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Optimal immunization strategies for Saanen goats against goatpox

Hossein Esmaeili[1,3*], Seyed Mehdi Joghataei[1], Zeinab Hamidiya[2], Ehsan Baghal Arani[1], Amir pasha Shakeri[1], Zia Nosrat Rad[1] and Mohammad Mahdi Safari[1]

Abstract

Background Goatpox is a highly contagious disease caused by the *Capripoxvirus*, primarily affecting sheep and goats. Breeds like the Saanen are particularly vulnerable, especially in enzootic areas, and face risks not only from the disease itself but also from adverse reactions to live attenuated vaccines. This study compares inactivated and attenuated vaccines to find the safest and most effective vaccination strategy for Saanen goats against the Goatpox Virus (GTPV). In this study, 375 pure-breed Saanen goats were strategically divided into four groups to explore the most effective vaccination protocols for combating combinations of vaccines. In contrast, one group remained unvaccinated as a control. After vaccination, the goats were challenged by exposure to naturally infected animals to assess the vaccines' protective efficacy. PCR assays and the Virus Neutralization method were also conducted.

Results Group G1 exhibited no adverse reactions following two inactivated vaccines, with only mild and brief signs observed in a small number of goats (2%) after the live attenuated vaccine. Group G2, which received an inactivated vaccine followed by a live attenuated vaccine, had mild lesions in 18.66% of the goats after vaccination. In contrast, Group G3, which only received the live attenuated vaccine, showed a high morbidity rate of 82% and a mortality rate of 22%, with severe clinical signs and Pox lesions. Following the challenge, no signs of GTPV infection were observed in Groups G1, G2, and G3, whereas the control group exhibited 100% morbidity and 72% mortality, confirming the vaccine's protective efficacy.

Conclusion This study found that a protocol using two inactivated vaccine doses at one-month intervals, followed by a live attenuated vaccine and annual boosters, effectively immunizes vulnerable breeds against GTPV without causing adverse reactions. This approach prevents complications and supports breeding in GTPV-enzootic regions.

Keywords Saanen, Goats, Goatpox, Vaccination, Pox lesions, Live attenuated goatpox vaccine

*Correspondence:
Hossein Esmaeili
hesmaeli@ut.ac.ir
[1]Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[2]Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[3]Faculty of Veterinary Medicine, University of Tehran, Azadi Street, Postal code: 1419963114, Tehran, Iran

Background

Pox disease is an acute to chronic condition in sheep and goats, highly contagious and marked by widespread pox lesions. Goatpox is a viral disease of both sheep and goats, presenting with fever, widespread papules or nodules, occasional vesicles, internal lesions especially in the lungs, and potential death. The disease is caused by *Capripoxvirus* (CaPV) strains, which can infect both species. While most strains lead to more severe clinical disease in one species over the other, some strains affect both species equally. The causative agents of Goatpox are the Sheeppox Virus (SPPV) and Goatpox Virus (GTPV), which, along with the lumpy skin disease virus, comprise the genus CaPV within the Poxviridae family [1–4]. Goatpox is enzootic in regions such as Africa north of the Equator, the west of Asia, and Asia, with recent outbreaks in parts of Europe. Malignant forms of Goatpox are found in the west of Asia, Turkey, Greece, the Far East, and Africa north of the Equator [2, 5–7]. Various attenuated live and inactivated CaPV vaccines have been utilized to protect against GTPV. Importantly, this disease does not infect humans [8].

The clinical signs and post-mortem lesions of Goatpox vary depending on the host breed and viral strain. Indigenous breeds in enzootic regions are generally more resistant and tend to develop mild lesions, whereas exotic breeds are highly susceptible and may experience severe disease [1, 9, 10]. The introduction of exotic breeds, such as Saanen and Alpine goats, into enzootic areas presents a significant challenge, as these breeds often suffer from higher morbidity and mortality rates upon exposure to GTPV [11, 12]. Kids with waned maternal antibodies, isolated animals, and goats imported from non-enzootic regions are particularly vulnerable to Goatpox. Those introduced to enzootic areas from isolated villages face an even higher risk, especially after experiencing stress from long-distance travel and mingling with other sheep and goats. In such cases, the disease can become generalized and, at times, fatal [12–14].

Goatpox control strategies vary depending on regional disease prevalence. In non-enzootic areas, eradication is primarily achieved through culling infected and exposed animals [15]. In contrast, in enzootic regions, mass vaccination remains the most widely recognized strategy for disease prevention [12, 16, 17]. Despite extensive vaccination efforts, sporadic outbreaks continue to occur in regions such as Iran and other parts of western Asia, necessitating ongoing disease control measures [1, 3, 4, 12, 18]. The effectiveness of these measures is often influenced by factors such as animal movement, management practices, and vaccination protocols.

Routine vaccination using live attenuated or inactivated virus strains is essential for Goatpox prevention in endemic areas. Live attenuated vaccines are widely used due to their ability to induce long-term protective immunity in vaccinated animals. However, inactivated vaccines provide only short-term immunity and are generally not as effective [2, 4, 13]. Inactivated vaccines produced from tissue culture contain only the intracellular mature virion form of the virus and lack the biologically significant extracellular enveloped virion form. Consequently, they fail to stimulate immunity against the extracellular virion, resulting in suboptimal protection [12, 16, 19, 20].

Although live attenuated Goatpox vaccines are generally safe and effective, they can cause adverse reactions in certain susceptible breeds, such as Saanen goats [12, 13, 19, 21]. In enzootic regions, goats are typically vaccinated within their first year of life, and indigenous breeds rarely exhibit adverse reactions post-vaccination. However, in highly sensitive breeds, such as Saanen and Alpine goats, vaccine-induced side effects can lead to economic losses due to disease-related complications and reduced productivity [12–14]. Given these challenges, optimizing vaccination strategies is crucial to ensuring effective disease prevention while minimizing the risk of adverse effects.

Unprotected imported breeds of sheep and goats in enzootic countries typically experience high mortality rates following GTPV infection [12]. The increased susceptibility of imported Saanen and Alpine breeds to GTPV has been repeatedly observed in Iran [13, 20, 22]. Since Iran, like other enzootic countries, employs attenuated live vaccines to control Goatpox, unwanted side effects are common in sensitive breeds like Saanen after vaccination with this vaccine. The present study aims to introduce the best and safest protocol/strategy for protecting sensitive breeds such as Saanen against Goatpox using both inactivated and live attenuated vaccines. This field study seeks to propose the most effective vaccination strategy to protect this sensitive breed against Goatpox.

Results

Monitoring of adverse reactions

Based on the investigations conducted during clinical examinations and PCR tests before vaccination in all studied groups, no infection related to GTPV was detected in the animals. The PCR results showed no amplicon for the 390 bp fragment, confirming the absence of the infectious agent.

Notable results were observed among the studied groups following the implementation of different vaccination protocols (Fig. 1). In Group G1, no reactions were noted after receiving the two inactivated vaccines; however, after receiving the live attenuated vaccine at six months, very mild signs were observed in only three goats (2%). Only limited and temporary skin lesions were observed beneath the tails of these animals (Fig. 2 -(a)).

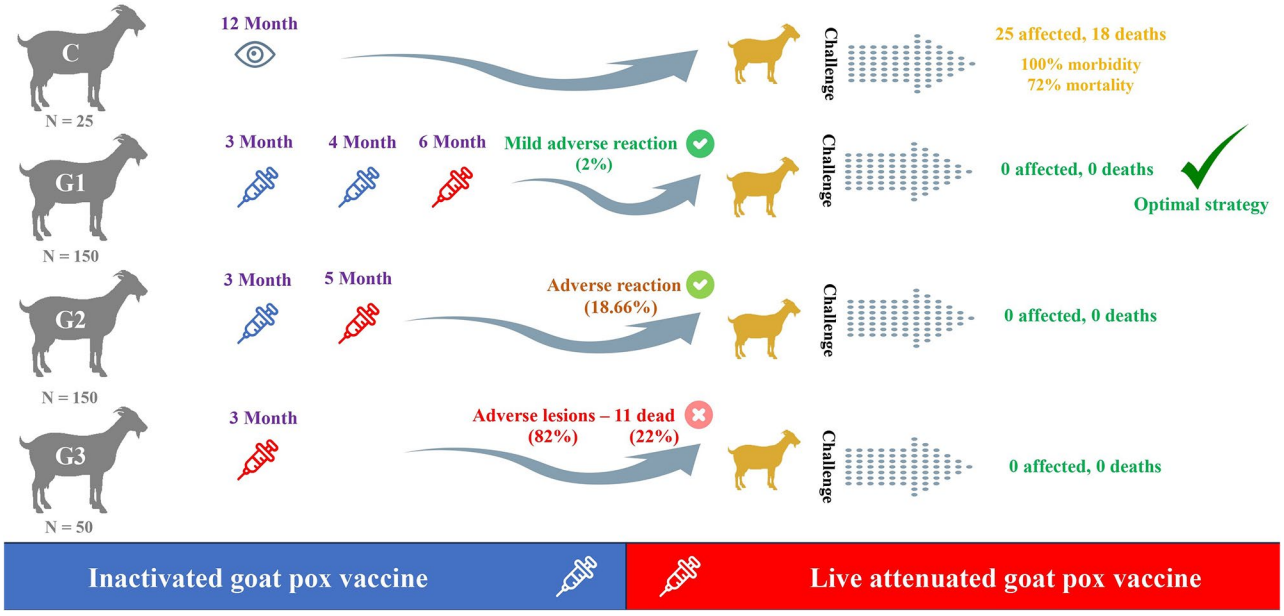

Fig. 1 A schematic illustration depicting the experimental groups and the methodology employed in the study

These reactions were transient and subsided gradually without affecting the animal's health. In these three animals, the rectal temperature increased slightly for 48 h and then returned to normal. The aforementioned mild lesions were observed 2 to 5 days after the increase in body temperature. In the subsequent years, this group of goats received an attenuated live vaccine with no adverse reactions post-inoculation. Impressively, no GTPV infections were recorded. The number of surviving animals in the subsequent years was 140 in the first year, 132 in the second year, and 124 in the third year. Deaths in all groups during these years were due to causes unrelated to GTPV.

In Group G2, which received an inactivated vaccine at three months and a live attenuated vaccine at five months, 28 goats (18.66%) showed mild lesions. In this group, the lesions were observed under the tail and were mostly at the pustular stage (Fig. 2-(b)). The lesions healed over the next three weeks. In this group, a mild increase in body temperature was observed after vaccination, lasting until 72 h post-vaccination before returning to normal. In the subsequent years, the herd size dwindled to 138 in the first year, 130 in the second, and 119 in the third.

In Group G3, which received only the live attenuated vaccine, 41 animals exhibited signs of Goatpox (morbidity 82%), with 11 goats dying (mortality 22%). Adverse reactions in this group began 18 to 25 days after vaccination, with new lesions continuing to develop over the next two weeks. In this group, a slight increase in body temperature was observed after vaccination, lasting for 5 to 7 days before returning to normal, accompanied by a transitory inflammation at the vaccination site. Affected animals were depressed, lacked appetite, and, in severe cases, experienced laboured breathing. Other clinical findings were harsh lung sound, rhinitis, conjunctivitis, and nasal mucopurulent discharge. Extensive and characteristic lesions of Goatpox in Saanen goats of this group initially appeared on the hairless parts of the body (under the tail, inside the ears, around the mouth and eyes, and sometimes inside the buccal cavity and external genitalia) then, in more severe cases, these lesions spread throughout the body (Fig. 2-(c)).

In Group G3, mortality began one week after the first appearance of lesions. Post-mortem examinations revealed pox lesions on the surface of the lungs, the mucosa of the trachea and bronchi, and the mesentery. Pneumonia was a predominant feature in all necropsied animals. PCR assay of the samples obtained from the lesions detected only the presence of the 218 bp amplicon, which represents the vaccine strain, and no amplicon associated with the presence of the field virus was observed. Over the following years, the herd size reduced to 31 in the first year, 26 in the second, and 21 in the third. In the subsequent years, when the healthy and surviving animals from the G1, G2, and G3 groups received an annual live attenuated booster, no adverse reactions were observed, and there were no cases of GTPV infection among the healthy goats.

Serology

The serum-neutralizing antibody titers of all 40 samples taken before vaccination were negative, but neutralizing antibodies were detected after immunization (Fig. 3).

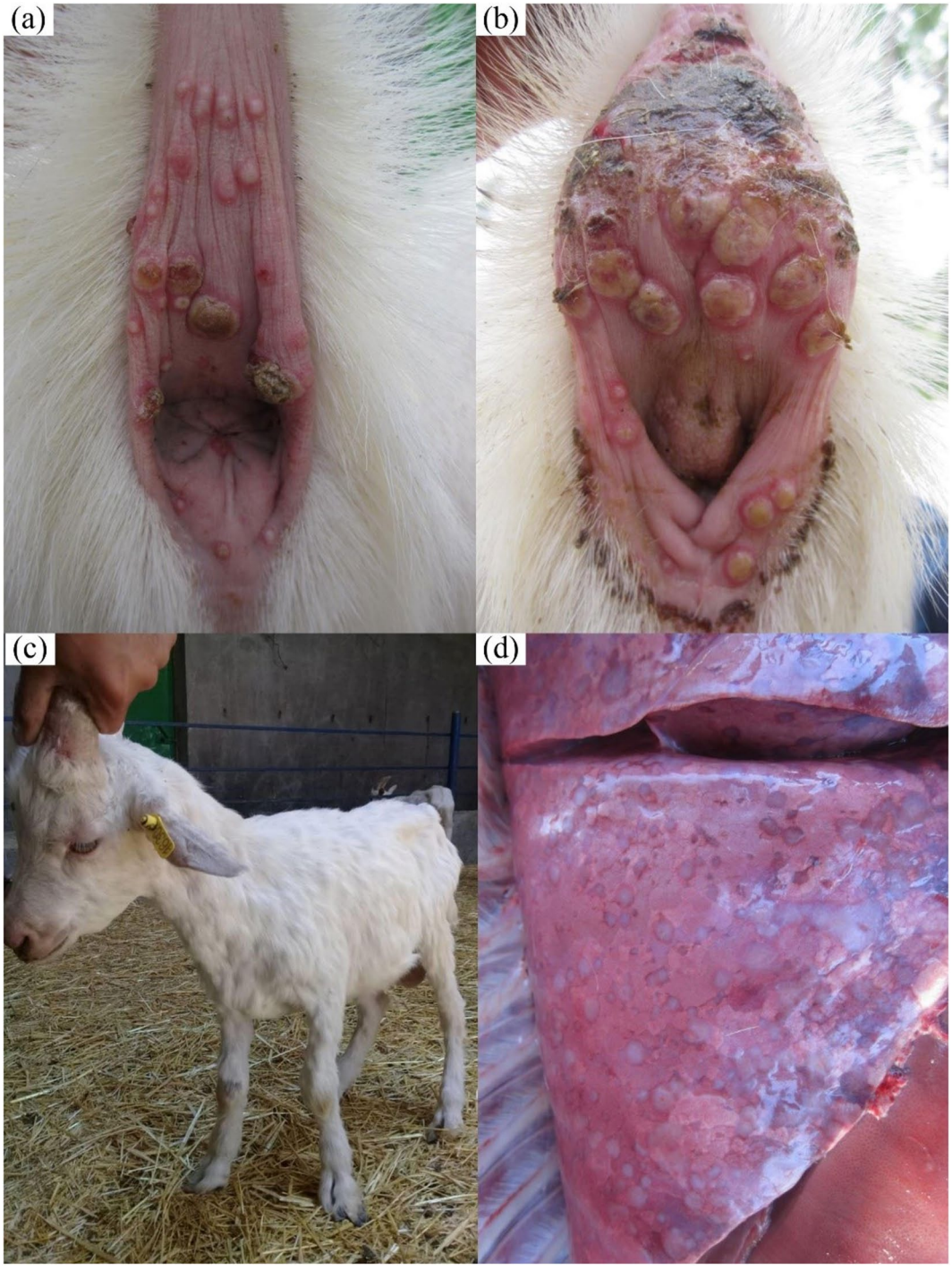

Fig. 2 (**a**) Photograph of mild lesions under the tail of a Saanen goat from the G1 group, which had received two inactivated vaccines followed by a live attenuated vaccine. The lesions observed were confined to this area. (**b**) A Saanen goat in Group G2 exhibited mild pox lesions under the tail after receiving both inactivated and live attenuated vaccines. Pay attention to the presence of pus inside the pustules. (**c**) A Saanen kid from Group G3 displayed widespread pox lesions following the administration of a live attenuated vaccine. (**d**) A necropsy photo showing remarkable lung lesions in a Saanen goat from the control group followed an experimental challenge and infection with the field strain. Multifocal nodular pneumonia is evident, characterized by nodules of varying colors—pale or red—distributed throughout the lung tissue, ranging from several millimeters to 2 to 4 cm in diameter

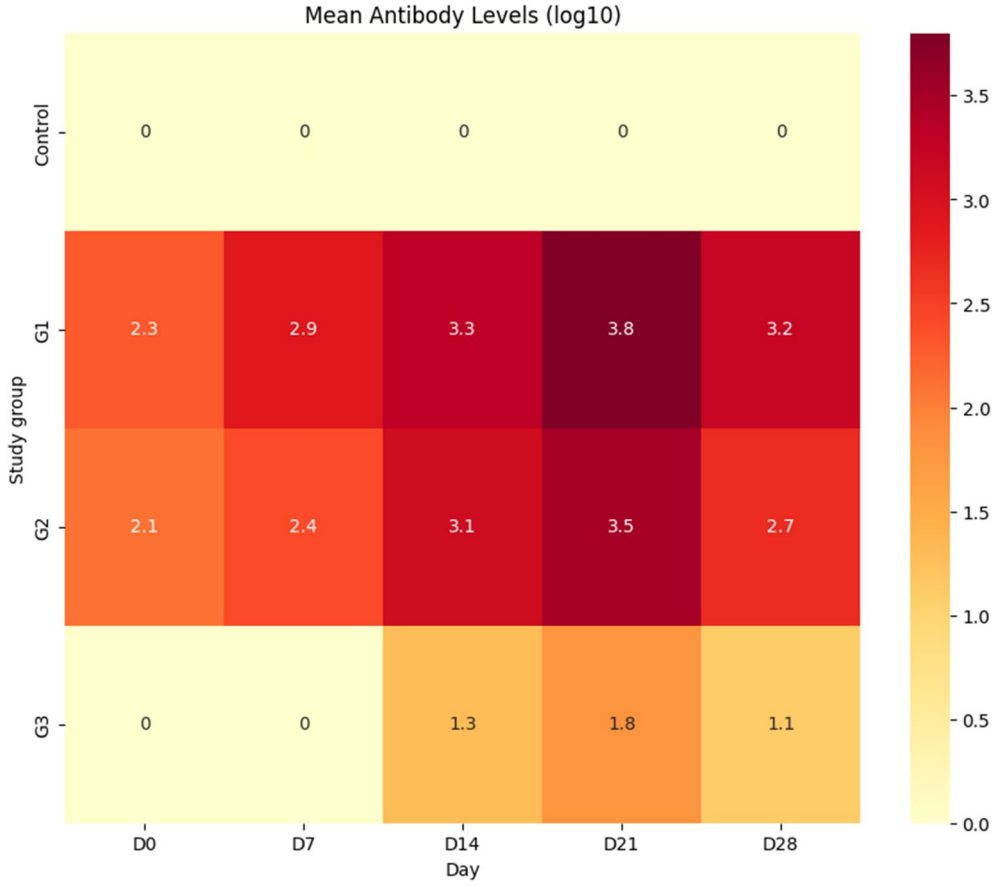

Fig. 3 Heat map illustrating changes in mean antibody levels over time across different study groups. Each cell in the heatmap represents the mean antibody level (log_{10}) for a specific group and day

After vaccination, Group G1 showed the highest antibody levels, reaching 3.8 log_{10} by Day 28. Group G2 peaked at 3.5 log_{10}, while Group G3 reached 1.8 log_{10} by Day 21 before declining to 1.1 log_{10} by Day 28. Overall, the data suggests that the combination of inactivated and live vaccines (as seen in G1 and G2) resulted in a more robust and sustained increase in antibody levels compared to the group that received only the live vaccine (G3) (Fig. 4).

Experimental infection

In the control group, clinical signs of GTPV infection were observed in the animals starting from the ninth day after the challenge (Fig. 5). The morbidity rate in this group was 100%, with 25 cases. The mortality rate was 72%, resulting in the deaths of 18 animals. The PCR assay results detected the 302 bp amplicon in the infected animals, indicating that these animals were infected with the GTPV field strain. Preceding the onset of the challenge, the control group exhibited no evidence of Goatpox infection. Subsequently, this group received an annual booster of attenuated live vaccine. In groups G1, G2, and G3, no signs associated with infection with GTPV were observed among the goats after the challenge, and all the animals remained healthy until the end of the experiment (Fig. 1).

Necropsies of dead animals in the control group revealed that skin lesions were less pronounced in those with the acute form of the disease compared to live animals. The majority of the body's lymph nodes were significantly swollen and exhibited noticeable edema. Pale areas approximately 1 cm in diameter were seen on the surfaces of the kidney, liver, and testicles. Multiple hard lesions, up to two cm in diameter, were commonly found in the lungs, particularly in the diaphragm lobes (Fig. 2-**(d)**). In some cases, papules, occasionally injured, were noted on the mucous membrane of the udder and sometimes on the walls of the rumen and large intestine, as well as on the tongue, hard and soft palate, trachea, and esophagus.

Discussion

In the current study, we have identified an effective and safe strategy to help sensitive Saanen goats adapt to the live attenuated Goatpox vaccine, significantly reducing adverse reactions after vaccination. These findings not

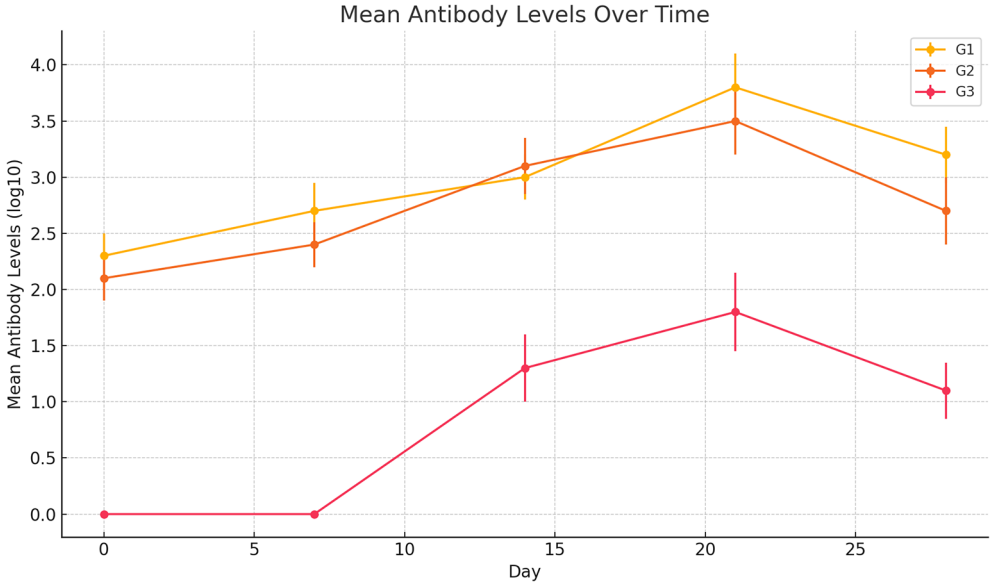

Fig. 4 Chart depicting mean antibody levels over time for study groups G1, G2, and G3. The y-axis represents the mean antibody level (log_{10}), and the x-axis shows the measurement day

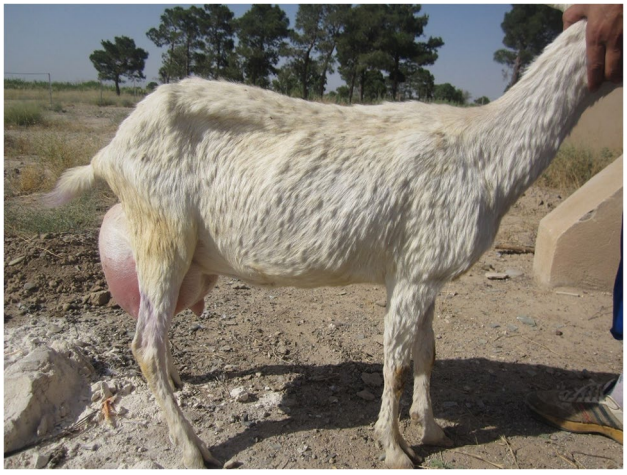

Fig. 5 The pox lesions are prominently visible across the entire body of a Saanen goat from the control group following exposure to infected animals (after the challenge). This indicates widespread manifestation of the disease characterized by visible lesions affecting various parts of the body

only pave the way for enhanced disease management in this specific breed but also establish a foundation for future research into veterinary immunization strategies for other sensitive breeds.

The results obtained from the G3 group in the present study align with the findings of numerous previous studies, which have consistently indicated that the live attenuated Goatpox vaccine can lead to the spread of GTPV-related infection in Saanen purebred [1, 13, 14]. This underscores the importance of carefully planning vaccination strategies, especially for livestock introduced into enzootic areas. The G1 group protocol, combining inactivated and live attenuated vaccines, proves effective in immunizing goats and preventing adverse reactions. Annual boosters with the live vaccine also cause no complications, and no GTPV cases are reported in this group in the following years.

When an animal sensitive to a live vaccine or virus is exposed for the first time, it often exhibits signs due to an overactive immune response. This sensitivity occurs because the animal's immune system encounters the virus without prior experience, leading to a delayed or inadequate production of specific antibodies. The immune system is essentially unprepared to fight off the virus effectively, resulting in the manifestation of disease signs [23]. However, this sensitivity can be significantly reduced or eliminated when the vaccine is administered twice, or when an inactivated vaccine is used prior to a live attenuated vaccine [24, 25].

Administering an inactivated vaccine twice or using it as an initial step, as demonstrated in this study, is an effective strategy for reducing sensitization in animals that are initially vulnerable to the virus. The first dose of the inactivated vaccine introduces a killed form of the virus, stimulating the immune system to produce antibodies and memory cells that recognize the pathogen. Mild clinical signs may occur, but the first dose helps train the immune system. The second dose boosts this response, enabling quicker and stronger virus neutralization. Priming with an inactivated vaccine offers a safer way to expose the immune system to antigens, reducing sensitivity and improving the effectiveness of later live vaccine doses [23, 26, 27].

Findings from the G2 group show that giving a live attenuated vaccine after a single inactivated dose leads to adverse reactions. Although no GTPV signs appear

during annual vaccination, this highlights how vaccine type and sequence can affect immune response and effectiveness [28, 29]. Comparatively, the protocol used for the G1 group, which included two doses of inactivated and one dose of live attenuated vaccines, resulted in significantly fewer adverse reactions ($p < 0.05$). This suggests combining inactivated and live attenuated vaccines may confer a more balanced immune response while minimizing adverse effects. Such a comprehensive vaccination strategy could be particularly beneficial in populations susceptible to GTPV infection, as it may provide enhanced protection without compromising safety.

Furthermore, numerous studies indicate that inactivated vaccines are generally less effective than live attenuated vaccines and are insufficient for long-term immunity against sheep and Goatpox. Live attenuated vaccines produce more potent and longer-lasting immunity with fewer doses than inactivated vaccines [12, 30]. For instance, Bhanuprakash et al. (2006) highlight the necessity of multiple doses or booster shots of inactivated vaccines in enzootic areas to achieve optimal immunity [30]. Similarly, Kitching and Carn (1999) emphasize that inactivated vaccines may require several doses to reach the desired level of protection compared to the robust immunity provided by live attenuated vaccines [31]. The WOAH Manual (2024) elaborates that inactivated vaccines for sheep and Goatpox can confer immunity, but they often necessitate a series of initial doses followed by regular boosters to maintain effective immunity [12]. Moreover, Zewdie et al. (2021) review control measures for Goatpox and sheeppox, underscoring that inactivated vaccines are generally less effective than live vaccines unless administered with an appropriate dosing schedule that includes multiple initial doses and regular boosters [16].

The G1 vaccination protocol effectively protects the sensitive Saanen breed against GTPV without adverse reactions. This is crucial due to the breed's susceptibility and the risks of live attenuated vaccines, which can cause lesions, scarring, fly strikes, and secondary pneumonia. Severe oral lesions may lead to anorexia and reduced productivity. G1 avoids these issues, offering a safer and more effective immunization strategy [12, 15, 32]. The G1 protocol, by combining inactivated and live vaccines, avoids these complications while ensuring strong and sustained immunity. It also reduces the logistical burden of repeated doses and boosters required for inactivated vaccines alone. Data from this study confirm that the combined strategy used in G1 and G2 results in higher and longer-lasting antibody levels compared to the group that received only the live vaccine (G3), making the G1 protocol a safer and more strategic choice for immunizing Saanen goats [12, 15, 32].

The reported incubation period (IP) for GTPV infection typically spans 12 days but can range from 4 to 14 days following contact between infected and susceptible animals. This IP has been consistent across various experimental and natural studies. In the present study, signs and lesions associated with GTPV infection were observed within this same period after the challenge (group C), aligning with previously reported data [1, 12, 33]. Another key finding is that in the G3 group, unwanted signs and lesions appear 18 to 25 days after administering the live attenuated vaccine, suggesting a prolonged incubation period. This aligns with a 2022 study by Ghorani and Esmaeili, which reports adverse signs 21 to 27 days post-vaccination, especially in Saanen goats [20].

In a study by Taghavi Razavizadeh in 2013, after vaccinating non-indigenous Saanen goats with a live attenuated vaccine (RVSRI, Iran), signs associated with GTPV infection were induced in these animals [19]. In a 2022 study by Ghorani and Esmaeili, after administering a live attenuated vaccine to pure Alpine and Saanen goats, similar to the recent study, undesired signs and pox lesions appeared two to three weeks post-vaccination in these goats [20]. The clinical signs observed in this study align with previous findings. Adverse reactions following live attenuated vaccination, as seen here, have been reported in several studies. Boumart et al. similarly documented lesion development in Timahdite sheep after receiving a live attenuated vaccine, consistent with the present study's findings [13]. In a 2021 study by Hamdi et al., eight six-month-old North African male goats were challenged with GTPV. The clinical signs observed, including pox lesions and rectal temperature changes (averaging 41.3 °C), were consistent with the findings of the present study. Additionally, the serological method employed in that study was similar to the one used in this research [34].

In a 2009 study by Babiuk et al., 6-month-old Merino sheep and Boer cross goats infected with the Yemen strain of GTPV develop a 2–3 cm red nodule at the inoculation site by day 4, with pox lesions appearing around day 7. The timing and clinical signs observed align with those reported in the present study [35]. The morbidity and mortality reported in the present study were similar to the studies conducted on GTPV infection. In contrast, most studies emphasized that morbidity and case fatality rates can reach 100% in stressed and sensitive animals. It is emphasized again that the Saanen breed has been introduced as a breed with a very high sensitivity to GTPV contamination [2, 16, 36, 37].

As previously mentioned, research has shown that inactivated vaccines offer only short-term immunity [1, 38–40]. In the present study, two doses of the inactivated vaccine are used to prepare animals for the live

attenuated vaccine, as shown by the G1 group results. Awad et al. report that neutralizing antibodies appear in the first week after inactivated sheeppox vaccination and persist up to four weeks post-challenge, with immunogenicity lasting up to six months. Similarly, antibodies are detectable from the first week after live vaccine administration in the G1 and G2 groups, unlike in G3, indicating the benefit of prior inactivated vaccination. The serological method used aligns with that of Awad et al. [38].

Serological analysis in this study shows that the G1 vaccination regimen is more effective than others in generating neutralizing antibodies against GTPV. This supports the strategy's role in reducing adverse effects and enhancing immune response. Inactivated vaccines, developed as safer alternatives to live attenuated ones, show promise in safety, efficacy, and immunity duration. Serum neutralization tests remain essential for accurately evaluating antibody responses. While live attenuated vaccines are widely used against SPPV, ongoing safety concerns continue to drive interest in inactivated options [41, 42].

Several studies have discussed the successful use of inactivated GTPV vaccines despite their inadequacy when used alone for susceptible breeds. In the study by Boumart et al., the developed inactivated Romanian SPPV vaccine demonstrated the potential to replace attenuated vaccines in controlling and preventing sheeppox in disease-free or enzootic regions [13]. Another field study confirmed the safety and efficacy of an inactivated oily adjuvanted vaccine based on the Neethling strain (LSDV) tested on cattle, which did not elicit any adverse reactions and induced a high level of antibodies for up to one year [43]. In research by Wolff et al., a low molecular weight copolymer-adjuvanted vaccine formulation was capable of inducing sterile immunity in animals after severe challenge infection. These findings strongly suggest the potential utility of inactivated vaccines against GTPV infections and highlight the significant impact of the chosen adjuvant on the level of protection [44]. Additionally, In the study by Kavitha and Chetty, goats were divided into three groups and given either an inactivated vaccine, a live attenuated vaccine, or no vaccine (control). ELISA analysis showed increased antibody levels in the vaccinated groups after 14 days. Both vaccines effectively protected against GTPV, while the control group showed signs of infection [45].

In enzootic regions like Iran and much of Southwest Asia, attenuated live vaccines are widely used to protect against GTPV by stimulating both cellular and humoral immunity. However, their safe use in non-enzootic countries remains a concern. Inactivated vaccines are considered less effective at inducing cell-mediated immunity, which is crucial against poxvirus infections. Due to concerns about mild illness and contamination risks, many countries reduce their use of live vaccines and increasingly consider inactivated vaccines as an alternative strategy [2, 6, 20, 46, 47]. The current study underscores the critical importance of conducting comprehensive evaluations of vaccination protocols to guarantee both their efficacy and safety, particularly in scenarios involving imported animal populations. The current study can be viewed as a remarkable contribution that addresses a research gap in the field, particularly in the comparison of various vaccination protocols within sensitive breeds like Saanen.

With the introduction of sensitive breeds to enzootic areas of Goatpox, particularly in the context of livestock imports from countries where the disease has been eradicated, the risk of contracting Goatpox and incurring economic losses is inevitable [9, 11, 31]. Implementing an effective vaccination protocol like the one used for the G1 group can mitigate these economic losses by ensuring better immunity and reducing adverse reactions. This can lead to increased productivity and stability in small ruminant farming, particularly for high-value breeds like the Saanen, which are crucial for income and food security in regions such as the West of Asia [16, 31, 48, 49].

Conclusion

Given the widespread use of live attenuated vaccines in many countries, including Iran, and the high susceptibility of sensitive breeds like the Saanen to adverse effects from these vaccines, it is crucial to develop an effective immunization strategy against Goatpox. A vaccination protocol that begins with two doses of an inactivated vaccine administered one month apart, followed by a live attenuated vaccine and an annual live attenuated booster, offers a promising solution. This approach could effectively protect these susceptible breeds in regions where Goatpox is enzootic, thereby facilitating the breeding of economically valuable but sensitive breeds, such as the Saanen, in these areas. Implementing this protocol would not only safeguard animal health but also enhance the sustainability and economic viability of breeding programs in environments prone to Goatpox outbreaks.

Methods

Animals and experimental groups

The study was conducted between 2016 and 2021 and involved 375 pure-breed Saanen goats imported from France to Iran. These goats were not vaccinated against GTPV and had no history of GTPV infection. Goatpox disease has been eradicated in the country of origin of these animals, and vaccination against this disease is not practiced there. They were healthy, locally quarantined, and kept in an intensive breeding system, ensuring uniformity and control. In terms of age, the goats in the present study were three months and twelve months old. The goats were divided into four groups to test the

hypotheses (Table 1 and Fig. 1). The study groups were kept at a distance from each other in separate sheds. All animals received the same feed and were kept under identical rearing conditions. During the study period, strict control and health precautions were enforced to prevent any communication between the studied groups regarding the movement of supplies and human resources.

The goats used in this study were of the Saanen breed and were imported to Iran from France. Due to the limited availability of animals across different age groups, younger goats (3 months old) were assigned to the vaccination groups to closely align with the recommended vaccination age, ensuring realistic conditions for evaluating immune responses and adverse reactions. Older goats (12 months old) were placed in the control group to serve as a valid comparison for disease susceptibility and immune response.

Goat vaccination protocol

Vaccination was carried out using two types of vaccines: inactivated and live attenuated Goatpox vaccine. The live attenuated vaccine was administered at a dose of 0.5 ml containing $10^{2.5}$ $TCID_{50}$ and the inactivated vaccine was given at a dose of 1 ml containing $10^{5.5}$ $TCID_{50}$ per goat, both using the Gorgan strain (Iranian). Vaccines were made by Razi Vaccine and Serum Research Institute (RVSRI) (Iran). Vaccination conditions and protocol varied among the four groups in this study (Table 1).

For vaccinating goats and kids, the required dose for each type of vaccine was injected subcutaneously in the scapula region as per the vaccine manufacturer's instructions. All immunized animals were monitored daily for 28 days for the rise in body temperature, the appearance of clinical signs typical for pox lesions (PLs), and inflammation at the injection site. Also, the animals were monitored after receiving the boosters. If any signs indicative of GTPV infection appeared, samples were collected for analysis. These samples underwent a PCR assay to detect the presence of either the field strain of the Goatpox virus or the vaccine strain, ensuring accurate diagnosis and monitoring of potential infections.

The vaccination protocol was as follows: Group G1 received an inactivated vaccine at three months, an inactivated vaccine booster at four months, and a live attenuated vaccine at six months. Group G2 received the inactivated vaccine at three months and the live attenuated vaccine at five months. Group G3 received a single dose of live attenuated vaccine at three months. Group C served as the control group (Table 1). After the challenge, healthy animals were reimmunized annually with a live attenuated booster for three years. Each year, after receiving the booster, they were monitored for 28 days.

Sampling for serology

In each group, after the last dose of vaccine/booster, blood samples from the vaccinated goats were collected weekly until the 35th day (before the challenge) to measure the antibody titer against GTPV using the virus neutralization index (VNI) method. To assess serology, 40 animals from each vaccinated group were randomly marked and sampled. Blood samples were taken from these 40 animals in all groups before the primary vaccination. The collected samples were labeled and allowed to clot for 15–30 min at room temperature. The sera were then transferred to cryovials under a laminar airflow hood to avoid contamination and stored at -20 °C until processed.

Experimental challenge infection

In this study, to replicate the natural conditions of infection transmission that typically occur in herds, two goats naturally infected with the wild strain of GTPV and showing disease signs were adjacent to the study groups to serve as a source of infection for the challenge

Table 1 Protocols for administering vaccines, group sizes, and incidences of deaths and adverse reactions

Groups	Group size		Age (month)	Vaccination Protocol	Adverse signs	Number of Deaths
	Male	Female				
C	10	15	12	without being administered a vaccine	-	-
G1	75	75	3	At three months, the Inactivated vaccine [a] At four months, the inactivated vaccine At six months, Live vaccine	Minor signs were observed in three animals (2%)	0
G2	75	75	3	At three months, the Inactivated vaccine At five months, Live vaccine	Twenty-eight cases (18.66%) of mild lesions	0
G3	25	25	3	At three months, Live vaccine	41 animals (82%) exhibited signs related to GTPV [*]	11 (22% [**])
All groups	-	-	-	Healthy animals are reimmunized annually with live attenuated booster for three years.	Not seen	Not seen
Total	185	190				
	375					

This table presents the recorded adverse reactions and number of deaths following the administration of distinct vaccines within each group. [a], The vaccines utilized were sourced from the Razi Vaccine and Serum Research Institute in Iran; [*], Goat Pox virus; [**], mortality rate

purposes. Close contact with infected or recovered animals is probably the most important mechanism for transmitting GTPV [1, 12, 39].

The animals employed in the challenge were diagnosed with GTPV infection, exhibiting clinical signs such as anorexia, depression, skin lesions, fever, respiratory issues, and conjunctivitis. The infection was confirmed using the PCR assay. This challenge was conducted to evaluate the effectiveness of the vaccination protocol in preventing infection among the goats in all groups (Table 1). Thirty-five days after receiving the final vaccine or booster vaccine, goats and kids in all groups (Except for the G3 group) were challenged by proximity to infected animals. Infected animals were housed for four weeks alongside the goats from each group, under feeding and housing conditions similar to those of the experimental group, sharing the same water and feed containers. The G3 group, which received the live attenuated vaccine, was challenged after three months. The three-month interval was necessary to allow the kids time to recover from the adverse signs caused by the live attenuated vaccine administration in this group.

After the challenge, the goats and kids were monitored daily for clinical signs, rectal temperature, and any signs related to Goatpox. In the event of animal deaths, necropsies were conducted promptly using standard methods. GTPV infection in animals showing signs was confirmed by PCR. Skin scabs and vesicle swabs were collected aseptically for PCR. Skin lesions and scabs serve as primary sources of the virus [1].

Confirming the diagnosis of goatpox by PCR

Sampling and preparation

One gram of biopsy sample from pox lesions was ground in approximately 5 ml of PBS containing antibiotics using a sterile glass tissue grinder. The medium was frozen and thawed twice, then clarified by centrifugation at 4000 rpm for 45 min. The resulting suspension was aliquoted and stored at -20 °C until needed.

DNA extraction

Total DNA was extracted from the samples following the manufacturer's instructions for the SinaPure™ Viral kit (Sinaclon Co., Iran).

Primers and amplification reaction (PCR)

To confirm the diagnosis of Goatpox in infected animals for the challenge, a PCR assay was conducted using B68 and B69 primers, following the method described by Heine et al. [50]. Additionally, to distinguish between field strains and vaccine strains, the method reported by Chibssa et al. was employed. Specific primers were used to amplify amplicons of 302 bp in GTPV field isolates and 218 bp fragments for vaccines (Gorgan strain) [51] (Table 2). The PCR protocol was executed following the reported conditions and the specified number of cycles. Each amplified product was subsequently subjected to gel electrophoresis on a 1.5% low electroendosmosis agarose gel in 1% TBE buffer for 80 min at 100 V. Visualization of the agarose gel was conducted using a UV transilluminator.

Table 2 Primers were used in the present study

Primer name	Sequences	Length	Amplicon size
DIV_Fow	5′-ATCTGCTACAAGTTTTAACGAACTTA-3′	26 bp	218 bp (GTPV vaccines)
DIV_Rev	5′-TGAATGTGATCTCATATCCTTATTG-3′	25 bp	302 bp (GTPV field isolates)
B68	5′-CTAAAATTAGAGAGCTATACTTCTT-3′	25 bp	390 bp (P32 gene)
B69	3′-CGATTTCCATAAACTAAAGTA-3′	21 bp	

Serological investigation

Serum samples were tested for the development of specific antibodies using the VNI method as described in the World Organization for Animal Health (WOAH) Terrestrial Manual (WOAH Chaps. 2.7.11 and 2.7.14). In WOAH, 2024, the VNI is the gold standard method to detect neutralizing antibodies against GTPV [12, 52, 53]. This test involved serial ¼ dilutions of heat-inactivated sera and a set amount of a reference strain of GTPV (100 $TCID_{50}$) (RVSRI, Iran). The VNI is the log titer difference between the titer of the virus in the negative serum and the test serum. The neutralizing antibody titer was calculated according to the Reed and Muench method [54].

Statistical analyses

If the data distribution was normal, a paired t-test was used to compare the means within the same group across different time points. If the distribution was not normal, a Wilcoxon signed-rank test was applied instead. A one-way ANOVA was utilized to compare differences between the groups when the data were normally distributed; otherwise, the Kruskal-Wallis test was performed. Given that all differences were statistically significant ($p < 0.05$), we confidently described the variations observed. Analyses were performed using IBM SPSS Statistics V.29.

Abbreviations

CaPV Capripoxvirus
SPPV Sheeppox Virus
GTPV Goatpox Virus

RVSRI	Razi Vaccine and Serum Research Institute
VNI	Virus neutralization index
PCR	Polymerase chain reaction
$TCID_{50}$	50% tissue culture infectious dose
TBE	A buffer solution containing a mixture of Tris base, boric acid and EDTA
WOAH	World Organisation for Animal Health
SPSS	Statistical package for social sciences
ELISA	Enzyme-linked immunosorbent assay

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Author contributions
H.E. and S.M.J. were responsible for the conceptualization and design of the project, developing the main conceptual ideas, and outlining the overall methodology. H.E. played a significant role in project administration and supervision, ensuring effective management throughout the study. H.E., Z.H., and E.B.A. handled the technical details, with Z.H., A.P.SH., and E.B.A. focusing specifically on software and data management. Z.N.R., S.M.J., and M.M.S. were responsible for data curation. All authors, especially H.E. and S.M.J., participated in the investigation and execution of the experiments, as well as in data analysis. S.M.J. drafted the original manuscript and integrated feedback from all co-authors, while H.E. and S.M.J. critically revised the manuscript for important intellectual content. All authors provided critical feedback, contributed to shaping the research, analysis, and manuscript, and approved the final version for submission.

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Data availability
The data supporting the findings of this study will be made available upon request.

Declarations

Ethics approval and consent to participate
The protocol for this study was approved by the Internal Ethics Committee of the Faculty of Veterinary Medicine, University of Tehran, under Protocol number IR28786/2. Informed consent was obtained from the owner of the imported goats after a comprehensive explanation of the vaccination procedures and research objectives. All animal care and experimental procedures were conducted in accordance with institutional guidelines, adhering to the ARRIVE guidelines, the U.K. Animals (Scientific Procedures) Act, 1986, EU Directive 2010/63/EU for animal experiments, and the National Research Council's Guide for the Care and Use of Laboratory Animals. No invasive procedures were used during the study to ensure the welfare of the animals. The study involved both male and female goats, and the potential influence of sex on the results was considered throughout the research.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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