BMC Veterinary Research

RESEARCH

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Live attenuated goatpox vaccination in pregnant Murcia-Granada goats: dosage implications and outcomes

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Abstract

Background Infectious diseases, particularly the Goatpox virus (GTPV) from the *Poxviridae* family, significantly impact livestock health and agricultural economies, especially in developing regions. Recent GTPV outbreaks in previously eradicated areas underscore the need for effective control measures, with vaccination being the most reliable strategy. This study investigates the effects of administering standard and double doses of live attenuated goatpox vaccine in pregnant Murcia-Granada goats, a non-native breed in Iran, to determine optimal vaccination protocols.

Results In 2018, 400 healthy and pregnant Murcia Granada goats imported from Spain were divided into groups of 200 and vaccinated with either a standard dose (0.5 ml) or a double dose (single 0.9 ml injection) of live attenuated goatpox vaccine. Post-vaccination, the goats were monitored daily for clinical signs of infection, with samples collected for PCR analysis to detect the presence of GTPV strains. In group A, which received the standard vaccine dose, no abortions or vaccine-related side effects were observed, and body temperatures remained normal. In group B, administered a double dose, 37% of the goats experienced abortions, displaying signs of GTPV infection, such as skin lesions (pox lesions) and increased body temperatures. Molecular analysis confirmed the vaccine strain of GTPV as the infection source, ruling out external contamination. Statistical analysis showed no significant differences in abortion rates concerning gestational age or t he age of the pregnant goats.

Conclusion The study highlights the importance of adhering to standard vaccine dosages in pregnant Murcia Granada goats to prevent adverse outcomes like abortions. This study emphasizes the necessity to review and revise vaccination protocols tailored to specific breeds and varying maintenance conditions, including pregnancy and outbreak scenarios. These findings stress the necessity for cautious and tailored vaccination strategies to ensure the safety and efficacy of vaccines in different goat breeds.

Keywords Murcia Granada goats, Goatpox, Live attenuated Goatpox vaccine, Pox lesions, Abortion

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Background

Infectious diseases, particularly those caused by viruses within the *Poxviridae* family, pose significant threats to livestock health and agricultural economics. Among these, Goatpox virus (GTPV), a member of *Capripoxvirus*, is a prominent pathogen affecting sheep and goat populations, especially in developing regions of Western Asia and beyond. The economic impact of GTPV is profound, leading to decreased productivity, increased veterinary costs, and trade restrictions [1–3].

Clinical signs of Goatpox include fever, nasal discharge, and generalized pox lesions on the skin, particularly around the mouth, nose, and eyes. These lesions can also appear on the udder and inner thighs, leading to pain, secondary bacterial infections, and reduced productivity. In severe cases, the disease can cause respiratory distress, swollen lymph nodes, and death, especially in young or immunocompromised animals [4–7]. Transmission of the virus occurs through direct contact with infected animals, their secretions (such as nasal discharge), and scabs containing the virus. Indirect transmission is also possible via contaminated equipment, bedding, and feed, making the virus highly transmissible in both small flocks and large herds [7, 8]. The disease is of significant concern in regions where goats are economically important, as outbreaks can lead to substantial losses in productivity and animal welfare [9].

Recent reports from 2022 to 2023 have documented sheep and Goatpox outbreaks in regions such as Spain, Russia, Greece, and Bulgaria, where the disease had previously been eradicated. These outbreaks can lead to up to 100% mortality in infected sheep and goats, causing significant economic losses, particularly in cases involving abortion [10]. Notably, some goat breeds demonstrate varying levels of susceptibility to this disease. Native breeds tend to be more resistant, while Australian cross and American merino breeds show lower susceptibility than Rambouillet breeds. These findings underscore the importance of enhancing control measures for this disease [11].

Vaccination is widely recognized as the most effective strategy for controlling and preventing outbreaks of Goatpox. Live-attenuated vaccines, which are derived from virus strain through serial passage in tissue cultures, have been shown to provide long-term immunity and are generally considered safe [9, 12, 13]. However, the efficacy of these vaccines can vary significantly based on factors such as the dosage administered, the specific strain of the virus, different pure or mixed breeds, and the physiological status of the animals, including their pregnancy status [7, 11, 14, 15].

Vaccination with live attenuated Goatpox vaccine is widespread in Iran, where Goatpox is enzootic. According to the protocol of the Iranian Veterinary Organization (IVO), during outbreaks of Goatpox, goats should receive a double dose of the vaccine in a single injection, which is equivalent to twice the standard dose in one administration [11, 16–18]. It is important to note that pregnant goats are especially vulnerable to infectious diseases due to the immunosuppressive effects of pregnancy, which can complicate the administration and efficacy of vaccines, particularly under epidemic conditions. Understanding the optimal vaccine dosage for this demographic is crucial to ensure the vaccine's safety and its offspring's survival [6, 19]. Given this context, the present study aims to investigate and report the outcomes and effects of administering a standard dose and a double dose of live attenuated Goatpox vaccine in pregnant Murcia Granada goats. This breed, which is not native to Iran, has been imported in recent years, necessitating a thorough examination of its response to vaccination protocols.

Iran has a high potential for goat breeding, and various breeds are raised within the country [20, 21]. The import of foreign breeds, like the Murcia Granada goats, is common to improve production rates or meet market demands, similar to practices in other countries [11, 22, 23]. In recent years, Iran has been developing its systems for the intensive breeding of dairy goats, and three prominent dairy breeds from around the world have been imported: the Saanen and Alpine breeds in previous years and, more recently, the Murcia Granada breed. Given the sensitivities of this breed and the disease prevention policies in Iran, it was necessary to evaluate the conditions of Goatpox vaccination in this specific breed, which is why it was included in this study.

Results

In group A, where goats received the standard dose of 0.5 ml, no abortions or vaccine-related side effects were observed, and the goats maintained normal body temperatures post-vaccination. Conversely, in group B, where pregnant goats were administered a double dose of 0.9 ml, 74 goats (37%) experienced abortions between 24 and 29 days after vaccination (Fig. 1). A statistically significant difference was observed in the abortion rates between group A and group B, with a p-value of less than 0.0001. This result demonstrates that the variation in abortion rates between the two groups is highly significant. There was no statistically significant correlation between the age of the pregnant goats and the abortion rate.

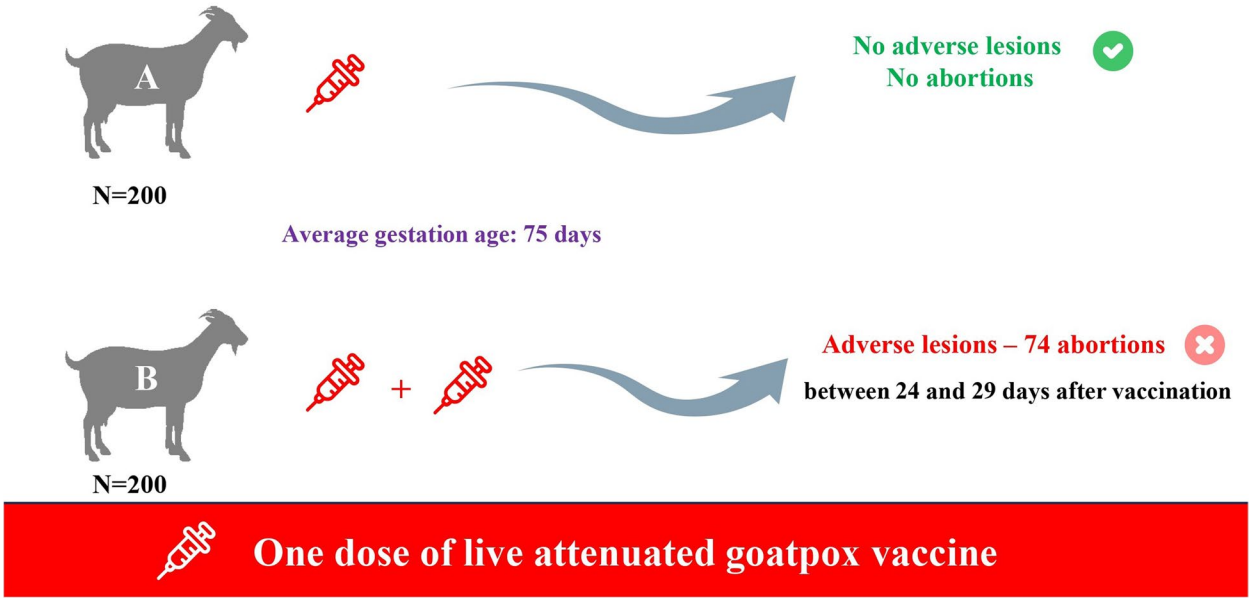

Fig. 1 Schematic image of the vaccination process and the results for Groups A and B

In Group B, 31 abortions were recorded during the third month of pregnancy, while 43 abortions occurred in the fourth month. However, statistical analysis revealed no significant difference in the number of abortions based on gestational age, with a p-value of 0.157, indicating that gestational age does not have a significant impact on the abortion rate following the administration of a double dose of the vaccine. Ten aborted fetuses and their placentas were subjected to examination and necropsy among these fatalities. No mortality was recorded among the pregnant goats. Out of the 74 aborted goats, only 36 (48.7%) exhibited pox lesions.

Between 19 and 25 days post-vaccination, signs associated with GTPV infection began to manifest in Group B, with new lesions developing over the following two weeks. The goats exhibited an increase in body temperature ranging from 41 to 42.5 °C, lasting between 6 and 8 days. Affected animals showed signs of depression, loss of appetite, and, in severe cases, labored breathing. Additional clinical findings included harsh lung sounds, rhinitis, conjunctivitis, and nasal mucopurulent discharge. The characteristic Goatpox lesions in Murcia Granada goats initially appeared on hairless areas such as under the tail, over the udder, inside the ears, around

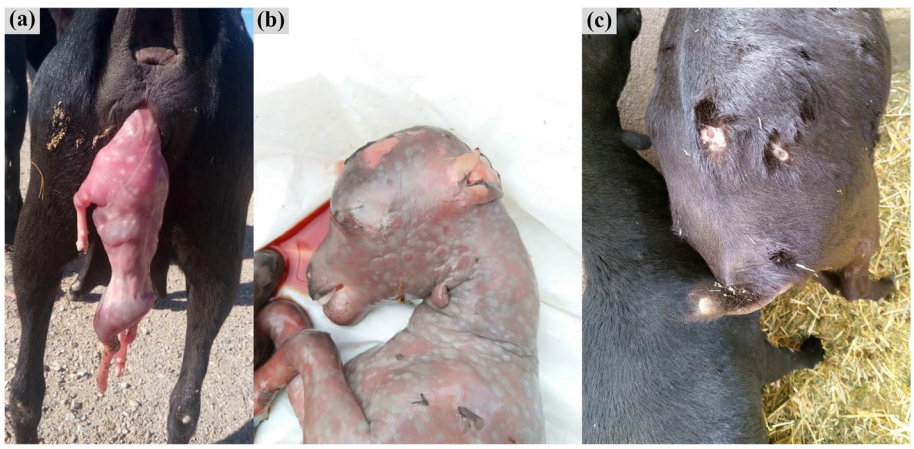

Fig. 2 Clinical manifestations in pregnant goats from Group B following vaccination with a double dose of the live attenuated Goatpox vaccine. **a** Premature termination of pregnancy and abortion in a goat, with pox lesions clearly visible on the aborted fetus. **b** An aborted fetus was showing distinct pox lesions on its skin. **c** Severe pox lesions on the body of a recently aborted Murcia Granada goat

the mouth and eyes, and occasionally within the buccal cavity and external genitalia. In more severe cases, these lesions spread throughout the body (Fig. 2– (c)).

Aborted fetuses appeared underdeveloped and showed signs of distress before abortion (Fig. 2– (a)). Characteristic pox lesions were present on their skin, appearing as nodules, pustules, or scabs (Fig. 2)– (b). These lesions, similar to those seen in adult goats, often occur on hairless body parts. Examination of internal organs revealed signs of pneumonia or other respiratory distress in the lungs, including congestion and consolidation. The liver and spleen were enlarged and showed evidence of necrosis or inflammation, indicating a systemic infection. The lymph nodes were also enlarged and exhibited signs of inflammation, further supporting the presence of a widespread infection. Lesions similar to those on the fetal skin were observed on the placentas, indicating intrauterine infection.

After conducting molecular analysis, the samples collected from goats displaying signs and from aborted fetuses were found to contain amplicons of 390 bp and 218 bp (Supplementary Fig. 1). These specific amplicon sizes indicate the vaccine strain of the GTPV, confirming that the vaccination caused the observed infections. Furthermore, the PCR assay did not detect the 302 bp amplicon, which is associated with the field strain of the GTPV. The absence of this 302 bp amplicon in all tested samples ruled out the possibility that the herd was contaminated with the field strain of GTPV. This molecular evidence clearly indicated that the source of the infection was the vaccine and not an external field strain.

These findings collectively pointed to the double dose of the vaccine as the cause of the abortions. The presence of pox lesions on the skin, internal organ pathology, and placental involvement all indicate that the vaccine strain of the GTPV was responsible for the infections and subsequent abortions in the pregnant goats.

Discussion

The findings from this study provide significant insights into the differential effects of live attenuated Goatpox vaccine dosages in pregnant Murcia-Granada goats, highlighting crucial considerations for vaccination protocols in goat herds. The results from group A indicate that the standard dose of the live attenuated Goatpox vaccine from RVSRI, used in Iran, was effective and safe for pregnant goats of the imported breed, as no abortions were observed. In a different study, Murcia-Granada goats that were not pregnant did not show any sensitivity to the vaccine administered [11]. Conversely, the findings from group B highlight that increasing the vaccine dose, regardless of fetal development stage or maternal age, can negatively impact pregnancy, leading to a high rate of abortions. These adverse effects suggest breed-specific sensitivities and emphasize the need for careful consideration when administering higher vaccine doses to non-native breeds.

Research and reports reveal that imported breeds of small ruminants, especially goats, are more susceptible to GTPV infections and adverse reactions from vaccinations with live attenuated vaccines. These records, along with findings from studies like the present one, underscore the importance of careful management and the potential need to revise vaccination protocols for imported animals [6, 7, 12, 13, 24–26].

For instance, a study by Ghorani and Esmaili in 2022 found that Saanen and Alpine goats exhibited greater sensitivity to the live attenuated Goatpox vaccine than Iranian native breeds. The lesions and their persistence observed in these goats were consistent with the current study's findings [11]. In a similar vein, research by Taghavi Razavizadeh et al. reported unwanted clinical signs in Saanen goats vaccinated with the TC-Gorgan strain of GTPV, which closely resembled the signs observed in the current study [17]. Additionally, Esmaeili et al. (2021) reported a significantly higher rate of ecthyma (caused by the *Parapoxvirus*) in imported breeds (87.30%) compared to indigenous breeds (39.30%), further highlighting the increased susceptibility of imported breeds to such infections [26].

According to the IVO protocol, healthy animals exposed to Goatpox outbreaks can be vaccinated with up to twice the manufacturer's recommended dose, i.e., 0.9 ml. This approach is common among native Iranian goat breeds, which typically have no adverse reactions [18]. However, the results of the present study indicate that pregnant Murcia-Granada goats exhibited sensitivity to the double dose of the vaccine, resulting in abortions and skin lesions. This sensitivity difference should be considered when breeding this goat breed in Iran.

Currently, no approved vaccines are available in non-enzootic countries to protect against *Capripoxviruse*s. The SPPV vaccine is restricted to enzootic regions such as Central and North Africa, West Asia, Türkiye, Iraq, and Iran [9]. In West Asia, the RM65 SPPV vaccine from Yugoslavia has been used in cattle at ten times the dose recommended for sheep. In Egypt, the Romanian SPP and Kenyan sheep and Goatpox virus vaccines have been used for cattle [27–30]. Additionally, in Türkiye, the Bakirkoy SPPV vaccine has been administered against lumpy skin disease virus (LSDV) at three to four times the recommended dose for sheep [31–34]. In a study by Uzar et al., it was demonstrated that while the sheep and Goatpox vaccine virus propagated in MDBK cells is safe for administration in cattle, it does not provide full protection against LSDV.

The difference in challenge titers between vaccinated and unvaccinated animals was less than log 2.5, and viremia occurred in vaccinated animals. In contrast, the Penpox-M vaccine provided better protection, with a titer difference greater than log 2.5, and no viremia was observed. This issue highlights the importance of choosing the appropriate vaccine strain and dose for adequate protection against genus *Capripoxviruses*, as seen with the Penpox-M providing superior immunity in comparison to the SGP vaccine produced in MDBK cells [34, 35].

Although live attenuated vaccines have shown more considerable and longer-lasting immunity against Goatpox, some studies have reported successful use of inactivated *Capripoxvirus* vaccines. Boumart et al. demonstrated that an inactivated Romanian SPPV vaccine they developed could potentially replace attenuated vaccines for controlling and preventing sheeppox, particularly in disease-free or enzootic countries [12]. Another study confirmed the safety and efficacy of an inactivated, oily adjuvanted vaccine based on LSDV, showing no adverse reactions and inducing robust immunity [36]. A herd of Damascus goats received the live Goatpox vaccine without exhibiting any clinical signs. In contrast, a separate study by Abo-Shehada reported that imported Saanen goats experienced 100% morbidity and 40.7% mortality after being administered the same vaccine and dose [25].

The molecular detection method used in this study was akin to those in related research and is a well-established technique [37–39]. PCR assays conducted on samples from affected goats and aborted fetuses conclusively identified the presence of the GTPV, with amplicons of 390 bp and 218 bp confirming the vaccine strain as the causative agent. The absence of the 302 bp amplicon, which would indicate the field strain, ruled out external contamination. These molecular diagnostics highlight the importance of accurate tools in understanding vaccination's impact and confirming the etiology of observed infections.

Conclusion

Given the heightened susceptibility of imported breeds to genus *Capripoxvirus* infections, it is recommended that targeted vaccination programs against Goatpox be implemented in Murcia-Granada goats residing in countries where the disease is enzootic. However, it is crucial to exercise caution regarding the vaccine dosage, ensuring it remains at the standard level and avoiding its administration during the last months of pregnancy.

Methods

Animals

In the present study, carried out in 2018, a total of 400 Murcia Granada goats were included. These goats were imported from Spain to Iran by a non-governmental farm three months before the study was conducted. They were healthy, quarantined locally, and maintained in an intensive breeding system to ensure uniformity and control. No livestock entry or exit occurred on the farm until the end of the study, and there were no other breeding farms within a 20 km radius around the farm, and no herds had moved in or out of the area during the study. The goats involved in this study were between 12 and 24 months old. None of the goats had been vaccinated against GTPV, nor did they have any history of GTPV infection.

Since estrus synchronization was part of the breeding policy for these goats, they had nearly the same gestation length. The gestation period for the goats in the study ranged from 60 to 90 days, with an average of 75 days, and all the animals were pregnant nannies.

Study design

To assess the impact of administering a single versus a double dose of the live attenuated Goatpox vaccine in pregnant Murcia Granada goats, researchers divided the animals into two groups of 200 each. The groups were housed separately in distinct halls to ensure controlled conditions for the study [7]. According to the vaccine manufacturer's instructions, a standard dose of 0.5 ml was injected into group A, while group B received a double dose, equivalent to twice the standard dose, in a single 0.9 ml injection administered subcutaneously in the shoulder region to vaccinate the pregnant goats. All animals in the study were vaccinated using the same batch-numbered vaccine. Each dose of this vaccine, produced by the Razi Vaccine and Serum Research Institute (RVSRI) in Iran, contains $10^{2.5}$ $TCID_{50}$ of the GTPV Gorgan strain.

Immediately after vaccination, the goats were monitored daily for any rise in body temperature, the appearance of clinical signs typical of pox lesions, abortion or signs of abortion, and inflammation at the injection site. If any signs of GTPV infection appeared, samples would be collected for analysis. Skin scabs and vesicle swabs were collected aseptically for PCR, as skin lesions and scabs serve as primary sources of the virus [6]. These samples would then undergo a PCR assay to detect the presence of the field strain or the vaccine strain of the GTPV, ensuring accurate diagnosis and monitoring of potential infections. In the event of an abortion, a necropsy was performed following standard procedures.

Molecular investigation

Sample preparation

One gram of biopsy sample from pox lesions was homogenized in approximately 5 ml of PBS containing antibiotics using a sterile glass tissue grinder. The homogenate

Table 1 Primers were utilized in the current study

Primer Name	Sequences	Length	Amplicon size
DIV_Fow	5′-ATCTGCTAC AAGTTTTAA CGAACTTA-3′	26 bp	218 bp (GTPV vaccines)
DIV_Rev	5′-TGAATGTGA TCTCATATCCTT ATTG-3′	25 bp	302 bp (GTPV field isolates)
B68	5′-CTAAAATTA GAGAGCTAT ACTTCTT-3′	25 bp	390 bp (P32 gene)
B69	5′-CGATTTCCA TAAACTAAA GTA-3′	21 bp	

was then frozen and thawed twice, followed by clarification via centrifugation at 4000 rpm for 45 min. The clarified suspension was aliquoted and stored at −20 °C until further use.

DNA extraction

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

Amplification reaction (PCR)

A PCR assay was performed using B68 and B69 primers to confirm the diagnosis of Goatpox in the animals, following the method described by Heine et al. [40] (Table 1). Additionally, the method described by Chibssa et al. was used to differentiate between field strains and the vaccine strain. Specific primers were employed to amplify 302 bp fragments in GTPV field isolates and 218 bp fragments in the vaccine strain (Gorgan strain) [37] (Table 1). The PCR protocol followed the reported conditions and cycle numbers. Each amplified product was subjected to gel electrophoresis on a 1.5% low electroendosmosis agarose gel in 1% TBE buffer for 80 min at 100 V. The agarose gel was then visualized using a UV transilluminator.

Statistical analysis

The data were analyzed using IBM SPSS Statistics 25 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were generated to summarize the data. Inferential statistical analysis was conducted using the Student's T-test to compare means between groups. A P-value of less than 0.05 was considered to indicate statistical significance, ensuring that the results were not due to random chance. This threshold was chosen to balance the risk of Type I and Type II errors, providing a rigorous assessment of the differences observed between the groups.

Abbreviations

GTPV	Goatpox virus
IVO	Iranian Veterinary Organization
RVSRI	Razi Vaccine and Serum Research Institute
$TCID_{50}$	50% tissue culture infectious dose
SPSS	Statistical package for social sciences
PBS	Phosphate-buffered saline
TBE	A buffer solution containing a mixture of Tris base, boric acid and EDTA
SPPV	Sheeppox Virus
LSDV	Lumpy skin disease virus

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-024-04395-z.

Supplementary Material 1. Supplementary Figure 1. PCR results on a 1.5% agarose gel using a 100 bp DNA ladder (M). Wells A1 to A3 represent samples from aborted fetuses, showing the presence of a 390 bp band. Wells B1 to B3 contain samples tested for the presence of a 218 bp band. The negative control (C-), a reaction without template DNA, shows no amplification.

Acknowledgements

The authors would like to express their gratitude to the workers and experts of the Murcia Granada goats Farm for their invaluable cooperation and support throughout the study process. Their assistance was instrumental in the successful completion of this research.

Authors' contributions

H.E. and M.G. devised the project, developed the main conceptual ideas, and outlined the proof. H.E., S.V., and D.L handled most of the technical details and performed the numerical calculations for the proposed experiment. All authors conducted the experiments and analyzed the data. H.E, S.M.J., and M.G. critically revised the manuscript for important intellectual content. S.M.J. wrote the manuscript, drafted the initial text, and integrated feedback from all co-authors to produce the final version. All authors provided critical feedback and contributed to shaping the research, analysis, and manuscript.

Funding

This research received no specific grant from any funding agency.

Data availability

The data produced and/or analyzed during the present study can be obtained from the corresponding author 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 (Protocol number IR28786/2). Additionally, all procedures involving the handling and care of the goats adhered to the international guidelines outlined in the Terrestrial Animal Health Code [41]. Informed consent was secured from the owner of the imported goats following a comprehensive explanation of the vaccination procedure and research goals.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Received: 20 August 2024 Accepted: 21 November 2024
Published online: 02 December 2024

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