies in which case rabies can be ruled out. The present waiting time of 21 days following primary vaccination is considered too short to ensure that vaccinated dogs do not incubate rabies (EFSA, 2006). This has resulted in understandable concern in cases where people have been bitten by recently imported rescue dogs. Postexposure prophylaxis has been needed, and systematic pre-exposure prophylaxis for veterinarians is considered. It is worrying that the relaxation in movement policy has led to increased adoption of rescue dogs from member states where rabies is still prevalent.

The majority of rescue dogs came from Romania where the number of reported rabies cases during 2012 was 318 in wild animals and 159 in domestic animals, including 52 dogs and 30 cats (FLI 2014). In these cases, the non-negligible risk that those dogs may have been exposed to the rabies virus before capture and rabies vaccination should be considered. In addition, these results suggest that the level of compliance with the regulation may be low. Goddard and others (2012) showed that a 20 per cent non-compliance to the present regulation decreased the predicted number of years between rabies introduction to the UK from 211 (90% CI 177 to 247) to 144 (90% CI 125 to 163), compared with full compliance. The present results suggest that compliance could be even lower. Low compliance with the regulation raises concern about other health issues as well, such as the treatment for Echinococcus multilocularis before entering free countries.

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