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## Negligible risk of rabies importation in dogs thirty days after demonstration of adequate serum antibody titer

Todd G. Smith<sup>a,1</sup>, Anthony R. Fooks<sup>b,c</sup>, Susan M. Moore<sup>d</sup>, Conrad M. Freuling<sup>e</sup>, Thomas Müller<sup>e,c</sup>, Gregorio Torres<sup>f</sup>, Ryan M. Wallace<sup>a,c</sup>

<sup>a</sup>Poxvirus and Rabies Branch, Division of High-consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases, Center for Disease Control and Prevention, 1600 Clifton Rd NE, Atlanta, GA, 30333, USA

<sup>b</sup>Animal and Plant Health Agency (APHA, Weybridge), Surrey KT15 3NB, UK

<sup>c</sup>World Organisation for Animal Health (OIE) Reference Expert and Laboratory for Rabies

<sup>d</sup>Kansas State University, Veterinary Diagnostic Laboratory, Rabies Laboratory, Manhattan, KS, 66502, USA

<sup>e</sup>Institute of Molecular Virology and Cell Biology, Friedrich-Loeffler Institute, Südufer 10, 17493, Greifswald - Insel Riems, Germany

<sup>f</sup>World Organisation for Animal Health (OIE), Science and New Technologies Department, Paris, France

### Commentary

Importation of one rabid dog from areas where rabies virus (RABV) is circulating into a country or zone that is declared free of rabies poses a threat of rabies re-introduction into the resident dog population (if “herd” immunity is insufficient) or congeneric species. To prevent rabies importation, the World Organisation for Animal Health (OIE) Terrestrial Code (8.14.7) currently recommends that rabies-vaccinated animals, individually identifiable and not showing any rabies clinical signs, have a minimum waiting period of three months between proof of adequate rabies serum antibody titer ( $\geq 0.5$  IU/mL) and entry into a rabies free country. Alternatively, dogs may undergo a 6 month quarantine prior to import. The waiting period ensures that a dog with RABV neutralizing antibodies (rVNA) detected through OIE recommended methods is not incubating RABV, which can induce rVNA in late stages of disease. We propose reducing the waiting period to 30 days.

Two potential risks, apart from subversion of these regulations, have been previously described [1]. Type A risk describes a dog infected with RABV prior to vaccination (i.e. is vaccinated during the RABV incubation period), which produces antibodies in response to vaccination, but succumbs to rabies after importation. Type B risk describes an apparently

<sup>1</sup>Corresponding author: Tel: 404-639-2282, Fax: 404-639-1564, tgsmith1@cdc.gov.

Conflicts of Interest

The authors have no competing interests.

healthy dog, which does not produce antibodies in response to vaccination (due to immune-status of the dog or sub-standard vaccine), instead develops antibodies due to natural infection occurring after vaccination, survives the post-titer waiting period, and becomes symptomatic after importation (Figure S1). Since nearly all healthy dogs respond to appropriately administered rabies vaccine, and nearly all animals that produce antibodies due to natural infection succumb within several days [2], Type B risk is generally considered negligible [1]. Despite this consideration, we did consider both Type A and B risk in our evaluation.

We collected information from the literature used to make prior OIE Terrestrial Code standards, keyword searches using PubMed, cross-references of previous publications, and subject matter experts to identify peer reviewed articles that had information relevant to rabies challenge, vaccination, postexposure prophylaxis, and serology in dogs. We found the three-month waiting period is not supported by current evidence and is unnecessarily restrictive which could increase the risk of non-compliance such as falsification of vaccination certificates and serology reports. Both the data identified during this review as well as over thirty years of monitoring rabid dog importation events in the United States and Europe, reaffirm the conclusions of Aubert nearly 30 years ago [3], that international movement of dogs should be based on adequate rVNA as measured by a qualified assay, permanent individual identification of the animal, and certification of rabies vaccination in the identified animal. After these conditions are met a 30-day waiting period is more than sufficient to mitigate RABV risk.

Type A risk presents the most likely scenario in which a failure of international guidance would result in an imported rabid dog, and quantifying this risk requires an understanding of the immunologic processes that could result in a Type A risk failure. After inoculation into a host, rabies virus enters a peripheral nerve and remains hidden from host immune cells until it reaches the central nervous system (CNS) and begins replicating in neurons. It is not until this point in the infectious process that the host displays any signs of illness, nor do they pose a threat of onward transmission. It is also not until the virus has reached the CNS that immune mediated processes of antibody production begin, notably detection of rabies virus neutralizing antibody in the serum. Host death can be attributed directly to the autonomic disturbances arising from rabies virus infected neurons, or from antibody-mediated neuronal cell death. A process referred to as “early death phenomena” has been described [4], in which apparently healthy-infected hosts experience death sooner as a result of antibody-mediated neuronal cell death due to vaccination. Regardless of the process, host death typically occurs within 14 days of initial symptom onset.

Notably, few studies have assessed rabies serum antibody responses over time in dogs post-RABV infection (Table 1). Cho and Lawson [5] vaccinated dogs starting 6-hours post-infection, representing Type A risk. Of 34 dogs (mixed-breed, 10 months old, observed for 180 days post-infection) that received either vaccine or immune globulin, 16 (47%) had detectable serum antibody levels by mouse inoculation test but still succumbed to rabies (the others survived challenge). These dogs died on average 15 days (range 12-20 days) post-infection and on average 8 days (range 5-13 days) after serum antibody detection. A 30-day waiting period represents over a two-fold increase in the maximum time necessary to

properly assess dogs and prevent importation when considering Type A risk (Figure S1). The observation of “early death” in animals incubating rabies that are then vaccinated [4] would apply to Type A risk; thus, dogs under Type A risk would tend to have shorter incubation periods than unvaccinated dogs.

Two studies [6, 7] measured immune responses in unvaccinated dogs, representing Type B risk (Table 1); 73% (11/15) of the dogs died, and one of 11 (9%) that died developed rVNA due to the virus challenge. For this animal the response was  $< 0.5$  IU/mL (0.3 IU/mL), and it succumbed to rabies within 7 days of serum antibody detection. The four dogs that survived all developed robust serum antibody responses, never developed rabies clinical signs, and were negative for rabies diagnosis 90 days post-infection. A 30-day waiting period represents over a three-fold increase in the maximum time necessary to properly assess dogs and prevent importation when considering Type B Risk (Figure S1).

In addition to experimental infections in dogs, there are numerous case reports of rabies in humans [8]. These reports support that few people develop rVNA early in the clinical course and those that do are within a few days of symptom onset. Instead, most people develop rVNA very late in the clinical course only a few days before death.

The international standard of 0.5 IU/mL is recognized as an adequate immune response post-rabies vaccination. Published reports of vaccinated animals with  $< 0.5$  IU/mL rVNA that succumb to RABV challenge are exceptionally rare [9]. Both the current OIE international standards and the proposed 30-day waiting period require proof of adequate rVNA prior to importation. Healthy dogs are highly likely to satisfy this requirement between day 8 and day 30 post-vaccination when administered highly-potent, rabies vaccines [2]. When the parameters for Type A and B risk from Table 1 are considered chronologically, a 30-day waiting period is sufficient to prevent rabies importation for both Type A and Type B risk (Figure 1). Because timing of the rVNA test is not controlled, the scenarios depicted in Figure 1 show the three most likely scenarios of day 8, 30, and 90 post-vaccination serum collection. Ideally, the rVNA test would occur on day 30 post-vaccination when the dog is most likely to have a robust serum antibody response, has complied with rabies vaccine manufacturer’s instructions, and when Type A risk is greatly reduced.

Several quantitative risk assessments have been published in which the risks of importing a rabid dog into a rabies free country are calculated [1, 10, 11]. These studies were the first to define Type A and Type B risk and utilized robust modelling methods. However, two critical issues were noted that impact the conclusions from these risk models.

First, these models have over-estimated the rabies virus incubation period (38 days), which has led to inflated estimates of the frequency of rabid dog importation. [1]. In Table S1, we show the results of 33 studies accounting for 203 animals, which demonstrate a much shorter average incubation period of 19 days in experimentally-infected, unvaccinated dogs ( $\pm 9.3$  days standard deviation). The 75<sup>th</sup> percentile was 21 days, and the 95<sup>th</sup> percentile was 33 days (Figure S2, range 4 – 92 days, showing a dose-dependent relationship). While we did not attempt to re-run these existing models with this more robust and reduced incubation

period, based on the equations this revised parameter would result in a significant reduction in the frequency of expected rabid dog importations.

Second, these models were tailored to importation rates seen in the UK and an overly conservative interpretation of risk. Goddard et. al. published risk model results that indicate reducing the waiting period from 90 to 30 days would result in a 1.8-fold increase in the rate of rabid dog importation (Table 5 in Goddard et. al. [10]). Effectively, this would reduce the interval between importation events into the UK from 211 years to 117 years. Statistically, this is a significant increase in the risk of rabid dog importation when applying a 30-day waiting period, however Goddard et. al. [10] note that importation on this centennial scale represents a low risk to canine rabies-free countries. Despite these models using an inflated value for a critical model parameter (incubation period), the risk of importation under a 30-day waiting period is still expected to occur less than once every 100 years.

Our evaluation assumes that OIE recommendations regarding proper vaccination and documentation are followed. However, the impact of non-compliance, another type of risk, must be considered when developing guidance on animal importation requirements. Goddard et. al. [10] showed there was a 20-fold increase in Type A risk when compliance dropped 10%, and Kwan, et al. [11] recommended promoting compliance by reducing the waiting period. We postulate that compliance with a 30-day waiting period would be greater than the current 90-day period, representing yet another benefit of adopting this evidence-based waiting period reduction.

From 2007 to 2019, seven imported rabid-dogs have been reported in the United States. In each case, falsification of rabies vaccination documentation was either confirmed or highly suspected [12]; in addition, falsified rabies serology documents have also been detected. Similar fraudulent imported rabies cases (N=33) have been reported in the European Union during the past 30 years through failure of border controls, ignorance of importation rules, or active subversion of these rules [13]. It is highly notable that none of the 40 imported rabid dogs reported by the United States and Europe would have constituted a failure of a 30-day waiting period. These failures of dog importation requirements are not attributable to Type A or B risk but rather non-compliance. National policies and international standards should be evidence based while also considering the inverse relationship between compliance and importation risks [10].

The preponderance of experimental, biological, and natural infection evidence supports a reduced waiting period from 90 to 30 days following proof of adequate rVNA in a healthy dog. A review of historical data has shown that dogs with detectable rabies virus serum antibody will either succumb to rabies within two weeks or will remain healthy (an indication of appropriate vaccination). Literature reviews show that the average incubation period for rabies in unvaccinated dogs is half of what was used for previous quantitative risk assessments (19 days vs 38 days). A review of thirty years of dog importation data has shown that all imported rabid dogs were the result of intentional or unintentional lack of compliance with national and international standards. A 30-day waiting period would ensure negligible increased Type A risk while decreasing risk of non-compliance, which has proven to be the greatest threat to importation of a rabid dog. Reduction of the post-titer waiting

period is not without precedent. The rabies-free islands of Hawaii conducted a risk assessment and in 2018 adopted a 30-day waiting period (decreased from three-months). Out of an estimated 7400 dogs imported in the two years since this change, no rabies importations have been identified in Hawaii. While beyond the scope of the current work, we think additional reductions in the waiting period for dogs receiving routine, rabies-booster vaccination could also be considered in the future.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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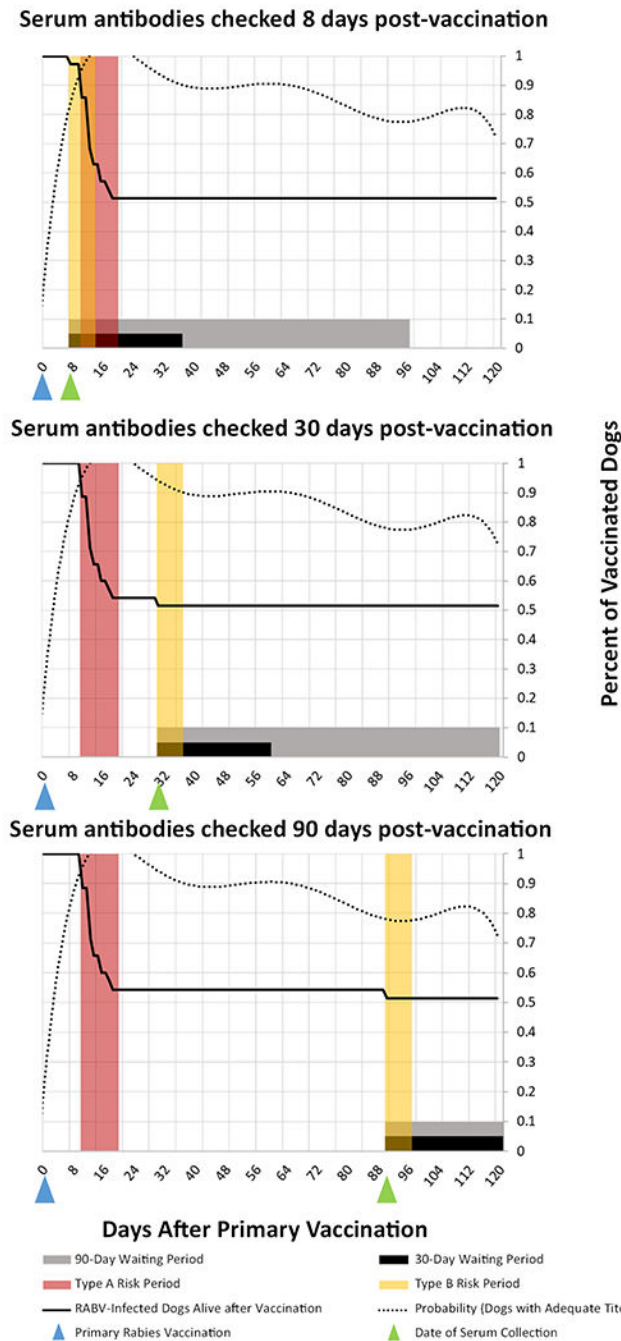
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**FIGURE 1. Comparison of Type A and Type B risk in relation to vaccination, antibody production, and waiting period before entry into a canine rabies-free country.**

Intervals for Type A and Type B risk are modeled based on Table S1 showing that death among dogs infected prior to vaccination occurred within 20 days post-vaccination and 13 days post-antibody detection (Type A risk, red); and that death occurred within 7 days of rabies antibody production in inadequately vaccinated dogs (Type B risk, yellow). A 90-day post-titer waiting period (gray) is compared to a 30-day post-titer waiting period (black). The survival curve (solid line) is representative only of dogs presented in Table S1 and not

survival rates of all dogs in the general population. The probability of dogs having an adequate titer (dotted line) is derived from Wallace et. al. [2].

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**TABLE 1.**  
Serology results and outcomes of experimental rabies virus infection in vaccinated and unvaccinated dogs, representing Type A and B risk.

Risk	Number of animals	Treatment post-infection	Antibody detection (day)	Outcome (day)	Days from antibody detection to death	Reference
Type A	13	Vaccine + RIG <sup>a</sup>	100% (7)	Survived (NA) <sup>b</sup>	NA	[5]
	5	Vaccine + RIG	100% (7)	Died (12-20)	5-13	
	5	RIG	100% (7)	Survived (NA)	NA	
	3	RIG	100% (7)	Died (12-17)	5-10	
	8	Vaccine	100% (7)	Died (12-15)	5-8	
Type B	4	None	100% (14-28)	Survived (NA)	NA	[6] [7]
	11	None	9% (14) <sup>c</sup>	Died (14-58)	0-7	

<sup>a</sup>Rabies immune globulin (RIG)  
<sup>b</sup>Not applicable (NA)

<sup>c</sup>Antibody detected on day 14 was <0.5 IU/mL (0.3 IU/mL). Another dog (ID 31) had "transient" serum antibody detected on day 14 but was subsequently negative for serum antibodies before death [7]. We interpret this animal to be sero-negative because, if serum antibody was due to RABV challenge, it would not decrease before death.