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An assessment of shedding with the oral rabies virus vaccine strain SPBN GASGAS in target and non-target species

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ABSTRACT

A safety requirement for live vaccines is investigating possible shedding in recipients since the presence of replication competent vaccine in secretions could result in direct and indirect horizontal transmission. This is especially relevant for oral rabies vaccine baits that are deliberately distributed into the environment. In the current study, survival of an oral rabies virus vaccine, SPBN GASGAS, was examined in excretions from different target and non-target species; red fox, raccoon dog, small Indian mongoose, raccoon, striped skunk, domestic dog, domestic cat and domestic pig. Saliva – and (pooled) fecal samples collected at different time points after oral administration of the vaccine strain were examined for the presence of viral RNA (rt-PCR). All PCR-positive and a subset of PCR-negative samples were subsequently investigated for the presence of infectious virus by isolation in cell culture (RTCIT). Up to 7 days post vaccine administration viral RNA could be detected in 50 of 758 fecal samples but no infectious virus was detected in any of the examined PCR-positive fecal samples. In contrast, RNA-fragments were detected in 248 of 1053 saliva swabs for an extended period (up to 10 days) after vaccine administration, but viable virus was only present during the first hours post vaccine administration in 38 samples. No infectious vaccine virus was isolated in saliva swabs taken 24 h or more after vaccine administration. Hence, no active shedding of the vaccine virus SPBN GASGAS after oral administration occurred and the virus isolated during the initial hours was material originally administered and not a result of virus replication within the host. Thus, potential horizontal transmission of this vaccine virus is limited to a short period directly after vaccine bait uptake. It can be concluded that the environmental risks associated with shedding after distributing vaccine baits containing SPBN GASGAS are negligible.

1. Introduction

Oral rabies vaccination has been developed into a highly effective strategy to control and eliminate the disease in some of the reservoir species like red foxes (*Vulpes vulpes*) and coyotes (*Canis latrans*) [1,2]. Concerns about vaccine safety and lack of efficacy in certain species of the currently available oral rabies vaccine baits support the need to develop alternative vaccine candidates [3,4]. One of these candidates is the vaccine virus SPBN GASGAS, a genetically modified rabies virus derived from the parental SAD B19 vaccine virus strain (SAD – Street Alabama Dufferin). Although highly attenuated through site-directed mutagenesis, SPBN GASGAS remains a live replication-competent virus. Hence, regulatory guidelines require that part of the safety evaluation of such live vaccine candidates should include potential shedding by testing of among others feces and oral secretions for the presence of the organism in vaccinated animals [5,6]. Per se, environmental shedding of orally administered live virus vaccines cannot be avoided and can sometimes lead to possible horizontal transmission as observed in humans vaccinated against polio, influenza and rotavirus [7–10]. Shedding of live vaccine virus from vaccinated animals in the context of oral vaccination against rabies is of special interest due to the potential of adverse reactions in non-target species [11]. Adverse reactions in non-target species, including humans, can result from direct contact with vaccine baits distributed or indirectly through contact with secretions of an animal vaccinated; for example, by being licked or bitten by a dog that consumed a bait. Actually, the only two reported adverse events associated with human contact and oral rabies vaccine baits

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involved dogs, although the baits distributed were targeted at raccoons [12,13]. To examine possible survival and shedding of SPBN GASGAS, saliva swabs and feces were collected during a number of studies where different target and non-target species received the vaccine construct by the oral route. This information will help to determine the potential risk of deliberate release in the environment of this construct in comparison with alternative oral rabies vaccines available.

2. Material & methods

2.1. Animals and ethical statement

Shedding was investigated in 8 different target and non-target species during several safety and immunogenicity studies; red fox (*Vulpes vulpes*), raccoon dog (*Nyctereutes procyonoides*), small Indian mongoose (*Herpestes auropunctatus*), raccoon (*Procyon lotor*), striped skunk (*Mephitis mephitis*), domestic dog (*Canis lupus familiaris*), domestic cat (*Felis catus*) and domestic pig (*Sus scrofa domesticus*) (Tables 1 and 2). Animals were obtained from commercial sources, except the small Indian mongoose; these animals were obtained through the Croatian Veterinary Institute in Zagreb and were captured on a rabies-free island off the Croatian coast.

All in vivo work was performed at IDT Biologika GmbH (IDT) according to European guidelines on animal welfare and care pursuant to the Federation of European Laboratory Animal Science Associations (FELASA). Study protocols were evaluated and approved by the responsible authorities (Landesverwaltungsamt Sachsen – Anhalt, Referat Verbraucherschutz, Veterinärangelegenheiten) in the federal state of Saxony-Anhalt, Germany; approval numbers AZ 42502-3-669 IDT, AZ 42502-3-658 IDT, AZ 42502-3-762 IDT. If possible, animals were housed together in small groups in the experimental animal facility at IDT Biologika GmbH. This was not always possible and consequently animals were kept in individual cages during several studies (see Table 2 column: Sample). In case animals were sedated during vaccine administration and sampling, a combination of ketamine-hydrochloride (Ketmanine 10%, Serumwerk Bernburg AG, 06406 Bernburg, Germany) and medetomidine-hydrochloride (Cepetor®, CP-Pharma GmbH, 31303 Burgdorf, Germany) was administered. Samples collected were examined at IDT, Friedrich Loeffler Institute (FLI) and/or Sate Office for Consumer Protection, Veterinary Medicine (LAV).

2.2. Vaccine

The vaccine construct SPBN GASGAS is derived from SAD L16, a cDNA clone of the oral rabies virus vaccine strain SAD B19. SPBN GASGAS lacks the pseudogene (ψ). Also, all three nucleotides were changed at amino acid positions 194 and 333 of the glycoprotein; position 194 – AAT [Asn] → TCC [Ser], position 333 – AGA [Arg] → GAG [Glu] [14]. As a result of the genetic modification at position 333 of the glycoprotein the construct is apathogenic for immunocompetent mice after i.c. administration. The site-directed mutagenesis at position 194 prevents reversion to virulence. Furthermore, the construct contains a second identical glycoprotein gene with modifications as described above. It was predicted that the overexpression of the rabies virus glycoprotein increased not only its efficacy but also its safety profile by reducing potential risk of reversion to virulence and increase of apoptosis [15,16]. Hence, the name SPBN GASGAS is derived from the introduced cloning sites (SmaI, PacI, BiswI and NheI), the two base exchanges (GA – glutamic acid, S – serine) and the expression of the two modified glycoprotein genes. Doses in the range of $10^{6.0}$–$10^{9.1}$ FFU/ml SPBN GASGAS were used in the studies listed in Tables 1 and 2. The virus material was produced according to the protocol given by Vos et al. [17]. Material for overdose studies with titers >$10^{8.0}$ FFU/ml was concentrated via tangential flow filtration using ultrafiltration flat sheet cassettes with a Molecular Weight Cut Off (MWCO) of 300 kDa. The vaccine was administered by the oral route; some animals received the vaccine by direct oral instillation and other animals were offered a vaccine bait.

2.3. Sampling

Saliva swabs were collected by using commercially available sterile dry swabs (Heinz Herenz Medizinalbedarf GmbH, Rudorffweg 10, 21031 Hamburg, Germany). The swabs were inserted into the oral cavity and gently moved back and forth in awake or anesthetized animals. Saliva samples taken on the day of vaccine administration were collected as close to the predefined times as possible. At each sampling point, 2 samples were taken and stored in medium (MEM; in-house produced modified Minimal Essential Medium Eagle) at −65 °C to −80 °C. The results obtained with the fecal samples are based on pooled samples in case the animals were housed in groups as no individual sampling was feasible. The

Table 1
Overview of the studies where saliva swabs were collected for shedding study purposes (Nr. – number of animals; d – day; h – hour); with 'mongoose' the small Indian mongoose is meant.

Animal species	Purpose	Nr.	Sampling schedule
Red fox	Screening[a]	4	0 h, 0.5 h, 1 h, 2 h
Red fox	Dissemination[c]	12	0 h, 2 h, 4 h, 1 d, 2 d, 3 d, 5 d, 7 d, 10 d
Red fox	Repeated dose	8	0 h, 2 h, 4 h, 1 d, 2 d, 3 d
Red fox	Immunogenicity	9	0 d, 1 d, 2 d, 3 d, 7 d, 14 d
Raccoon dog	Dissemination[c]	12	0 h, 2 h, 4 h, 1 d, 2 d, 3 d, 5 d, 7 d, 10 d
Raccoon dog	Repeated dose	8	0 h, 2 h, 4 h, 1 d, 2 d, 3 d
Raccoon dog	Immunogenicity	9	0 d, 1 d, 2 d, 3 d, 7 d, 14 d
Mongoose	Dissemination[c]	12	0 h, 2 h, 4 h, 1 d, 2 d, 3 d, 5 d, 7 d, 10 d
Mongoose	Immunogenicity	27	0 d, 1 d, 2 d, 3 d
Mongoose	Overdose	14[b]	0 d, 1–10 d, 14 d, 21 d, 28 d, 84 d, 140 d
Dog	Overdose	12	0 h, 1 h, 2 h, 4 h, 1 d, 2 d, 3 d
Dog	Efficacy	8	0 h, 1 h, 2 h, 4 h, 1 d, 2 d, 3 d
Cat	Overdose	12	0 h, 2 h, 4 h, 6 h, 1 d, 2 d, 3 d, 7 d, 8 d, 9 d, 13 d, 24 d, 28 d
Raccoon	Immunogenicity	8	0 h, 2 h, 1 d, 2 d, 3 d, 7 d, 10 d
Striped skunk	Screening[a]	4	0 h, 0.5 h, 1 h, 2 h
Domestic pig	Dissemination[c]	12	0 h, 2 h, 4 h, 1 d, 2 d

[a] The results shown for fox –screening and striped skunk – screening were previously reported in Vos et al. [4].
[b] Only from 12 animals samples were taken on day 84 and 140 post administration.
[c] On selected days animals were sacrificed to investigate the dissemination of the vaccine virus, hence not all animals were available anymore for the saliva swab collection schedule as listed in the table.

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