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Mini Review

A Review on Safety and Potency Testing Of Capripoxvirus Vaccines

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Abstract

Capripoxvirus-induced diseases are commonly described as the most serious poxvirus diseases of production animals, as they have a significant impact on national and global economies. Therefore, they are classified as notifiable infectious diseases to the World Organization for Animal Health (WOAH) which need to be immediately declared due to their considerable and substantial economic impact in endemic regions. Prevention and control of capripoxvirus infections is mainly based on vaccination of susceptible animals. As the 3 capripoxvirus share major neutralization sites, cross-immunity has been reported. A variety of live attenuated and inactivated vaccines have been used for the control of capripoxvirus. In this review, we report safety and efficacy results on animals for different vaccines used against capripoxvirus. We focus precisely on capripoxvirus diseases, namely sheep pox, goat pox, and lumpy skin disease which have a high potential for infection, causing damage to small ruminants and cattle, economic loss due to trade restrictions, limitations on animal movement and implementation of vaccination campaigns.

Keywords: Animal testing; Capripoxvirus; Efficacy; Potency; Safety; Vaccine

Introduction

Vaccination is the most effective tool for the prevention and eradication of a wide range of infectious diseases [1]. Veterinary vaccines cannot only be used to protect animal health, but also to protect human health from zoonotic infections through animal vaccination, as exemplified by the vaccination of wildlife against rabies [2,3]. The field of vaccinology has yielded several effective vaccines that have significantly reduced the impact of some important diseases [4]. Vaccination has been instrumental in the world, such as eradication of smallpox disease in humans and rinderpest in cattle [5,6]. Following the dramatic outbreak of foot-and-mouth disease in the United Kingdom in 2001, and to a lesser extend in France and in The Netherlands, the European Union lightened its regulation and is nowadays more prone to consider emergency vaccination as an alternative to slaughtering [7,8]. It is estimated that veterinary vaccines are available for more than 400 diseases affecting mammals, birds and fish, including farm animals, pets and wildlife [9]. However, the development of veterinary vaccines is a challenging task, in part, due to a variety of pathogens, hosts, and the uniqueness of host-susceptibility to each pathogen, in addition to the animal testing and associated costs which is required for vaccines control [6].

Veterinary vaccines, like those produced for human use, are authorized according to high standards of quality, safety and efficacy [10]. Only animal trials are applicable to control the safety and efficacy of veterinary vaccines. The control of vaccines is regulated by monographs and guidelines, published by international pharmacopoeias and organizations (e.g. World Organization for Animal Health, European Medicines Agency),

to ensure the quality, safety and efficacy of the product. Animal testing is mandatory, but it is not easy to apply.

Special care should be taken in animal studies to ensure animal welfare [11,12]. Housing and husbandry should be appropriate for the purpose of the study and comply with local animal welfare regulations [13]. Euthanasia and necropsy of moribund animals is recommended [14]. Animal testing provides variable data with high rates of invalid testing, which increases the number of animals required and leads to repeated testing and animal suffering [15]. For this reason, the ethical guideline for animal experimentation is based on the principle of the 3Rs (replace, reduce, and refine). Animal testing is performed when no other suitable technique or method is available (replace), with a minimum number of animals (reduce) with less intensive methods (refine) [16,17]. The cost of animal testing is high due to the conditions and the difficulty of maintaining sufficient numbers of animals for several months. Although the 3Rs-concept has been widely accepted as a fundamental principle, the number of approved alternatives for *in vivo* testing is still limited [18]. In this review, we report on different methods used to control safety and potency testing in animals for vaccines against Capripoxvirus (CaPV). We have focused on Lumpy Skin Disease (LSD), Sheeppox (SPP) and Goat Pox (GTP), as they are commonly described as the most serious pox diseases of cattle, sheep and goats respectively, causing in endemic areas significant economic losses to the livestock industry [19,20].

Safety Control

Vaccine safety is of major importance to animal health and welfare. Safety must fundamentally determine that the benefits of the product outweigh any potential risks, not only to the target species being vaccinated, but also to the vaccine user, the environment, and the consumer in the case of animals from which food is derived [10]. CaPV vaccines must be safe for use in all ages, both sexes, and all breeds and species (WOAH Terrestrial Manual, 2021b). An ideal vaccine should be safe and not cause clinical disease or spread to unvaccinated animals. In addition, the vaccine should be inexpensive and thermostable [21-24]. Safety control is mandatory and follows the standards and norms recommended for vaccines in general. Safety monitoring is performed 4 hours after vaccination and daily during 14 days post vaccination (dpv) [25]. Therefore, body temperature is measured daily starting at-3 dpv. In addition, the injection sites are examined for adverse reactions. The local skin reaction at the vaccination site should be accepted, as it indicates that the vaccine virus is replicating and thus producing a good humoral and cell-mediated immunity in the vaccinated animals [26]. Live attenuated vaccines can be very effective because they induce both cellular and humoral immune responses [27], [28]. However, a major concern that is associated with the potential risk of reversion [29]. Inactivated vaccines are safer, but may be less effective than attenuated vaccines [4]. Adverse effects derived from vaccination should be minimally acceptable [6].

Live Attenuated Vaccines Against LSD

There are five vaccine strains available to control LSD: two from cattle (Neethling and SGPV Kenya), two from sheep (RM65 and Romania) and one from goats (Gorgan) [30-35]. Previously, the Romanian strain of the SPP vaccine was used to control the LSD outbreak in Egypt. However, heterologous vaccines are sometimes associated with local severe reactions in exotic cattle [36,37]. Therefore, homologous vaccine with Neethling strain of LSDV is now being widely used in cattle for protection against LSD [38]. Field experience with the use of LSD homologous vaccines has shown that vaccines cause side effects only when used for the first time. These can include temporary reduction in milk yield, risk of contamination by adventitious agents, local skin reaction at the vaccination site or generalized small size skin nodules referred to as “Neethling disease” [39-46]. Booster vaccination does not cause adverse reactions, even if the initial vaccine used was a heterologous vaccine [40].

The safety one dose and overdose (10x) of MEVAC vaccine (Neethling strain) was evaluated in different categories of animals. Other major physiological parameters such as rumination index, health index and milk yield were automatically monitored by specialized cow health management software [47-49]. Field studies conducted in Egypt and Vietnam confirmed the laboratory results, the vaccine was well tolerated by vaccinated animals with no or insignificant skin reactions and no change in health indices and milk yield. Abortion was reported in 0.3% of pregnant animals at 2-9 dpv, skin swelling in 0.6%, and local hyperreaction in 1.9% [49]. The administration of live attenuated LSD vaccines is not recommended in countries previously free of the disease or in the late stages of the disease, as their use compromises the “CaPV virus-free” status of the respective country [50].

LSD Inactivated Vaccines

The inactivated vaccines are completely safe because they consist only of dead pathogens [5]. Their non-replicative properties prevent transmission of the vaccine virus to cohabiting animals, reversion to virulence and assortment with virulent field strains [50,51]. To our knowledge, only two recent publications have reported the use of two inactivated vaccines against LSD in cattle. Hamdi et al. (2020), tested an inactivated LSD vaccine (Neethling strain) in cattle and it was safe and did not cause any adverse reaction. In addition, a field study in 181 cattle from 4 dairy farms in different regions of Bulgaria confirmed the safety of the vaccine [50]. The findings of Hamdi et al. (2020) were validated in a study by Wolff et al. 2021, where they confirmed that an inactivated vaccine against LSD did not induce any local adverse effects or fever and is therefore safe for administration in cattle [52].

Live Attenuated Vaccines Against SPP

Several locally produced SPP vaccines are available, particularly in the Indian subcontinent. Perego, Bakirkoy, Algerian, SGPV, Kenyan, Karnal, Pendik, RM/65, SPV/RH, Mathura SP8, Jaipur, Ranipet, Hyderabad, Mauritanian, Roumanian-Fanar, Kazakhstan, Chitinsk, Mongolian and Niski strains vaccines have been used with variable success. Indigenous strains are the right choice for control and eradication of the disease in endemic countries [53]. Live SPP vaccines have been shown to be safe in pregnant ewes and result in normal lambing. There was no virus shedding from the vaccinated ewes [53]. Following vaccination, live SPP vaccined animals showed a transient rise in temperature and produced local inflammation at the site of vaccination indicating the viability of the vaccine virus. Slight rise in body temperature is a physiological phenomenon and is triggered by any antigen. The potential to cause severe local reactions is considered one of the disadvantages of using live poxviruses [54]. However, there is no correlation between the formation or size of the reaction and protective immunity [55,56]. The side effects caused by SPP vaccine in naive calves are rarely seen compared to those caused by attenuated LSD vaccines. However, it has been shown that administration of a high dose of SPPV RM65 vaccine can cause typical vaccine side effects, such as generalized skin lesions in cattle [26].

SPP Inactivated Vaccines

An inactivated vaccine and a live attenuated Romanian SPP vaccine were compared for safety and efficacy [51]. The developed inactivated SPP vaccine was safer in vaccinated animals than the live attenuated vaccine. No increase in body temperature and no clinical signs with transient local inflammation were observed in a few vaccinated animals [51]. Another inactivated vaccine based on a local Egyptian strain was tested for safety and efficacy in lambs. The vaccine was found to be safe with no adverse reactions in vaccinated lambs [57].

Live Attenuated Vaccines Against GTP

A GTP vaccine using an indigenous strain (Uttarkashi/78) has been developed and commercialized in India. The vaccine was safe in both experimental and field trials. Vaccinated animals present a small skin reaction at the injection site with a slight rise in body temperature but, no adverse reaction even at the highest dose, and no horizontal transmission from the immunized to in-contact animals was observed. However, the vaccine is not recommended for use in pregnant animals as a precautionary measure [58]. Live and inactivated GTP vaccines were compared and tested for safety and efficacy in goats. The results showed that both vaccines were safe [59]. In Iran, live attenuated GTP vaccines are routinely used (Gorgan strain). The susceptibility of three pure breeds (Saanen, Alpine, and Murcia-Granada) and two hybrid breeds (Saanen-Mahabadi and Alpine-Mahabadi) vaccinated with live attenuated GTP vaccine was compared [60]. Pure Saanen goats were the most affected with 89.9% morbidity and 27% mortality. Only pure Saanen and Alpine breeds showed reactions to the GTP vaccine at 3 to 4 weeks pv. The Saanen were much more responsive to the GTP vaccine than the Alpine breed. This sensitivity to live GTP vaccines suggests that safer vaccines should be used in some breeds [60]. It is necessary to use the inactivated GTP vaccine in these pure imported animals.

Safety in Laboratory Animals

Safety testing of CaPV vaccines is performed on the target species. There is no alternative method in laboratory animals. Non-specific safety or residual toxicity testing in laboratory animals (mice and guinea pigs) is carried out for inactivated vaccines to detect possible extraneous toxic contaminants from the manufacturing process [61,62]. Animals were subcutaneously vaccinated with the vaccine and clinically observed for 7 dpv.

Potency Control

Models of experimental infection

LSDV: Several models have been described in the literature to evaluate the efficacy of vaccines against LSD [52,63]. In the first experimental study reported in Table 1, only half of the infected cattle developed clinical disease, although all of the infected animals became viraemic [64-67]. In addition, silent infections without skin lesions are known to occur in field outbreaks of LSDV [68]. The presence of asymptomatic viraemic animals capable of transmitting the virus via arthropod vectors complicates the control and eradication of LSDV [24].

Study	N° of animals	Age	Strain/ virus titer	Volume/ Route	Observation duration/ monitoring	Clinical findings	Reference
1	5	1-2 years	LSDV field isolate 2×10^5 log $TCID_{50}$/ml	IV (2ml)	1 month: body temperature, clinical signs and serology	60% of animals were clinically sick with high fever, ocular and nasal discharge, enlarged superficial lymph nodes, circumscribed large swelling at the site of inoculation, pox lesions on the skin and nasal and oral mucosa.	[32]
2	6	9-10 months	LSD Neethling vaccine strain $10^{7.8}$ $TCID_{50}$/ml	IV (3ml) SC (1ml)	28 days : body temperature, clinical score, serological and molecular tests (EDTA blood, serum, nasal and oral swabs)	No fever and no generalized skin lesions. 2 cattle showed massive reactions at the site of SC inoculation.	[69]
	6	9-10 months	LSDV-Macedonia 2016 field strain $10^{7.4}$ $TCID_{50}$/ml	IV (3ml) SC (1ml)	28 days : body temperature, clinical score, serological and molecular tests (EDTA blood, serum, nasal and oral swabs)	50% of inoculated calves developed severe clinical symptoms (fever and generalized skin lesions) and high virus loads in collected samples.	
3	6	4-6 months	LSDV-Macedonia 2016 field strain 10^2 $CCID_{50}$ 10^4 $CCID_{50}$ 10^6 $CCID_{50}$ 10^7 $CCID_{50}$	IV (6ml)	3 weeks: clinical symptoms, viremia and viral shedding	Animals with moderate to severe clinical signs. Characteristic pox-like skin lesions, sporadic or were generalized in some animals. Virus titer of 10^5 to 10^6 $CCID_{50}/_{mL}$ of "Macedonia 2016" provides a robust and sufficient challenge model.	[70]
5	5	4-6 months	LSDV Israeli field isolate ($10^{6.5}$ $TCID_{50}$/100 µl)	IV (5ml) ID (1ml)	3 weeks: body temperature, clinical signs and viral DNA in blood and oral swabs.	60% of the animals showed a severe disease with generalized nodules over the whole body between 7 and 8 dpi, with viremia and positive oral swabs. Typical LSD lesions were observed at necropsy and confirmed positive by PCR	[50]
6	20 (5 per group)	6 months	LSDV Israeli field isolate ($10^{7.5}$ $TCID_{50}$/100 ml)	IV (5ml) ID (121ml)	3 weeks: body temperature, clinical signs, serological analysis and viremia	Two to three animals in each of the four control groups developed typical LSDV skin nodules with the onset between 6 and 8 dpi. All animals developed fever and viremia was detected only in animals with nodules.	[63]

7	6	4-6 months	LSDV-Macedonia 2016 field strain 10^7CCID_{50}/ml	IV (3ml) SC (1ml)	28 days: body temperature, clinical reaction score, serological and molecular analysis (nasal swabs)	Fever, moderate to severe clinical signs with clinical reaction scores between 4 and 10. High viremia in all animals. High viral genome loads in swabs.	[52]
7	3	4-6 months	LSDV (LSD/ KN1/2020) 5×10^6 $TCID_{50}$	ID (1 ml) neck	2 weeks: Body temperature and clinical signs	Fever and severe LSD symptoms (swelling between 3.5 and 5.0 cm in diameter at the injection site).	[49]

*IV: intravenous; SC: subcutaneous; $CCID_{50}$: cell culture infectious $dose_{50}$; $TCID_{50:}$ tissue culture infectious $dose_{50}$

Table 1: experimental studies reporting LSDV challenge model.

In the second experimental infection model using the protection index (PI) (Table 2), skin swelling at the viral injection site equal to or greater than 0.5 cm in diameter was considered a positive reaction. The infectious titers for each animal and the group average were calculated. If the difference between the challenge virus titers of the vaccinated and control groups was 0.75 or less, the animal was not considered immune. If the difference was between 0.80 and 1.4, the animal was considered to have very low immunity. The difference between 1.5 and 2.5 indicated moderate immunity, and a difference of 2.6 log or more indicated strong immunity [49,71,72].

Study	N° of animals	Age	Strain/ virus titer	Volume/ Route	Observation duration	Clinical findings	Reference
1	3	6-12 months	LSD Dermatitis nodulares/2016/ Atyrau/KZ" -field strain 6.25 log $TCID_{50}$/ml 10^{-1} to 10^{-4}	ID (0.25ml), four replicates	3 weeks: body temperature, general clinical reaction and skin reaction at the inoculation sites.	Fever, enlarged superficial lymph nodes, swelling at the inoculation sites, skin nodules and pox lesions on the nasal and oral mucosa. The average viral titer was 5.9 log ID_{50}/0.25 mL	[73]
2	2	Dairy cows	LSDV field isolate- $5x10^6TCID_{50}$ 10^{-1} to 10^{-4}	ID (0.1ml), four replicates	3 weeks: body temperature, clinical signs and skin reactions at inoculation sites	Edematous swelling at inoculation sites, but replicates receiving the most diluted inoculate showed little to no response.	[49]

Table 2: experimental studies reported the LSDV challenge model using the protection index.

In the third experimental model, calves were inoculated by two different routes, either by needle inoculation (IV + ID routes) or by LSDV-positive blood-feeding arthropods (*S. calcitrans* and *Ae. aegypti*), a route which is more representative of virus transmission in the field [67]. Following infection of calves by the IV and ID routes, 41% of needle-inoculated calves developed clinical disease characterized by multifocal necrotic skin nodules and lymphadenopathy. In comparison, 80% of the arthropod-inoculated calves developed clinical disease characterized by fever, swelling and necrosis at the inoculation sites, enlarged lymph nodes, and early viremia (3 dpi). A variable LSDV-specific IFN-g immune response was detected in the needle-inoculated calves, with no difference between clinical calves and non-clinical calves. In contrast, a robust and uniform cell-mediated immune response was detected in all clinical arthropod-inoculated calves, with little response detected in the non-clinical arthropod-inoculated calves. Comparison of the production of anti-LSDV IgM and IgG antibodies revealed no difference between clinical and non-clinical needle-inoculated calves, however a strong IgM response was evident in the non-clinical arthropod-inoculated calves but absent in the clinical arthropod-inoculated calves [74].

SPP/ GTP

The most commonly used challenge model to infect small ruminants with SPPV or GTPV is virus titration by ID injection of serial dilutions of the strain into the flank of animals (Table 3) [53,75-77]. The development of a hypersensitivity response after challenge is

an indication of protection in vaccinated animals, and this response is attributed to cytotoxic T cells [78,79]. PI in vaccinated animals ranging from 4.7 to 5.2 indicate complete and long-lasting protection in these species against SPPV and GTPV infections [80].

Species	Number	Age	Virus	Dose/route	Observation duration	Results	Reference
Sheep	8	3-6 months	Field isolate M'sila, $10^{5.05 \pm 0,45}$ ID_{50} /0,2 ml	ID route in the flank of the animals at ten fold dilutions (10^{-1} to 10^{-6}) (4 sites)	14 days: rectal temperature and the development of inflammation in each of the injection sites	Fever, local and general reactions. Infectious titer: 5,43 ± 0,12 $IDCC_{50}$	[81]
Sheep	4	3-4 months	Virulent SPV-R virus	SC injection with 10^4SID_{50}	14 days: temperature, clinical signs and general reactions	Hyperthermia, local reaction, and severe clinical signs of SPPV	[82]
Goats	3	3 months	virulent field virus GTPV	10^4GID_{50}/0.2 mL at the rate of 0.2 mL ID on the ventral aspect of the tail.	14 days	Fever, mucopurulent nasal discharge, cough, cutaneous pock lesions at the inoculation site and all over the body	[83]
Sheep	3	1-1.5 year	SPPV (Srin 38/00 strain), $10^{6.5}$ SRID50/ml	8 dilutions in triplicates inoculated ID at 0.1 ml/site along the abdomen	14 days	Fever, progressed to characteristic SPPV lesions. The $SRID_{50}$ was $10^{6.5}$	[84]
Sheep	2	6-8M	virulent field SPPV strain (HELD)	ID route in the flank of the animals at ten fold dilutions (10^{-1} to 10^{-6}) (5 sites)	14 days: rectal temperature, local inflammation reaction and clinical signs.	Fever, local reactions at the injection sites. Typical SPPV skin nodules. The virus titers obtained were 5.5 and 5.9 log10 ID_{50}/ml	[85,86]
sheep	10	6-12M	Virulent SPPV $10^{4.5}$ $TCID_{50}$/ml	ID in the area under the tail fold	14 days : rectal temperature and clinical signs.	Fever, local reaction and typical clinical signs of SPPV. Clinical score of 15.2.	[87]
Goats	8	6M	virulent isolate of GTPV (Vietnamese)	$10^{5.4}TCID_{50}$ per mL 0.5 ml SC	14 days: temperature and clinical signs based on clinical score.	All inoculated goats displayed clinical signs, including varying degrees of hyperthermia, loss of appetite, inactivity, and skin lesions. Some infected animals developed a severe disease, while other goats exhibited moderate to mild clinical signs.	[88]

Goats	5	6-12M	Virulent GTPV 2×10^6 $SRIC_{50}$%	0.1ml ID per point (five sites on each site of abdomen)	14 days: rectal temperature, appearance of local skin lesions, generalized infection and serological analysis.	Fever, severe local skin reaction. Generalized infection.	[89]

*GID: goats infective dose $CCID_{50}$= 50% cell culture infective dose SID_{50}= 50% skin infective dose

SRID50 = 50% skin reaction infective dose $TCID_{50}$: 50% tissue culture infective dose

Table 3: experimental studies reported SPPV and GTPV challenge model.

Efficacy test

Efficacy testing is performed to ensure that vaccines induce protective immunity after administration. Challenge studies with virulent strains are the most accurate way to measure the protection. Efficacy testing of live vaccines is mainly determined by *in vivo* titration by vaccinating animals with a fixed dose and challenging them with variable doses of a virulent strain. However, potency testing of inactivated vaccines is often based on vaccination with variable doses of vaccine and challenge with a fixed dose of virus, as is done for foot-and-mouth disease vaccines to determine the 50% protective dose..gree of sequence conservation, and therefore may cross-protect to a different degree than homologous virus, allowing the hypothesis that there is a potential for using a single vaccine to protect against all CaPV infections [90-92].

Vaccines Against LSD

The efficacy and immunogenicity of the homologous LSD vaccine is known to be excellent, providing good protection of cattle against virulent field strains [22,93-95]. The field efficacy of the live attenuated vaccines was demonstrated between 2016 and 2017, when LSD outbreaks in southeastern Europe were successfully eliminated by mass vaccination with homologous Neethling strain vaccines [93]. Importantly, the efficacy of the Neethling strain vaccines was experimentally evaluated in a challenge study by the LSD reference laboratory in Sciensano, Belgium [63]. The Neethling strain based vaccine was used in six Balkan countries in 2016-2017, and the average percentage of its effecacy was 79.8% (range=62.5-97%) [96]. Efficacy of Mevac vaccine (Neethling strain) was evaluated using two different tests, PI and challenge test. The PI obtained was ≥ 2.5 log10, indicating protection [49,72]. The challenge experiment was conducted in Vietnam and animals were challenged 28 dpv (5×10^6 $TCID_{50,}$ ID). Vaccinated calves were protected, and the PI value was 3.5 log10 [49].The efficacy of the KS1 O-180 vaccine strain against natural LSD infection under field conditions has been published. Over 60% of the herd owners reported that the vaccine had a low efficacy in protecting animals against clinical LSD with no adverse reactions. The severity of the disease was significantly reduced in vaccinated animals compared to unvaccinated animals [97]. In 2020, Hamdi et al. and Wolff et al. published their results, demonstrating the efficacy of the inactivated LSD Neethling vaccine compared to live vaccine in cattle [50,52]. Complete clinical protection was achieved with the inactivated vaccine with no viremia or viral shedding, but low levels of viral DNA were found in skin samples from a few animals with very high Ct values [50]. Wolff et al. achieved only partial protection against LSDV. There were local reactions at the site of virus inoculation and some animals showed mild viremia and virus shedding in collected swabs [52]. The results obtained confirm that control of LSDV with an inactivated vaccine, similar to SPPV [57,98] and GTPV [59,99,100], may be possible. However, there are publications claiming only a short duration of protection after vaccination with inactivated CaPV virus vaccines [50-52,101].

Vaccines against SPPV

A number of SPPV strains have been used with variable success Perego, Bakirkoy, Algerian, SGPV, Kenyan, Karnal, Pendik, RM/65, SPV/RH, Mathura SP8, Jaipur, Ranipet, Hyderabad, Mauritanian, Roumanian-Fanar, Kazakhstan, Chitinsk and Mongolian (USSR) and Niski [102]. The efficacy of live attenuated SPV vaccine (Ranipet strain) was tested in lambs. The vaccine was safe and effective in the field study and all animals were protected against virulent challenge [82]. In the study by Yogisharadhya et al. 2011, the efficacy and potency of two live attenuated SPV vaccines (SPPV-Srinagar and SPPV-Romanian-Fanar) were tested by challenge with PI. Vaccines were found to be immunogenic and efficacious, with PI greater than log $10^{4.0}$ and log $10^{3.25}$ for SPPV-Srin and SPPV-RF vaccines, respectively [84]. The Romanian SPPV and the Yugoslavian RM65, are widely used in endemic countries to protect sheep against the disease [36,103,104]. The Romania SPP strain has also been used to vaccinate goats with controversial results. Adapted SPV vaccine (Kenyan strain) protects goats against virulent GPV [105,106]. In Saudi Arabia, Abuelzein et al. 2003 noted the occurrence of

GTP disease in animals vaccinated with the local Romanian SPP vaccine [107], while the same authors recommended vaccination of goats with the same vaccine at 3 months of age with an annual booster [108]. Rao et al. (2000) and Abdelfatah et al. (2018) reported that the vaccine did not protect of goats against GTPV [54,109]. Abdelfatah et al. 2019, observed that vaccination of goats with Romania strain induced cell-mediated immunity with a satisfactory PBMC and lymphocyte proliferation levels [110]. In the study by Hamdi et al. 2020, sheep and goats vaccinated with Romania SPPV vaccine were fully protected against challenge with virulent SPPV and GTPV strains, respectively. However, small ruminants vaccinated with LSDV Neethling vaccine showed only partial protection against challenge with virulent SPPV strain [86]. Attenuated SPPV vaccines, such as KSGP O-240, Yugoslavian RM65 and Romanian SPPV strains, have been used against LSDV [91,111]. The Saudi Arabians vaccinate their cattle every six months with a dose ten times higher than that used in sheep [111,112]. However, several studies reported incomplete protection against LSD in cattle vaccinated with all SPP vaccines [111,113-115]. In the study of Ayelet et al. 2013, a field study showed that the Kenyan SPP vaccine strain used to control LSD did not provide the expected protection. Out of a total of 476 animals observed, 22.9% and 2.31% cattle were found sick and dead due to LSD, respectively. This finding is in agreement with the report from Egypt [116,117] and Israel [91] who reported the occurrence of LSD outbreaks after vaccination of cattle with SPP vaccine. Partial protection have been also recorded in cattle vaccinated with Romania SPPV [86]. As an initial response to the recent LSD outbreaks, Turkey, Georgia, and Azerbaijan have used a Turkish Bakirköy SPPV strain vaccine to vaccinate cattle at doses three to ten times higher than those used in sheep [118].

In Kazakhstan, a local vaccine based on the Niskhi SPPV strain was tested in cattle. The SPPV strain provided complete protection for experimental calves with an average protection index of 5.3 ± 1.4 ID_{50}/0.25 ml [71]. The elimination of LSD using SPP vaccines was neither as complete nor as effective as the success of the homologous vaccine in the Balkans [23]. The study of Boumart and co-authors (2016), published the results of their detailed study comparing the efficacy of a live attenuated and an inactivated SPPV vaccine. After challenge infection, sheep vaccinated with inactivated SPPV showed no clinical signs typical of SPPV infection, excet for elevated body temperature for two days and a hypersensitivity reaction at the inoculation site of the challenge infection were observed. In addition, the PI of the inactivated SPPV vaccine was comparable to that of the live vaccine [51]. The results of the present study are consistent with other studies, demonstrating the efficacy of inactivated SPP vaccines in protecting sheep against challenge [57,82]. The inactivated SPV vaccine using the local Egyptian strain of SPPV was safe and inducing protection in vaccinated lambs after challenge with the virulent SPPV at 6 months pv. Specific antibodies appeared from the first week pv and remained until the 4th week post challenge [57].

Vaccines against GTP

Live and inactivated GTP vaccines have been reported using different strains of GTPV in goats [100,119,120]. Few attempts have been made in the past to develop live attenuated vaccines for GTP [120]. An attenuated live GTP vaccine has been developed by the Indian Veterinary Research Institute and the vaccine has been tested in laboratory and field trials. The vaccine provides complete protection of vaccinated goats against high dose of challenge even at low dose [58,121]. In the study of Barman et al. 2010, vaccination with live attenuated GTP vaccine produced at the Institute of Animal Health & Veterinary Biologicals, Kolkata, showed that vaccinated goats challenged 3 weeks pv (with 1:16 serum neutralizing antibody titre) were fully protected. The protective titre of 1:16 can be considered as achieved early (21 dpv) and maintained up to 1 year pv [83]. It has been reported that sheep vaccinated with GTP were protected against SPP and vice versa in goats [122], although, some claim otherwise [123]. However, an earlier study reported the failure of SPP vaccine to protect goats against GPV, while GTP vaccine provided solid immunity against both SPV and GPV in sheep [124]. In 2015, the efficacy of the Gorgan GTP strain vaccine against LSD was evaluated in Ethiopian cattle, using challenge and monitoring of the immune responses in vaccinated animals in the field. The vaccine provided good protection and seroconversion in cattle against clinical signs of the highly virulent LSD field strain [32]. In Kazakhstan, a local vaccine based on the GTPV strain (G2-LKW) was tested in cattle. The GTPV strain showed a stronger protective response and provided complete protection against LSD in calves with an average protection index of 5.9 [71]. In the study of Abitaev et al., 2022, the GTPV vaccine (G20-LKV strain) was tested in cattle by challenge with PT. All the vaccinated animals resisted to the challenge without showing any clinical signs of the disease [125]. In a study of Bhanuprakash et al. 2022 an attenuated live GTP vaccine (GTPV/ Uttarkashi/1978 strain) was evaluated for duration of immunity following a single dose vaccination in goats during 52 months pv. Long-term immunity was evaluated by serological monitoring and challenge. The rise in the level of antibodies reached a maximum at 21 dpv and were maintained for 2 years pv, with a steady decline. On challenge by ID route at 12, 24, 42, and 52 months pv, protection was evident in all vaccinated animals (100%). [89].

Serological Testing

LSD Vaccines

Antibodies are thought to play an important role in the early stages of pi. Seroconversion measured by either VNT or

ELISA, starts approximately 10-15 dpv and reaches the peak levels around one month pv, after which titers gradually decline. These results are consistent with previous reports [46]. It is known that not all vaccinated animals seroconvert despite being fully protected against LSD [30,78,126]. Therefore, the measurement of antibodies alone may not provide sufficient data on the protection status of the vaccinated animals, which must be taken into account when evaluating the effectiveness of vaccination campaigns in vaccinated herds.

The inactivated vaccine induced a higher humoral antibody response compared to what is normally observed with the live vaccine. In fact, serological response to live vaccine is around 50% positive animals which is in agreement with other authors. One study reported VNT antibodies in 50% of vaccinated cattle [50], while another study reported a seroconversion rate between 34% and 65% with live attenuated LSD vaccine [126,127].

Serological monitoring of cattle vaccinated with Mevac vaccine was carried out during 42 dpv by using ELISA (ID Screen Capripox Double Antigen ELISA kit) and VNT [49]. Immunogenicity studies showed a mean positive ELISA of 51.7±30.6%, while the mean positive titers by VNT (≥1.2 log10) was 78.38±15.18% [49]. Overall, the observed low serological responses have been explained by the significant role of cellular immunity in protecting against the disease [128,129]. Milovanović et al. (2019), showed that only 33% of vaccinated cattle remained ELISA positive 11 months pv, while VNT was positive in 35.06% of vaccinated animals. A booster vaccination was given 12 months after the initial vaccination. Five months after the booster vaccination, 57% of the vaccinated animals remained positive. Approximately 27% of cattle did not seroconvert after both the first and the second vaccinations [126]. A field study was conducted with the inactivated LSD vaccine in 181 cattle in Bulgaria and animals were sampled for serology during 360 dpv and tested by both VNT and ELISA. The response showed that 80% of the cattle seroconverted at 28 dpv and 68% seroconverted at 120 dpv using the ELISA test. Using VNT, the percentage recorded was of 70% [50].

SPP Vaccines

Neutralizing antibodies play a role in long-term protection against SPPV, and have been shown to be long-lasting in follow-up studies of animals vaccinated against SPPV [76,86,130]. Immunogenicity and potency of six strains of SPPV (Istanbul, Djelfa, RM 65, Romania, KSG and IPA) were tested in sheep. Two strains, among those studied present immunogenic characteristics (Djelfa and Romania strains). Antibodies detected were 1.39 and 1.61 at 6 months pv, and 1.10 and 1.26 at 12 months pv, respectively [81]. Serological monitoring of an inactivated Romanian SPPV vaccine was assessed in comparison with a live attenuated Romanian SPPV vaccine using VNT [51]. In animals vaccinated with the inactivated vaccine, antibodies appeared at 7 dpv. Compared to the live vaccine, they registered similar values on D14 and D21, but reached a significantly higher value of antibody neutralizing titer (2.1 log10) on D28. In animals vaccinated with live vaccine, the increase of antibodies was noted later on 14 dpv and showed a slight decrease on D28 to reach a value of 1 log10. Most sheep vaccinated with inactivated vaccine showed an increase in antibody titre after the booster. Immunity persisted for at least 9 months pv and stabilized at 1 to 2.1 log10 [51]. The humoral response of the commercial vaccine SPPV RM/ 65 used in Algeria was evaluated in sheep during 360 dpv using VNT [131]. Neutralizing antibodies obtained at 1 month pv ranged from 0.87 to 0.98. They then increased at 90 dpv with an average between 1.02 and 1.22. At day 365, neutralizing titers ranged between 0.73 to 1.22. The results obtained show that the neutralization index never reaches the recommended value (1.5). However, the neutralization index obtained is similar to that observed by Achour et al. (2000) after vaccination with RM/65 strain [131]. A single vaccination of sheep with a combined PPR (N75/1) and SPP (NISKhI) vaccine provided reliable protection of animals against two simultaneous infections for one year pv. Using VNT, antibodies against PPRV persisted for up to 12 months, with slight fluctuations. From 7 to 21 dpv, there was an increase in the average anti-SPP antibody titers in the sera of vaccinated sheep, reaching 3.0-5.2 log2. The antibodies developed were maintained for up to 6 months pv with insignificant fluctuations. Furthermore, a steady decrease in titer was observed, reaching only 1.9 log2 at the end of the experiment [87].

GTP Vaccines

Vaccination of goats with GTP vaccine showed a uniform increase in serum neutralizing antibody titre (1:16) at 21 dpv, which peaked at 3 months pv (1:32) and persisted up to 1 year pv [83]. Live GTP vaccine was tested in goats by serological monitoring using VNT. The vaccine was able to induce immunity within 7 dpv, peaking at 21 days. The titre remained at a protective level in the range of 1.82 ± 0.05 to 2.11 ± 0.05 throughout the one-year study [132]. A neutralization index of ≥ 1.5 was considered as positive [132,133]. The duration of immunity after single-dose vaccination with live attenuated GTP vaccine (Uttarkashi/1978 strain) was evaluated in goats for 52 months pv. The study showed that the vaccine could induce immunity against the disease within 7 dpv and reached a peak at 21 days, with 4- to 64-fold increase in serum neutralizing antibody titers. SN titers declined over time, with a 4- to 32-fold difference at 1 year pv and a 2- to 32-fold difference at 24 and 42 months pv. Persistence of antibodies was evident at 52 months pv, with 8- to 16-fold difference in SN titer [89].

Cellular Immunity

Several studies examining immune responses to CaPV vaccination indicate that both a cell-mediated and a humoral immune response are generated [69,76,77,86,126,130,134]. Animals that recover from a virulent CaPV infection generate lifelong immunity (humoral and cellular) that protects the animals against all CaPV isolates [30,135]. The unique characteristic of CaPV is that most of the progeny virus remains within the infected cells, releasing few virions that results in the low levels of extracellular virus. Circulating antibody limits the spread of virus in affected animals, but does not prevent replication at the site of infection. Local cell-to-cell spread of infection effectively protects the virus from circulating antibodies. Maternal immunity provides virus protection for up to 3 months [136,137]. The role of cell-mediated immunity (CMI) in LSD is particularly poorly understood [31,138,139]. This immune response, driven primarily by T lymphocytes, results in the production of key cytokines including type II IFN (IFN-g), which is produced by CD4+ helper T cells, CD8+ cytotoxic T cells, ɣd T cells, natural killer T cells, and NK cells [140]. IFN-g and other cytokines induced by the CMI response have a number of functions, including the activation of NK cells and macrophages and the inducation of class switching of immunoglobulins from activated plasma B cells [141-143].

The role of antibodies in protection against CaPV was demonstrated by passive transfer of sera from infected sheep, which protected the recipient sheep against CaPV challenge, suggesting that antibodies alone are sufficient for protection [144]. A field study was conducted with a live attenuated SPV vaccine (Ranipet strain) in 660 sheep. Blood samples were collected from 10% of the vaccinated animals during 6 months pv. the blood samples were tested to study the humoral and cellular responses. Humoral response was measured by VNT and cellular response was measured by Glucose Utilization Test (GUT). A significant difference was found between the vaccinated and control sheep in both measures of immunity [82]. Cattle vaccinated with Gorgan GTP vaccine, showed strong cellular immune responses at the vaccination site as measured by delayed-type hypersensitivity reactions, indicating a high level of immunogenicity [32]. To evaluate the cell-mediated immune response in animals vaccinated with LSDV inactivated vaccine, interferon gamma (IFN-γ) levels were examined in heparin blood using the Bovigam TB kit The IFN-γ present in the plasma supernatant of each blood sample was determined using a sandwich ELISA. Thirteen among 15 vaccinated animals responded to vaccination with IFN-γ, 9 strongly and 4 moderately [50]. More recently, complex immune assays have quantified IFN-g, a key biomarker of the CMI response, in cattle that had been vaccinated or challenged, or both [63,145,146], with evidence suggesting the involvement of CD4+ and CD8+ T cells in the production of IFN-g [145]. However, the kinetics and magnitude of the CMI response to LSDV and its role in disease protection are not yet understood. The immune response of calves to LSDV inoculation was evaluated by measuring the levels of the pro-inflammatory cytokine IFN-g and the anti-inflammatory cytokine IL-10 in using an ELISA test. No IFN-g or IL-10 was detected in the serum of inoculated calves at any time point, demonstrating that LSDV infection does not induce high systemic levels of these cytokines in either clinical or non-clinical animals [74].

Conclusion

SPPV, GTPV, and LSDV are the most notifiable transboundary diseases on the World Organization for Animal Health (WOAH) list. There is always a constant threat of CaPV spreading to new geographical areas though trade in livestock and their products. Vaccination is the most effective way to control and eradicate infectious diseases. Control programs should be monitored by a well-organized vaccination, using sufficient coverage and effective vaccines. The vaccines produced are subject to strict safety and quality control standards. For these reasons, in this work we report the safety and efficacy animal testing for the control of CaPV vaccine.

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