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Oral Rabies Vaccines (ORVs), an Alternative to the Parenteral Vaccination Strategy for Rabies Elimination in Stray Dogs - Risk and Benefits in Indian Perspectives

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ABSTRACT

Oral rabies vaccines (ORVs) have successfully eradicated rabies in wildlife, providing significant benefits over parenteral vaccination by reducing human resources, costs, and time demands. Despite these advantages, ORV use in domestic dogs presents unique challenges due to the higher risk of human exposure, potentially leading to vaccine-associated rabies cases. Limited genomic surveillance, inadequate screening of non-target animals, and insufficient funding for comprehensive surveillance impede the detection and reporting of such cases. Simulation models indicate that human risk from 1st and 2nd generation vaccine-associated rabies is significantly higher—approximately 19 times—when ORVs are used in dogs compared to wild animals. Regulatory and procedural adaptations are essential to address these barriers and enhance ORV safety for domestic dogs. Recommendations include encouraging manufacturers to secure central licensure for ORV use in dogs, and for the *World Organisation for Animal Health (WOAH)* to support regulatory convergence among member countries. Additionally, WOAH and the *United Against Rabies* initiative should adopt a structured, prequalification process to validate ORVs specifically for canine use, thereby enhancing global oversight and harmonizing standards. Establishing a global regulatory science agenda, spearheaded by the WOAH and WHO, could further advance ORV deployment, facilitating a standardized and safe application of ORVs in canine rabies control efforts.

HIGHLIGHTS

- Oral rabies vaccines (ORVs) have successfully eradicated rabies in wildlife.
- ORV use in domestic dogs presents unique challenges due to the higher risk of human exposure

Keywords: Dog rabies, wildlife rabies, oral rabies vaccine, parenteral rabies vaccine, safety of ORVs

A global target has been set to eliminate human death due to dog-mediated rabies by 2030, a nearly always lethal, however vaccine-preventable viral disease. Rabies is a zoonotic viral encephalitis that kills about 59,000 people around the globe every year. Although various modes of transmission occur, 99% of human rabies cases are due to a bite from a rabid dog (Chen *et al.,* 2023). Therefore, eliminating dog-mediated rabies is mandatory for any country to reduce the incidence of human rabies cases. Also in animals, it causes economic losses directly or indirectly affecting the local and national economy. As losses are relatively high for pastoral people in rural areas due to their major dependence on livestock. Mostly, rabies in livestock remains underreported in developing

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countries due to inadequate and inefficient reporting systems (Abdelmalik and Yahia, 2021).

Prevention, control, and eventual elimination of rabies in India, require a One Health approach with coordinated and concerted efforts by all stakeholders viz, human health, veterinary and wildlife sector, and urban and rural local self-government. Broadly the activities can be categorized as human health component (Pre and Post Exposure Prophylaxis (PrEP and PEP) against rabies virus), animal health component (mass dog vaccination and dog population management, proper solid waste management and other activities such as awareness generation and community engagement. Post Exposure Prophylaxis (PEP) for an individual with exposure to a potential rabid dog requires proper wound washing, multi-dose rabies vaccine, and instillation of rabies immunoglobulins in the wound as per the category of the wound.

PEP is the sole approach to prevent the death of any victim exposed to the Rabid dog. However, it will not impact the animal reservoir which can still transmit the disease and leave other members of the community vulnerable to acquiring the disease (Gibson *et al.,* 2022). On the other hand, mass dog vaccination is proven to be an effective strategy as vaccinating the domestic animal reservoir reduces the risk of rabies exposure to humans (Bucher *et al.,* 2023). The canine rabies vaccine was first introduced in 1915 where mass dog vaccination was implemented in the 1920s. Various countries have successfully eliminated dog-mediated rabies using this canine vaccination strategy. As the concept of herd immunity was familiarized in 1923, it was suggested not to vaccinate all the canine population; so, the required proportion of vaccination was derived (Coleman *et al.,* 1996). Thus, the World Health Organization (WHO) estimated that vaccinating 70% of the dog population of a particular region for seven consecutive years may control and potentially eliminate rabies which is cost-effective as well (Wallace *et al.,* 2017; Cleaveland *et al.,* 2018).

At present, parenteral vaccination of dogs is the only approach for eliminating dog-mediated rabies at a large scale. However, its implementation is very challenging due to the large inaccessible dog population, lack of trained manpower for vaccination, logistic challenges for scale-up, etc (Cliquet *et al.,* 2018). Parenteral vaccination by CVR techniques has led to tangible reductions in dog-mediated human rabies deaths in areas such as Bali, Indonesia, and Goa, India (Wallace *et al.,* 2017). The use of ORV has been projected as a complementary policy to the existing parenteral dog vaccination to upsurge overall coverage, particularly in large areas where animals are non-accessible. ORVs were successfully used in eliminating rabies in wildlife (Mahl *et al.,* 2014). However, in wild settings human and wildlife interface is very limited as vaccines contain live attenuated rabies virus, which may cause disease in humans; if not properly handled or administered. The incidence of human rabies caused by ORVs is extremely rare, but few are reported. In 2002, a 52-year-old woman in France died of rabies after being bitten by a dog that had recently received an ORV. The vaccine strain was identified as the cause of rabies. In 2009, a 53-year-old man in the United States died of rabies after being bitten by a bat that had been infected with a vaccine strain of rabies virus. The ORV had been distributed in the area where the bat was found (Blanton *et al.,* 2012). In 2011, a 32-year-old woman in Germany died of rabies after being bitten by a cat that had recently received an ORV. The vaccine strain was identified as the cause of rabies (Muhldorfer *et al.,* 2013). The feasibility of the ORV of stray dogs as a potential strategy for vaccinating larger canine population in urban settings needs to be scientifically investigated concerning the selection of vaccine candidate product profile, distribution methods, safety and efficacy, environmental factors, and potential risk of exposure of the vaccine virus to non-target species.

HISTORY OF ORAL RABIES VACCINES

Even though dog-mediated rabies was eradicated from European nations in the middle of the 19th century, wildlife started to emerge as a significant reservoir, which has since become a major public health concern (Freuling *et al.,* 2013). Hunting, trapping, and poisoning wild animals were used as part of the population reduction strategy to stop the transmission. In the 1970s, oral immunization of wild animals was investigated as a possible method of rabies control due to its controversial nature and potential influence on biodiversity (Mahl *et al.,* 2014). The types of ORVs that are now in use are genetically modified vaccines vector-based vaccines (VBV) and modified live virus (MLV), which are live attenuated vaccinations

(Cliquet *et al.,* 2018). Rabies virus strains used in the ORV for trailing in Dogs are given in Table 1.

Modified Live Vaccines (MLVs)

The Street-Alabama-Dufferin (SAD) strain of the rabies virus, which was first isolated from the salivary glands of a rabid dog in 1935 in the USA, is used in the majority of modern MLVs (Maki *et al.,* 2014). The SAD strain was extensively passaged in cell lines such as mice, pigs, chick embryos, and various cell lines for the production of an attenuated strain named Evelyn Rokitnicki Abelseth (ERA). Further, the SAD-Bern strain was produced through cells adapted in BHK-21 cell lines, which was the first field-trailed ORV (Fig. 1) (Steck *et al.,* 1982). Several strains such as SAD B19 and SAD 5/88 were derived from SAD-Bern. However, these 1st generation ORVs are associated with a potential risk for contamination as these

Table 1: Rabies virus Strain used in the ORV for trailing in Dogs

Type	Vaccine Strain	Vaccine Name and Manufacturer	Dog	
			Years in Use	**Used Countries**
Modified Live (1st generation)	**SAD Bern**	Lysvulpen, Bioveta, Czech Republic	1994	Tunisia
	SAD B19	Fuchsoral, Ceva, France	2001	Philippines
			1998	Turkey
	RV-97	Sinrab, FGBI ARRIAH, Russia	—	—
	VRC-RZ2	Kazakhstan laboratory	2017	Kazakhstan (laboratory)
	KMIEV-94	Institute of Experimental Veterinary, Belarus	—	—
Modified Live (2nd generation)	SAG 2	RABIGEN® Virbac, France	2007	India
			2020	Thailand
			2012	Morocco
Modified Live (3rd generation)	SPBN GASGAS	Rabitec® Ceva, France	2017	Haiti
			2020	Thailand
	ERA G333	Prokov, Russia	—	—
Vector–based (Vaccinia virus)	V-RG	Raboral V-RG® Boehringer Ingelheim, Germany	2000	Sri Lanka
			2005	USA (Laboratory)
Vector-based (Adenovirus)	AdRG1.3	ONRAB® Artemis Technologies Inc., Canada	2016	USA (laboratory)
			2007	China (laboratory)

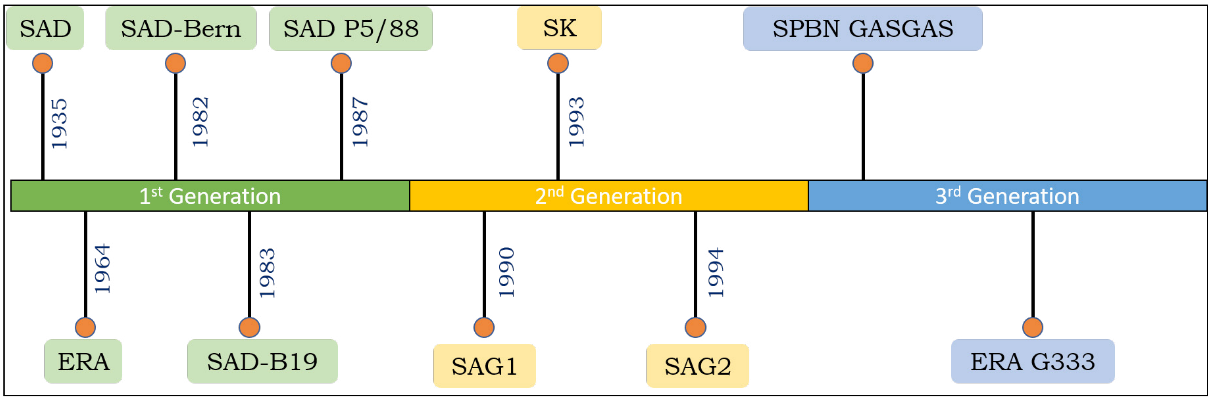

Fig. 1: Timeline of different generations of Modified Live Virus strains used in Oral Rabies Vaccines

strains still retained the residual pathogenicity in some animals (Hostink *et al.,* 2014). To produce safer ORVs, point mutations were introduced in the genome of SAD-Bern, as a result, 2nd generation ORVs such as SAG-1 and SAG-2 were produced (Muller *et al.,* 2015). Further, 3rd generation ORVs such as SPBN GASGAS and ERA G333 were developed by using reverse generation techniques. This was achieved by the site-directed mutagenesis in the G-Protein region, which is responsible for the pathogenicity of the virus (Wallace *et al.,* 2020; Kamp *et al.,* 2021).

Vector-Based Vaccines (VBVs)

People who are exposed to the ORV, there is a chance for vaccine-derived rabies, and need to receive the necessary PEP (Fehlner-Gardiner *et al.,* 2008). To address this problem, recombinant ORVs i.e. VBVs were developed to mitigate the risks associated with the introduction of live rabies viruses in the environment. VBVs are genetically modified vector viruses, which express the Rabies virus G-Protein through the encoded gene. Currently, two VBVs are approved i.e. Rabies vaccine strain V-RG and AdRG1.3, Vaccinia virus and Adenovirus based, respectively, for the control of wildlife rabies (Pastoret *et al.,* 1988; Rosatte *et al.,* 2009). The illness that these vector viruses can cause and their inability to effectively induce immunity in a subset of the population are possible drawbacks to these ORVs.

APPLICATIONS OF ORVS

Application of ORVs in Wildlife

The eradication of rabies from the wildlife population has been accomplished with the help of ORVs. Over the past forty years, the main concern in Europe and Canada has been fox-mediated rabies, which has been controlled through the widespread use of ORVs in forests. (Freuling *et al.,* 2013). The field trial of rabies vaccination through the oral route was first conducted in Switzerland in 1978 to control rabies transmission from red foxes (Steck *et al.,* 1982). Following this, other countries in the European Union (EU) such as Germany, France, and Belgium attempted the control wildlife rabies in the 1980s (Wandeler *et al.,* 1988). The EU provided partial funding to the member states that adopted ORVs as part of the National Rabies Elimination Programme, which significantly contributed to the elimination of fox rabies in Europe. Additionally, the EU assisted the non-EU neighbors that border Europe (Muller *et al.,* 2011). It is estimated that around 700 million ORV baits have been disseminated in the wild of about 30 European countries in the last four decades (Muller *et al.,* 2018). Since foxes in Finland, the Baltic states, and many other parts of Eastern Europe are potential rabies virus reservoirs, efforts to stop the virus's cycle of transmission using ORVs have been intensified (Mahl *et al.,* 2014).

To eradicate fox and raccoon rabies, recombinant VBVs were tested in the United States, Canada, and a few European and Asian nations. Because of the predominance of skunks and raccoons in the USA, as opposed to the wildlife rabies burden in red foxes from Europe and Canada. It is estimated that about 250 million doses of V-RG baits and 30 million AdRG1.3 baits have been distributed across the globe since its approval. Field application of this oral vaccine has successfully helped in the elimination of rabies from wildlife in a few European countries (Belgium, France, and Luxembourg) and of the dog/coyote rabies virus variant from the USA (Maki *et al.,* 2017).

Application of ORVs in domestic animals

Licensure of ORVs for dogs

While oral vaccines have never been used successfully in dog-mediated rabies control programs and are still an underappreciated tool for achieving dog rabies elimination, ORV has been a cornerstone in the over 40 years that the rabies virus has been eliminated from wildlife (Cliquet *et al.,* 2018; Yale *et al.,* 2022). This is majorly because, although various ORVs have been licensed for their application in wildlife, none of them were approved to be used in domestic animals (Wallace *et al.,* 2020). A national regulatory body carefully examines the product's safety on the target, potential non-target animals, and humans. Notably, vaccine licensing is not an international process since a product licensed in one nation may not be used in another without the required authorizations. Despite these, some researchers are advocating for the off-label use of ORVs for domestic dogs that have the highest level of safety and efficacy when used for wildlife (Yale *et al.,* 2022; Wallace *et al.,* 2020). However, this may not be feasible unless it is mandatory to obtain the prequalification for ORVs like human vaccines at the global level.

ORVs trailed on dogs

Most of the MLVs licensed for wildlife use have been trailed in dogs across the globe (Yale *et al.,* 2022). The 1st generation ORV has been trailed for the type of bait suitable for dogs, efficacy, cost-effectiveness, immunogenicity, and feasibility of using ORV baits in the field (Estrada *et al.,* 2001; Haddad *et al.,* 1994; Aylan *et al.,* 2000). The 2nd generation ORVs i.e. SAG-2 trailed in a few Asian and African countries on dogs. Although studies showed that it is feasible, safe, and efficacious, the sample size included was very small, which may not have statistical significance. SPBN GASGAS, a 3rd generation ORV, has been trailed in Haiti and Thailand. The initial studies show that this vaccine induces a comparable immune response over parenteral vaccination (Smith *et al.,* 2017; Leelahapongsathon *et al.,* 2020) However, the study suggests that the handout method is ideal as it reduces the risk associated with unintended contact with ORV. Unlike MLVs, the VBVs are trailed only at the laboratory level for their application in dogs, except for a single feasibility study conducted in Sri Lanka. Thus, more studies are required to analyze their efficacy and safety at the field level.

ORVs trails in India

Although being one of the highest rabies burden countries in the world, only a few ORV trials have been conducted in India. The safety and efficacy of the SAG 2 i.e. RABIGEN® Virbac were tested on Indian stray dogs in 2007 by Cliquet *et al.* Safety was tested by the absence of adverse clinical symptoms followed by vaccination. When they analyzed the efficacy of vaccinated dogs, all nine survived followed by a highly virulent street rabies virus challenge. The interesting fact is that out of nine only five were seroconverted. This shows that further research is required on this and, the sample size was too small to determine the safety and efficacy of this vaccine. However, no further trials were conducted in India on this vaccine. 11 years later in 2018 Ortman *et al.,* conducted a safety trial of SPBN GASGAS on Mongoose. This study found that animals were fond of being safe even after overdose, repeated doses, and different routes of administration. Similarly, there were no follow-up trials on this vaccine as well. Compared to other countries where ORV is used to control rabies either in wildlife or domestic animals, only a few trials have been conducted in India. Therefore, more trials in different contexts need to be performed to demonstrate safety, immunogenicity, and efficacy before approving them for field use.

SAFETY OF ORVS

Using widespread parenteral vaccination, nations confronting the canine rabies threat have so far been able to control the virus transmission. While ORVs have been instrumental in reducing wildlife rabies, safety regulations take precedence. While administering injectable vaccines to a specific number of animals involves more control, the use of ORVs may cause a purposeful release of live virus-containing baits into the environment, where they may be consumed by both target and non-target animals. Thus, the WOAH has formulated highly stringent international standards to determine the efficacy and safety of these vaccines. For ORVs that target dog populations, WOAH has set more extensive requirements than those of ORVs that applied in the wildlife sector, which indicates the importance of risk associated with it. This includes a human risk assessment that measures the probability of human coming into contact with ORVs and their potential health outcomes (WOAH, 2023).

A thorough assessment of the safety of target and non-target organisms, the potential for virus dissemination, field genetic stability, ecological impact and safety, and the mode of dissemination must be carried out before implementing any form of ORV dissemination.

Safety on Target Animals

All of the approved ORVs, including genetically modified and live vaccines against the rabies virus, are only used for wildlife in several nations, including the USA, Canada, Europe, Russia, Belarus, and Kazakhstan. However, trials have only been carried out on domestic animals, particularly on dogs (Cliquet *et al.,* 2018). Since dogs and humans are generally closely related, there is a high probability that young children and puppies may come into contact. Hence, vaccinations shouldn't result in rabies in humans, even if they are given in greater amounts than what is allowed in the field (WHO, 1995). The safety procedure for dogs is conducted primarily at the laboratory level followed by the field trail before implementing the actual bait distribution to the environment.

Several incidences of adverse reactions have been reported in dogs, which consumed baits containing 1st and 2nd generation live attenuated ORVs in a few countries. This includes gastrointestinal symptoms (vomiting, inappetence, constipation, or diarrhea) or behavioural symptoms (restlessness, listlessness, and unwillingness to continue hunting) (Nokireki *et al.,* 2016). It is to be noted that the same vaccine is safe for Raccoon dogs in wildlife (Cliquet *et al.,* 2006). Several cases of vaccine-virus-associated rabies cases were found in red foxes during the post-vaccination surveillance conducted in 2001, after the field distribution of about 97 million baits containing SAD B19 and SAD P5/88 in Germany and Austria in 1983 and 1986, respectively (Muller *et al.,* 2009). During the fox rabies elimination program in Slovenia, four vaccine-induced rabies cases were found in foxes with second-generation ORVs (Cerne *et al.,* 2021). This demonstrates unequivocally the occurrence of similar cases in which similar vaccine baits were distributed; however, these cases are significantly underreported due to the lack of effective service.

The 3rd generation ORVs such as SPBN GASGAS and ERA G333 have been studied through in vitro and in vivo trials among wild animals including red foxes, and raccoon dogs for their safety genetic stability, and efficacy in various countries. The details of studies related to the SPBN GASGAS on wild animals and dogs have been given in Table 2.

Similarly, various studies related to the safety and efficacy of recombinant ORVs in target animals have been conducted at both laboratory and field levels (Maki *et al.,* 2017; Muller *et al.,* 2020; Rosatte *et al.,* 2009). When it was administered to red fox and raccoon dogs, through multiple routes and even for a long time, the disease was not developed. These vaccines are being disseminated in the wild of the USA, Canada, France, Belgium, Luxembourg, Ukraine, Israel, and South Korea to control Rabies in raccoons, coyotes, grey foxes, red foxes, golden jackals, raccoon dogs, and striped skunks since 1987 (Maki *et al.,* 2017; Rosatte *et al.,* 2009). However, although it is not a live attenuated virus, there are other safety concerns associated with the vector virus i.e. vaccinia virus and adenovirus used in these ORVs. The major issue is the probable recombination of the vaccinia virus with other poxviruses in wild animals, which could lead to adverse events. Even though the cowpox virus has limited prevalence in the ORV target species, other orthopoxviruses have been detected in foxes, raccoons, skunks, etc. Moreover, the pre-existing immunity to the poxvirus might interfere with the immunogenicity of the ORVs, which may reduce the immunization rate (Root *et al.,* 2008). Except for one confined field trial, the use of recombinant ORVs in domestic dogs is restricted to laboratory trials. Further field trials are necessary to address the safety concerns related to the recombinant vaccine in dogs, even though it is safe and produces long-lasting immunity.

Safety on Non-Target Animals

Non-target animals are animals sharing the habitat with the target animals either in the wild or urban regions, which accidentally consume the baits containing ORVs. Like target animals, even in excess doses comparable to 10 times a concentration of field should not cause any disease to non-target animals (Mahl *et al.,* 2014). Because the field distribution will not limit the consumption of baits only by the target animals. In addition, before the field release of all types of ORVs, the safety of the vaccine should be demonstrated in local rodents and other non-human primates including chimpanzees, baboons, and rhesus monkeys (WHO, 2007). It is mandatory because several incidences of non-target injections of ORVs have occurred in the wild. For instance, during the field efficacy study of SPBN GASGAS in red foxes and raccoon dogs in Finland from 2017 to 2019, the anti-rabies antibodies were found in wild boar (*Sus scrofa*), indicating the consumption of distributed baits by non-target species (Vos *et al.,* 2021)

The 1st generation ORVs i.e. SAD Bern developed adverse events in wild rodents, wild and domestic carnivores, and baboons (Cliquet *et al.,* 2018). Similarly, SAD B19 had residual pathogenicity when offered to wild rodents and vaccine-induced rabies in nude mice (Vos *et al.,* 1999). The safety of 2nd generation ORV SAG2 was extensively studied in various non-target animals including, rodents

Table 2: Safety, and efficacy of 3rd generation ORV (SPBN GASGAS) on wild animals and domestic dogs

Genetic Stability study	Application	Target Species	Country	Immunogenicity	Safety
In vitro and *in vivo* passaging: 11 SNPs were detected with changes in the amino acids of N, G, and L genes of the Rabies Virus during the vivo passaging, without causing any virulence (Borutzki *et al.,* 2022).	Wildlife	Mongoose	India	—	The animal was fond of being safe even after overdose, repeated doses, and different routes of administration. The total study population n= 47 (Ortmann *et al.,* 2018).
		Red Fox	Germany	The anti-rabies antibody was found in animals even after one year.	None of them have died when challenged with the Fox Rabies virus. Total study population n= 48 (Freuling *et al.,* 2019).
		Red Fox, Raccoon dog	Germany	—	The vaccine did not cause any disease even when overdosed and repeated doses. It was not disseminated beyond the site of vaccination (Ortmann *et al.,* 2018).
		Red Fox, Raccoon dog	Finland	Field Study 2018-2019. It was found that the seroconversion rates are low owing to several factors (Vos *et al.,* 2021).	—
		Red Fox	Germany	A total of 22 captive animals were vaccinated (including 11 controls).	While all animals in the parenteral vaccination group were seroprotected and survived after the Rabies virus challenge, the ORV-treated group did not completely turn seroconverted and few deaths were noticed (Kamp *et al.,* 2021).
		Kudu	Namibia	A total of 10 animals were treated with ORVs and the seroconversion rate was very poor.	Out of 10 only 3 animals survived after treatment with the Rabies virus (Hassel *et al.,* 2018).
	Domestic	Dogs	Thailand	Study conducted in about 25 dogs and found anti-rabies antibody even after 1 year, although it is less compared to control parenteral group (Leelahapongsathon *et al.,* 2020).	—
		Dogs	Germany	Study conducted in about 35 dogs (including 10 controls) which treated with the ORVs. 13 out of 25 were seropositive (>0.5 IU/mL).	When challenged with Rabies virus after 6 months, 24 survived out of 25 (Bobe *et al.,* 2023).
		Dogs	Namibia	A total of 57 dogs were vaccinated orally (including 9 control) and about 78% of them were found to be seroconverted after 2 months (Molini *et al.,* 2021).	—
		Dogs	Indonesia	A total of 90 dogs (including 40 controls) were treated with ORVs were around 90% of the animals were seropositive (Saputra *et al.,* 2023).	—
		Dogs	Thailand	Around 1800 dogs from four provinces were vaccinated orally out of which about 1400 dogs were found to be seroconverted (Chanachai *et al.,* 2021).	—

(mouse, rat, vole, squirrel, gerbil, jerboa, meriones), carnivores (coyote, ferret, civet, mongoose, badger, genet), non-human primates (Chacma baboons), other mammals (hedgehog, wild boar, domestic goat, cow), and diurnal and nocturnal birds (crow, rook, buzzard, kite, owl) (Mahl *et al.,* 2014). This vaccine was inoculated orally, intramuscularly, and intracerebrally. However, the presence of live virus was detected even after 1 day of orally inoculated wild animals (Bingham *et al.,* 1997).

Similarly, the safety studies of 3rd generation ORVs in non-target animals have been studied in very few countries. In a study conducted in Germany, the safety of SPBN GASGAS was analyzed on selected non-target animals such as domestic cats, domestic pigs, and wild rodents (field mice, house mice, and guinea pigs). It was reported that no horizontal transmission was observed in the wild rodents, in non-target species, the virus was not disseminated from the site of entry and overdose did not cause any adverse events. However, a risk assessment needs to be conducted to identify possible non-target species for which additional safety studies need to be conducted in either wild or urban settings for this ORV (Ortmann *et al.,* 2018). Moreover, although the recombinant ORVs did not induce any adverse events in the non-target animals, the effect of the vector virus needs to be studied.

Safety on human and environmental concerns

In addition to safety studies conducted on both target and non-target animals, it is critical to look into the potential for disease transmission to humans through vaccinated animals and ORV spillage in the environment. The application of ORVs to domestic dogs is extremely difficult because of the increased likelihood of interaction between the vaccinated dog and human, even though it is widely accepted to use in the wild where there is less chance of human interference. Furthermore, it is extremely concerning that the live-attenuated rabies vaccine virus may leak into the environment (Cliquet *et al.,* 2018).

Humans may be exposed to the vaccine through contact with a freshly vaccinated dog either by licking or biting. Several cases of accidental exposure to ORVs in humans have been reported. When genetically modified ORV is applied in the wild of the USA to control raccoon rabies, 22 cases of accidental exposure to rabies have been reported, where 8 and 14 cases are human and domestic animals, respectively (BSN *et al.,* 2007). In addition, 83 and 55 human cases exposed to rabies vaccine-containing baits were reported from Ohio, USA, in 2011 and 2012, respectively (Kellogg *et al.,* 2012). Since these vaccines are genetically modified, no rabies-related symptoms were reported. However, human cases with an infection from vaccine vector virus, have been reported from other provinces of the country.

It is essential to rule out the absence of vaccine viruses in the saliva of the vaccinated animals. Because the viral secretion in saliva may indicate the local replication and consequently an increased risk of mutation, reversion to pathogenicity, and transmission. For instance, the presence of the vaccine virus was observed in the salivary excretion of dogs even after 3 days that consumed 1st generation ORVs (Haddad *et al.,* 1994). The 2nd generation SAG2 ORV was found in the salivary swabs of laboratory dogs provided with vaccine strain both intra-muscularly and orally (Cliquet *et al.,* 2018). In addition, the same strain was found in the tonsils and buccal mucosa of dogs till 96 hours might be due to the local replication (Orciari *et al.,* 2001).

The study conducted by Vos *et al.* in 2018 shows that even 3rd generation ORV i.e. SPBN GASGAS rabies virus strain detected in the salivary secretion after 4 hours of the 25% of the dogs analyzed after the ORV trial. This study analyzed the shedding of vaccine virus in various target and non-target species such as red fox, raccoon dog, small Indian mongoose, raccoon, striped skunk, domestic dog, domestic cat, and domestic pig. Interestingly, 50 out of 758 fecal samples were found to be viral RNA positive, however, no active virus was detected. In contrast, in about 248 of 1053 saliva samples, RNA fragments were detected for up to 10 days. Among those positives, 38 samples contained the actively replicating virus till 24 hours of the vaccination. This study indicates the presence of an active virus in the saliva of the vaccinated animal, which might be transmitted to the non-vaccinated candidates of the same species, non-target animals, and even to humans. The transmission of genetically modified ORVs from orally vaccinated wild animals viz red fox and raccoons to non-vaccinated counterparts was observed shortly after vaccination through a bite. Also, the horizontal transmission of ORV was observed between the raccoons and their progenies (Maki *et al.,* 2018). Therefore, Future research shall be targeted at determining the dynamics of oral vaccination, i.e. the primary sites of viral replication and the rapidity of clearance of candidate vaccines by using standardized procedures.

This evidence clearly shows the possibility of both horizontal and vertical transmission of the ORVs when applied to domestic dogs. Moreover, there is a chance of exposure to this ORV to humans by vaccinated dogs, as the dog menace is increasing day by day in the urban settings of the country. Considering this risk, the WHO recommended having PEP, in case of accidental exposure to the ORVs via mouth, nose, eye, or wound (WHO, 2007). Notably, unlike the MLVs, the genetically modified ORVs are not required to have the PEP. However, a few human cases of vector virus-associated infections have been reported in the past (Maki *et al.,* 2017). Thus, although, these vaccine type minimizes the risk associated with the MLVs, the adverse events caused by the vector virus further challenge their dissemination in the urban regions.

The other concern about the application of ORVs to domestic dogs is their environmental spillage. SPBN GASGAS when distributed in the wild of Finland, it was found that most of the bait consumed by Foxes and Raccoon dogs was seronegative owing to either the unpunctured capsules that contained the vaccine or spillover of the vaccine to the environment (Vos *et al.,* 2021). Notably, the package inserts from the manufacturer using this particular strain given that the stability of this vaccine virus in the environment is about 7 days. There was no study about the transmission of vaccine virus from the environment.

CONCLUSION AND FUTURE PERSPECTIVE

The use of ORVs to eradicate rabies in wild animals has proven effective in several nations. There is no doubt that the oral vaccination approach offers several advantages over parenteral vaccination in terms of human resources, economy, time, etc. in the prevention and control of rabies and the achievement of the global goal the "Zero by 30." However, it should also be remembered that when used in the field setting, there is minimal risk involved for target animals, non-target animals, and humans, irrespective of whether the ORV is genetically modified or live attenuated. Although only a few vaccine-associated rabies cases were reported from a few countries, despite the distribution of millions of baits containing rabies vaccine strains in the large geographical region for the past 40 years, however, it was clearly shown that these types of cases may be reasonably unreported. The key reasons for underreporting include (a). lack of effective surveillance post distribution of the baits, (b) lack of screening among the non-target animals that share the common habitat where the baits were distributed, (c) lack of technology or funding for the genomic surveillance to prove the vaccine-associated Rabies cases, and (d) minimal interaction with the human population. Therefore, the probability of vaccine-mediated rabies due to exposure to ORVs has not been exactly calculated to date.

According to international organizations, the application of ORVs to domestic dogs is more stringent than to wild animals. This is majorly due to the chance of human exposure to the vaccine virus. Moreover, as per the simulation models, the risk of 1st and 2nd generation-associated human deaths were 19 times greater when the ORV was applied to dogs than the wild animals. Although the 3rd generation ORVs are claimed to be safer than the previous generation vaccines, the existence of live viruses either in the saliva or environment may be associated with the risk. In addition, the research studies that have trailed these vaccines include only a minimal number of dogs, which may not produce significant results to generalize to a wider stray dog population of developing countries. Therefore, before considering the ORVs for the use of urban rabies control, the following suggestions may be fulfilled (a) The live vaccine from the secretion of vaccinated animals either orally or other routes will not cause disease to target animals, non-target animals, and humans, (b) the dynamics of the vaccine virus in the vaccinated animals with the shelf-life is extensively studied, (c) only the use of ORVs that are manufactured for domestic dogs, (d) the study results of environmental risk assessment that is fate of the ORVs released into the environment are available, and (e) an establishment of an effective pharmacovigilance system is in place to detect any possible human exposure to vaccine etc.

To overcome barriers to the licensure of oral rabies vaccines, several actions are recommended. First, although licensure can be a long, arduous, and expensive process, manufacturers should continue to seek central licensure for the use of their products in dogs. Second, WOAH should continue its efforts to promote the concept of vaccine regulatory convergence among WOAH member countries.

Third, although WOAH and WHO recognize the need for the use of animal vaccines off-label, a prospective approach for validating oral rabies vaccines, such as the WHO vaccine prequalification process, should be developed to provide more confidence in the use of oral rabies vaccines, both in field-trials and integration into mass parenteral vaccination programs. Fourth, prequalification should be a future requirement for any oral rabies vaccine to be used for dogs in projects funded or supervised by the United Against Rabies initiative, thereby creating an incentive for manufacturers to invest in this area. Finally, OIE and WHO should consider developing a global regulatory science agenda for oral rabies vaccines, like what is recommended for human vaccines.

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