The raccoon (Procyon lotor) as potential rabies reservoir species in Germany: a risk assessment

Terrestrial wildlife rabies has been successfully eliminated from Germany predominantly as a result of the distribution of oral rabies vaccine baits. In case that wildlife rabies would re-emerge among its known reservoir species in Germany, swift action based on previous experiences could spatially and temporally limit and subsequently control such an outbreak. However, if rabies emerged in the raccoon population in Germany (Procyon lotor), there are no tools or local experience available to cope with this situation. This is especially worrisome for urban areas like Kassel (Hesse) due to the extremely high raccoon population density. A rabies outbreak among this potential reservoir host species in these urban settings could have a significant impact on public and animal health.

Keywords: susceptibility, oral vaccination, vaccine, baits, raccoon, Germany

Zusammenfassung

Die terrestrische Tollwut wurde in Deutschland vor allem durch das Auslegen von oralen Impfstoffködern erfolgreich getilgt. Falls sich die Trägerspezies in Deutschland wieder mit dem Tollwutvirus re-infizieren, kann aufgrund der vorhandenen Erfahrungen schnell sowie gezielt reagiert und der Ausbruch rasch unter Kontrolle gebracht werden. Allerdings gibt es bislang keine Mittel und Erfahrungen mit der Bekämpfung von mit Tollwut infizierten Waschbären (Procyon lotor) in Deutschland. Dies ist insbesondere für Städte wie Kassel (Hessen) bedeutsam, in denen die Waschbären eine extrem hohe Populationsdichte erreichen. Ein Tollwutausbruch bei dieser potenziellen Trägerspezies in städtischen Gebieten stellt deswegen ein Risiko für die Gesundheit von Mensch und Tier dar.

Schlüsselwörter: Empfindlichkeit, orale Immunisierung, Impfstoff, Köder, Waschbär, Deutschland
Introduction

On World Rabies Day, 28 September 2008, Germany was declared rabies free; since 2006 no indigenous terrestrial rabies cases had been reported (Anonymous, 2008). The last sylvatic epizootic entered Germany from Poland when an infected animal crossed the Odra river in 1947 (Müller et al., 2004b). The outbreak was fox-mediated; 73% of all cases reported involved foxes (Vulpes vulpes). The highest annual number of cases reported occurred in 1983 with 9160 rabies cases (Müller et al., 2004b); the same year that the first field trails with oral vaccination of foxes against rabies took place in Germany. The distribution of these vaccine baits lead to the ultimate elimination of terrestrial rabies in this country. Although many animal species other than foxes became infected no other terrestrial animal species acted as reservoir during this outbreak. Recently, the raccoon dog (Nyctereutes procyonoides) has also emerged as a reservoir species in North-eastern Europe. Fortunately, the available oral rabies vaccine baits developed for foxes are also efficacious in raccoon dogs (Schuster et al., 2001; Cliquet et al., 2006). Oral vaccination of wildlife has changed the rabies landscape to a great extent and most of Europe is now considered free of terrestrial wildlife rabies. However, vigilance is still required. Terrestrial rabies can be reintroduced through direct entry from rabies-infected wildlife in neighbouring countries as recently occurred in Italy. Alternatively, raccoons can also be reintroduced through importation of infected animals incubating raccoons (Johnson et al., 2011). In the last decade, at least five cases of imported dog rabbits were reported from Germany (Johnson et al., 2011). Sometimes these importations occur through ignorance of regulations or active disregard of importation requirements (Le Roux and van Gucht, 2008). The re-introduction of rabies in Germany through infected wildlife or pets could lead to a spill-over infection into the reservoir species resulting in a new outbreak. Another possibility is a spill-over infection from bats infected with European Bat Lyssavirus Type 1 or 2 (EBLV-1 and -2). Such spill-over infections are relatively rare but have been reported on several occasions like in a stone marten (Martes foina) and domestic cats (Felis catus) (Müller et al., 2004a; Dacheux et al., 2009). Typically, these spill-over infections are so-called dead-ends and do not progress beyond the first infected terrestrial host. On rare occasions these spill-over infections develop into independent new sustainable infection cycles within the new host species, like recently has been observed in a skunk population in Arizona infected with a bat variant of the rabies virus (Leslie et al., 2006). Most likely these carnivores have preyed on bats and came in contact with an infected animal. Raccoons (Procyon lotor, Fig. 1) in (semi-) urban settings may use attics as resting – or den sites, which can also be occupied by bats. Considering that the serotine bat (Eptesicus serotinus), the reservoir host of EBLV-1 responsible for more than 90% of bat rabies cases reported in Germany, is a bat species that typically uses buildings as roosts, encounters between serotine bats and raccoons cannot be excluded. A raccoon outbreak among raccoons especially in extremely densely populated urban settings would pose a serious risk for public and animal health. The recent outbreak among raccoons in Central Park, Manhattan – New York City (USA), clearly underscores the potential risk and the difficulties resolving this problem; 121 raccoons found in and around Central Park were reported rabid in 2010 (source: http://www.nyc.gov/html/doh/html/cd/cdrab-cp.shtml). On first thought, one would suggest using the highly successful approach of oral rabies vaccination for raccoons. Unfortunately, this approach used so successfully for foxes cannot simply be copied for raccoons for several reasons. First of all, there is only limited experience with distribution of baits containing live replication competent virus vaccines in urban areas. Secondly, no local experience in distributing baits targeted for animals living at extremely high densities is available. Finally and most importantly, the licensed oral rabies vaccine baits in Germany are most likely not suitable for raccoons. Hence, the responsible authorities in Germany would not only be completely unprepared in case of emergence of raccoons in the raccoon population but would also have no or limited tools available to address such an outbreak. In this paper we will try to assess the risks associated with the raccoon population in Germany as a potential rabies reservoir species and to identify possible methods to intervene in case rabies would infect the raccoon population.

Raccoons in Germany

The first free-living raccoons were observed in Germany approximately 80 years ago. Today Germany accommodates the largest raccoon population within Central Europe. In the late 1920s raccoons were precious fur bearers and during this period escapes from enclosures and attempts to introduce the animals in the wild were documented for the first time (Müller-Using, 1956). However, these early escapes and deliberate efforts to introduce raccoons failed. The first successful introduction took place in 1934, when four raccoon (two females and two males) were abandoned at Edersee, Hesse. This
event is regarded as the “initiation” of the free-ranging raccoon population in Central Europe. The second introduction took place in Wolfshagen (East Brandenburg). During the turmoil of the Second World war 20–30 animals were released from a raccoon farm in 1945 (K. Allner, pers. comm.) and formed the basis for the raccoon population in Eastern Brandenburg and Western Pomerania (Stubbe, 1993). Raccoons are difficult to contain in captivity; hence, constantly additional captive animals escaped and entered the raccoon populations established by these two original events. In spite of this constant influx of new individuals into the German raccoon population genetic diversity is relatively low compared to several raccoon populations studied in the USA, underscoring the “founder-effect” in the German raccoon population (Gramlich et al., 2011). The main foci for range expansion are still limited to these original sites in the middle and northeastern part of Germany (Fig. 2). The reason for this is the relatively conservative dispersal behaviour. Raccoons disperse actively and exploratively with a distinct gender difference. Due to very strong philopatric behaviour the females remain near their birthplace their entire life, while male raccoons almost always leave the maternal area (Gehrt, 2003; Michler and Köhnemann, 2010).

In 1954, hunting efforts started with the aim of extirpation. However, despite efforts and the use of methods like steel traps or gassing of burrows, hunting never succeeded in having the desired effect. The annual hunting bag increased continuously and showed an exponential increase in the mid 1990s reaching over 50 000 killed raccoons (Fig. 3). Raccoons expanded their range during the last few decades and now occur at varying population densities in all 16 federal states in Germany. According to the Federal Nature Conservation Act (BNatSchG § 7 Abs. 2 Nr. 7), the raccoon is considered nowadays an indigenous species. Although being highly adaptable, raccoons are dependant on certain habitats. These are primarily mixed woodland and the presence of wetland (lakes, streams, swamps). Raccoons tend to urbanisation as almost no other wild animals. They are able to successfully utilise human settlement areas because of their extraordinary adaptability and complex and variable social system with distinct intraspecific tolerance (no territorial behaviour). Besides this, the climbing abilities and tactile manipulating skills of raccoons enable the use of resources that are not accessible to most other terrestrial wild animals. (Sub-) Urban habitats provide a markedly superior supply of resources than most woodland habitats, often allowing population densities of raccoons in (sub-) urban areas to reach up to ten times higher than in rural areas. In Germany, the population density in forested habitats is generally well below ten animals/km², but in certain urban areas population densities of approximately 100 animals/km² have been documented (Kassel – 90 animals/km² and Bad Karlshof – 110 animals/km²; Hohmann 1998; Hohmann et al., 2001; Michler 2004, 2007).

**Raccoon and rabies**

This New World species can be found almost everywhere in North and Central America, from southern Canada to central Panama. Also, raccoon rabies is widespread especially in the eastern USA; rabid raccoons have been reported from 25 states during 2009 in the USA (Blanton et al., 2010). In the same year, 2327 (34.8%) of the total reported 6841 rabies cases in the USA were raccoons, making it the most often reported rabies infected animal species in North America (Blanton et al., 2010). Rabies can cause high mortality rates during an initial epizootic, with up to 80% of the population succumbing to infection (Clavette, 1996). Some of the most common rabies symptoms or behaviours observed among infected raccoons are aggression, appearance of being sick or unhealthy, impaired mobility or ataxia, fighting with dogs, and abnormal vocalizations. However, extremely aggressive behaviour is far less common in rabies infected raccoons than in rabid foxes (Kappus et al., 1970), and some rabid raccoons display no abnormal behaviour at all. Several authors have indicated that in contrast to fox rabies there is no
distinct temporal pattern in raccoon rabies (Rupprecht, 1992). Torrence et al. (1992) reported on a bimodal pattern with peaks in late winter and early fall whereby the highest and lowest incidence was observed in late winter and the summer months, respectively. These two peaks are very similar to the fox rabies incidence and have been associated with the mating season (January to March) and dispersal of the juveniles in autumn, two periods with increased intraspecific contact rates and aggression.

**Raccoon rabies in Germany**

During the most recent rabies epizootic, only 15 rabies cases in raccoons were reported from former West Germany between 1960 and 1975 (Rojahn, 1977); most of these cases from the northern part of Hesse. Also several cases were reported from former East-Germany. However, these cases were all temporally-spatially isolated and the raccoon had no role in the emergence or spread of wildlife rabies in Germany (Tscharich, 2001). This low number of rabies cases in raccoons is rather remarkable. Some people claimed that rabid raccoons tend to withdraw in their hide-outs (Kampmann, 1975) and therefore are most of the times not found and reported. However, we know from the surveillance data in the USA that this is most likely not the case. Another explanation for the low rabies incidence among German raccoons was the low population density during the last fox rabies outbreak. The exponential increase did not occur until the mid 1990s when fox rabies was already restricted to some isolated residual foci. Therefore, in 1970s and 1980s during the peak of the raccoons outbreak the number of raccoons may have been too low for the development of an independent transmission cycle. However, one would have expected more isolated spill-over infections from foxes to raccoons or even self-limited local outbreaks among raccoons after such a spill-over event. There are two other possible explanations. First of all, raccoons may not be very susceptible for the fox rabies virus variant present in Germany. Interspecific variability in susceptibility for different rabies variants is well documented (Blancou, 1988). For example, during an experimental study, raccoons were about thousand-fold more resistant than foxes to infection with a rabies virus isolated from the salivary gland of a rabid fox (Winkler and Jenkins, 1995). Furthermore, it is suggested that raccoons can survive a rabies infection as indicated by the prevalence of seropositive raccoons in rabies endemic areas (McLean, 1971; Jenkins et al., 1988). Secondly, in experimental settings not all raccoons die when challenged with racibes virus; nor do all animals effectively seroconvert when vaccinated, suggesting that there is a significant level of variability in the immune response of raccoons to rabies (Hanlon et al., 2002; Szanto, 2009). It is not known if this variability has a genetic basis but genetic variation in susceptibility for certain viral diseases is well documented and has also been suggested for raccoons and rabies (Hedrick, 2002; Sommer, 2005; Srithayakumar et al., 2011). As mentioned above the genetic relatedness among German raccoons is very high. If raccoons in Germany are not (very) susceptible to rabies infection there is a less urgent need for contingency plans. In the framework of the Lyssavirus research network (http://lyssavirus.fli.bund.de/Home.aspx), the susceptibility of German raccoons for several rabies virus variants will be investigated in association with potential re-emergence of terrestrial rabies in Germany.

**Control of raccoon rabies**

Just as with fox rabies, intensified population reduction schemes alone do not seem to be a sustainable and effective control measure for raccoon rabies, but in combination with other tools it has shown to be highly effective. When raccoon-mediated rabies entered Ontario, Canada, from the USA in July 1999 an integrated emergency control programme was initiated (Rosatte et al., 2009a). Five kilometres around the location of a rabies case, animals were trapped and euthanized to reduce the raccoon population by 80% to 90%. Animals captured in an approximately 5 km radial area around the population reduction zone were vaccinated by the parenteral route using an inactivated vaccine and subsequently released. If more than 60% of the raccoon population in this TVR-zone (Trap-Vaccinate-Release) were vaccinated, the TVR-programme was discontinued. Around the TVR-zone an up to 50 km wide buffer zone was created by distributing oral rabies vaccine bait. As a result of these efforts, the last case of raccoon rabies was reported in Ontario on 23 September, 2005 (Rosatte et al., 2009a). This approach has later also been successfully implemented to control raccoon rabies in Quebec, Canada (Guerin et al., 2008). A special hurdle in implementing such a programme is the fact that the highest raccoon densities are reported from (semi-) urban areas. Population reduction is not only complicated and time and labour consuming, but would also meet considerable opposition from a large part of the public. In Ohio, USA, a similar approach using baiting and TVR but without population reduction was used (Slate et al., 2009). One of the major reasons for these enhanced control efforts, including TVR and population reduction, was that the distribution of the available oral rabies vaccine bait could not reach a sufficient high vaccination level in the raccoon population to control rabies. Furthermore, the efficacy of the oral rabies vaccine used was not as efficacious as the vaccine used for parenteral vaccination (Brown et al., 2011). Another complicating factor is the delivery of baits in urban areas, which is not without difficulties and
there is little or no experience available in Europe with the exception of anthelmintic baiting of foxes against *Echinococcus multilocularis* (Hegglin et al., 2003; König et al., 2008).

**Oral vaccination of raccoons**

**Vaccine**

Animals differ greatly in their relative susceptibility to oral vaccination (Hable et al., 1992), and raccoons are more refractory to oral vaccination against rabies than foxes and raccoon dogs. Therefore, it must be determined if the three oral racbles vaccine strains SAD B19, SAD P5/88 and SAG2 registered in Germany are also efficacious in raccoons. The most widely used vaccine in Germany, SAD B19, has been investigated in detail (Rupprecht et al., 1989). Also, the double mutant SAG2 has been tested in raccoons (Hanlon et al., 2002). Both strains showed protective activity in raccoons but at much higher doses than used for foxes. Furthermore, the efficacy was less pronounced when the vaccine was not administered by direct oral instillation but by offering animals vaccine in a bait (Rupprecht et al., 1989). Presently there is only one oral racbles vaccine licensed for use in raccoons, the recombinant vaccinia virus expressing the rabies glycoprotein (V-RG). During experimental studies this vaccine protected raccoons against a relevant challenge infection (Rupprecht et al., 1986). Unfortunately, field data are inconsistent with results from this and other laboratory efficacy trials where 80% to 100% of raccoons orally immunized with V-RG seroconverted.

The seroconversion rate determined after the distribution of V-RG baits is often much lower (Rosatte et al., 2008; Slate et al., 2009). This low seroconversion rate is not a result of poor bait uptake as indicated by the detection rate of the bait marker, tetracycline. On Parramore Island, Virginia, 1000 baits per km² were distributed during 1990 and only 52% of the raccoons seroconverted although 84% of the raccoons tested positive for the bait marker (Hanlon et al., 1998). Also, in other areas a large discrepancy between bait acceptance (detection of bait marker) and seroconversion rates in raccoons has been observed (Rosatte et al., 2008). In the USA, the average long term post-ORV antibody levels (> 0.05 IU/ml) in raccoons was approximately 30%, much lower in comparison with average levels reported for gray foxes and coyotes using the same vaccine virus and similar baiting strategies (Slate et al., 2009). However, the correlation between seroconversion in terms of the presence of rabies virus neutralizing antibodies (VNA) and protective immunity remains a topic of debate. Hence, animals that consumed a vaccine bait but do not develop detectable levels of VNA can still be protected against a relevant challenge infection (Hanlon et al., 2002).

Several other candidate vaccines have been tested in raccoons; including genetically modified rabies virus vaccines, recombinant raccoon pox virus and canine adenovirus type 2 both expressing the rabies virus glycoprotein (Blanton et al., 2007; Henderson et al., 2009; Esposito et al., 1988). Some of these constructs have shown promising experimental results but are most likely not suitable because of pre-existing immunity against the vector virus among the target population in the field (Root et al., 2008). Another not yet licensed product has already been widely tested in Canada; ONRAB® – a recombinant human adenovirus type 5 expressing the rabies glycoprotein (Rosatte et al., 2009b). Compared to V-RO, ONRAB® vaccine is very immunogenic in raccoons under field conditions (Rosatte et al., 2008). However, it can be expected that in Europe the intentional environmental release of replication-competent recombinant viruses expressing a foreign gene could encounter considerable resistance from the public, especially when it concerns a human pathogen.

**Bait**

Another possible explanation for the observed poor efficiency in raccoons in the USA is the bait types used. The first bait used was the fish meal polymer bait. During experimental screening experiments and field studies these fish-flavoured baits were preferred over for example baits with a fruit-based or corn oil coating (Linhart et al., 1991, 1994, 2002; Kavanaugh and Linhart, 2000). However, during other studies preference for fish-flavoured baits was less pronounced. In Ontario, Canada, cheese and vanilla-sugar flavoured baits were accepted better by raccoons than for example sea food flavoured baits (Rosatte et al., 1998). Also, initial experimental and field studies in Germany indicated that the present fox bait (fish meal) was not accepted as good as fruit or corn-based baits (unpublished results). Besides bait palatability there are many other bait properties that determine its efficiency in terms of vaccine delivery. For example, a large proportion of discarded sachets within the fish meal polymer baits were not punctured by raccoons after bait uptake (Olson and Werner, 1999; Linhart et al., 2002). In contrast to foxes, raccoons are selective omnivores displaying great ability of manual manipulation of food items, enabling them to selectively remove unfavourable parts of food items. Therefore, it is not uncommon to recover the vaccine capsule intact from a bait of which the matrix has been consumed (Hable et al., 1992).

Hence, other bait types targeted at raccoons were developed like the coated-sachet whereby the sachet containing the vaccine is no longer placed within a bait matrix but simply sprinkled with a fish-meal coating (Linhart et al., 2002). The latest bait developed and used for oral vaccination of raccoons is the UltraLight bait used for the distribution of the ONRAB® vaccine. It consists of an elongated plastic blister coated with a thin bait matrix containing different predominantly sweet attractants (Rosatte et al., 2009b). Both types largely eliminate the risk of selective separation of bait matrix and vaccine container.

Another important factor in determining bait uptake by raccoons is bait competition by non-target species. Especially in urban areas, bait uptake by pets can raise certain concerns by the owners and it may be necessary to develop species-specific baiting techniques. For example, bait distribution in urban settings targeted at red foxes was highly effective when baits were placed at selected sites attractive to foxes and that restricted access to bait competitors (Hegglin et al., 2003; König et al., 2008). The most important bait competitors for foxes in urban areas were dogs, rodents, snails and hedgehogs (Hegglin et al., 2004). Although cats were observed near the baits, they never removed the fish-flavoured baits (Hegglin et al., 2004). However, this does not necessarily apply to different flavoured bait types. It can be expected
that sweet-tasting baits will attract many other animal species.

Another relevant issue is the relationship between raccoon population density and the minimum density of vaccine baits necessary to confer herd immunity. Due to the higher population densities, much higher bait densities must be applied than were used during vaccination campaigns for red foxes in Canada, with a raccoon density of 13–20 animals/km² 400 baits per km² were distributed (Rosatte and Lawson, 2001). So, this would mean that for areas with a raccoon density of 100 animals per km² or more, an unrealistic number of thousands of baits per km² have to be distributed. Therefore, new, innovative bait delivery strategies that could enhance bait uptake efficiency should be developed. Rosatte et al. (2009a) suggest a staggered approach using these extremely high densities only in localized areas and using a lower bait density in surrounding buffer zones. Also cluster baiting at known feeding sites (Hadidian et al., 1989) habitat-targeted baiting (Boyer et al., 2011), or the establishment of artificial feeding stations that are regularly visited by raccoons have been suggested (Boulanger et al., 2008).

Conclusion

The raccoon is not considered a reservoir species for raciies in Germany based on the last terrestrial syphatic rabies outbreak. However, due to the continuously increasing size of the raccoon population this status could change upon re-emergence and spillover of rabies into raccoons in Germany. Unfortunately, the susceptibility of the German raccoon population for relevant rabies virus variants is unknown. Therefore, it is of utmost importance to determine susceptibility. If shown to be susceptible, all available control measures need to be assessed in detail. The most promising control method is oral rabies vaccination, but no vaccine bait licensed in Germany has been shown to be efficacious in raccoons. Hence, efficacious and safe oral raccoons vaccines for raccoons need to be identified and a raccoon-specific bait needs to be developed. Also, effective baiting strategies targeted for raccoons need to be formulated, especially for urban areas with extremely high population densities. Finally, consideration should be given to preparation of conceptual contingency plans to better ensure swift and appropriate intervention in a worst case scenario of emergence of (urban) raccoons in Germany.

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