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Article:

Impact of Rabbit Hemorrhagic Septicemia Vaccination on Comfort Behavior, and Activity Levels in Two Breeds Rabbit Housed in Cages.

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Abstract

This study evaluates the impact of rabbit hemorrhagic disease virus (RHDV) vaccination on the comfort, exploratory, Ingestive, and physical behaviors of two rabbit breeds Chinchilla and V-line housed individually in cages. Behavioral responses were assessed at baseline (24 hours before vaccination) and at 1, 8, 27 and 72 hours post-vaccination using frequency and duration measures. The results showed that comfort behavior, neither lying nor stretching was observed in any group, while V-line rabbits exhibited a significantly lower frequency of sitting compared to Chinchilla rabbits, although no differences were noted in the duration of sitting. Self-grooming (auto-grooming) behavior was significantly reduced in both frequency and duration at 8 hours post-vaccination across all groups. Exploratory behavior showed increased sniffing duration at 1 hour post-vaccination in V-line rabbits, while the frequency of investigating the enclosure decreased in all groups at 1 and 8 hours compared to control Chinchilla rabbits. Ingestive behavior analysis revealed a significant reduction in feeding duration in vaccinated Chinchilla rabbits at 8 hours post-vaccination and an increase in drinking duration in vaccinated rabbits compared to non-vaccinated counterparts. These changes in ingestive behavior suggest potential physiological responses to vaccination. Physical activity, assessed through hopping, showed no notable differences in jumping, and reduced hopping Overall, the results highlight breed-specific behavioral differences following vaccination, with notable effects on self-grooming, feeding, and hopping activities. These findings suggest that vaccination with RHDV may influence various behavioral patterns, potentially due to immune or stress responses, though breed differences may also play a significant role.

Keywords: Behavior, Rabbit, Viral Hemorrhagic Septicemia Vaccination.

Introduction

The behavior of rabbits is a crucial indicator of their health and well-being, particularly in environments such as cages, where they may face restrictions in movement and social interaction. Monitoring behavioral patterns, such as resting, comfort, exploration, and grooming, can provide valuable insights into their physical and psychological status [1]. These behaviors are often influenced by various environmental and physiological factors, including immune challenges like vaccination, which can have a significant impact on the rabbits' activity and welfare [2].

Vaccination plays a key role in disease prevention in rabbits, yet it may induce physiological stress and behavioral changes, especially when animals are housed in controlled environments such as cages [3]. Understanding

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how vaccination affects behaviors in different rabbit breeds can help inform best practices for their management and welfare. In particular, studying resting and comfort behaviors, as well as exploratory and grooming activities, provides critical data on how rabbits cope with vaccination-induced stress or discomfort [4].

Resting behavior, for example, is a fundamental activity for rabbits and reflects their overall comfort and contentment. Healthy rabbits typically spend a substantial portion of their time lying down or sitting in relaxed postures, which indicates a suitable cage environment [1,5]. When rabbits experience stress or discomfort—whether from their environment or immune challenges such as vaccination—their resting patterns may alter, signaling a shift in their well-being [6].

Exploratory and grooming behaviors are equally important in assessing a rabbit's health. Exploratory behaviors reflect the animal's cognitive engagement and curiosity about its surroundings, which are essential for its adaptation to the cage environment [7]. Grooming, on the other hand, serves as an indicator of both physical health and social bonding, as it helps maintain hygiene and fosters social connections [8]. Changes in these behaviors after vaccination could highlight the physiological or psychological impacts of the immune response [9].

This study aims to evaluate the impact of vaccination with rabbit hemorrhagic septicemia vaccine on various behavioral patterns—including comfort, exploratory, ingestion, and physical activity—of two rabbit breeds bred in cages. By analyzing the changes in these behaviors post-vaccination, we seek to provide insights into breed-specific responses to vaccination and their implications for animal welfare and management.

Materials and Methods

1- Animals

A total of 24 Chinchilla and V-line male rabbits of 10 weeks of age and weighting about 1.5–2 kg was used in this study, was seronegative for rabbit haemorrhagic disease virus antibodies. Each breed contain 2 groups (8 vaccinated and 4 control rabbits), was housed in an isolated battery under a specific management program, including feeding, watering, use of utensils, disinfection, and vaccination. This study was approved by the Veterinary Medical Research Ethics Committee, Faculty of Veterinary Medicine, Sohag University, Sohag, Egypt (soh.un.vet/00073 R).

2- Housing and Management

The experiment was conducted in the experimental animal unit of the Faculty of Medicine, Sohag University in a room measured 7×7×3 meters. The experimental room was equipped with two air suction fans (25×25 cm) and one air cooler system. The walls were constructed using aluminum and complemented by a ceramic floor [10]. The rabbits were housed in galvanized iron batteries, and the batteries were separated by a 1-meter gap to allow good ventilation and waste disposal. Each cage had dimensions of 60×40×30 cm, providing adequate space for each rabbit (one rabbit per cage). Each breed was housed in an isolated battery, equipped with automatic drinking systems and isolated feeders mesuring (15×15 cm) [11]. The cages were designed to be easy to clean, offering a safe environment for the rabbits to live and raise [12].

3- Microclimatic Conditions

Ventilation was maintained by the use of air conditioner, two air suctions, and shaded windows to keep the temperature and relative humidity within an acceptable level of (22ºC, 70%) respectively. And there was daily hygienic disposal of manure. Each battery was supplied with an automatic watering system of a drip nipple design (one nipple per cage). Rabbits were fed dry pellets ad-libitum from a commercial diet containing 17% crude protein, and 14% crude fiber serving as maintenance ration [13].

4- Commercial bivalent vaccine:

Commercial vaccine was used, the inactivated "SERVAC®" bivalent vaccine against RHDV1&RHDV2 (Batch No.23011), with a recommended dose of 0.5 ml by subcutaneous route. The vaccine was produced by the Veterinary Serum and Vaccine Research Institute (VSVRI).

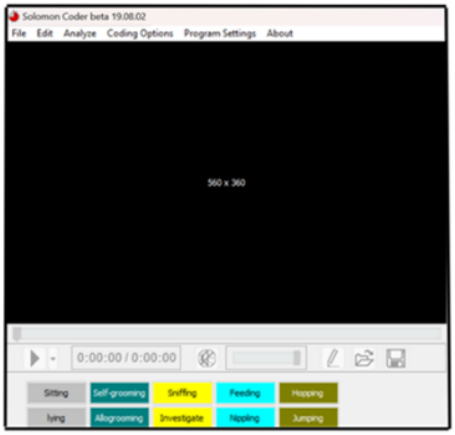
5- Behavioral measurements

Behavioral responses to immune stimulation were assessed in each group at baseline (before vaccination) and at 1-, 8-, 27-, and 72-hours post-vaccination, along a period of 3 days for both breeds. In individually housed rabbits, the behavioral patterns (Table.1), including resting, comfort, exploration, play, physical activity, and ingestion, were recorded in terms of frequency and duration within the home cage, using the battery system [10,14]. Video recordings of behavioral responses were analyzed using Solomon Coder software (Version: beta 19.08.02) [15]. These behaviors were specifically selected for observing the immune response represented by Comfort behavior and Activity levels, which triggered by vaccination involves various physiological and biochemical changes, including the release of stress-related hormones such as corticosteroids. This stress response can transiently weaken the immune system and impact the overall health, potentially making rabbits more susceptible to infections [16]. A Solomon Coder configuration file with behaviors of interest (Comfort, Activity levels) was first created prior to coding (Fig. 1). Video files were uploaded onto the Solomon Coder platform and annotated one at a time. Using the configuration file, coders annotated whether each of the behaviors (i.e., comfort, exploring and ingestive behaviors, body care and physical activity) was present at every 20s intervals in each video (i.e., at a rate of 5 Hz). From the annotations, we established a continuous measure of

behavior, by assigning the value "0" to time points where none of the behaviors was present, and a value of "1" for all other instances. The ethogram of the laboratory rabbit has been described in previous studies [17, 18]. The most common behaviors of individually housed rabbits included lying alert, grooming, sleeping, and eating. Individually housed rabbits display inactive behavior most of the time [18].

Statistical analysis

The interaction between breed and time was analyzed using a two-way ANOVA in GraphPad Prism software (GraphPad Prism. Version 10. 2023). Subsequent Post hoc comparisons between groups were performed using Tukey's test. Results were expressed as mean ± SD, with statistical significance set at $P < 0.05$.



1: Subject

Time	Comfort behavi	Body care	Exploratory beh	Ingestive behav	Physical activity
0.00	Sitting	Self-grooming	Sniffing	Feeding	Hopping
0.20	lying	Allogrooming	Investigate	Nippling	Jumping
0.40	Stretching				
0.60					
0.80					
1.00					
1.20					
1.40					
1.60					

Fig.1 A Solomon Coder configuration file (top: loading video screen; bottom: output of frequency and duration), used for coding different behavior patterns.

Table 1 Ethogram of experimental rabbit

Behavior Categories		Description
Comfort behavior	**sitting**	Upright stationary position, with rear end and forepaws on ground and ears down.
	lying	Limbs outstretched – resting with body trunk on ground, all four limbs outstretched, and belly exposed.
Body care	**grooming**	Auto grooming : fast-forward flicking of forelimbs whilst rabbit sits upright on haunches (Air-boxing) and passed over the head and ears, then licking of fur over the rest of the body
Exploratory behavior	**Sniffing**	Close olfactory investigation of objects.
	Investigate enclosure	Smell the surrounding environment, smell. Inherit in rabbits closely adapted to their instincts for survival and adaptation.
Ingestive behavior	**Feeding**	Taking food material into mouth and chewing and swallowing from food dispenser.
	Drinking	Lapping up water with tongue, with mouth in contact with drinking nipple(nippling)
Physical activity	**hopping**	Forward movement is achieved by alternate extension of fore and hindlimbs. Distinguished from running by its slower speed and shorter distance covered per forward jump.

Results

The obtained results outline the behavioral responses of Chinchilla and V-line rabbits in the context of vaccination against the rabbit hemorrhagic disease virus (RHDV), with a focus on assessing variations in comfort, exploratory, ingestive, and physical activities.

The behavioral responses of Chinchilla and V-line rabbits were assessed following vaccination against the rabbit hemorrhagic disease virus (RHDV). Significant breed-specific differences were observed in comfort,

exploratory, ingestive, and physical activities post-vaccination, suggesting that immune responses to RHDV may influence behavior differently across breeds. Notably, vaccinated V-line rabbits exhibited high sitting frequency and hopping duration compared to Chinchilla rabbits, highlighting breed-related differences in activity levels and rest patterns.

In relation to comfort behaviors (Fig. 2), no lying or stretching behaviors were observed in the vaccinated or control rabbits from either breed. However, the frequency of sitting (Fig. 2A) was significantly lower in both vaccinated and non-vaccinated V-line rabbits compared to Chinchilla rabbits ($P < 0.05$), though the duration of sitting (Fig. 2B) showed no significant differences between breeds.

In self-grooming (auto-grooming) behavior, there was a significant reduction in both frequency and duration in all groups at 8 hours post-vaccination compared to control V-line rabbits (Fig. 3; $P < 0.01$).

Exploratory behavior, assessed through sniffing and enclosure investigation (Fig. 4), revealed increased sniffing duration at 1-hour post-vaccination in V-line rabbits (Fig. 4B, $P < 0.01$), while a reduction in investigative behavior was noted at 1- and 8-hours post-vaccination in all groups compared to control Chinchilla rabbits (Fig. 4C, $P < 0.05$).

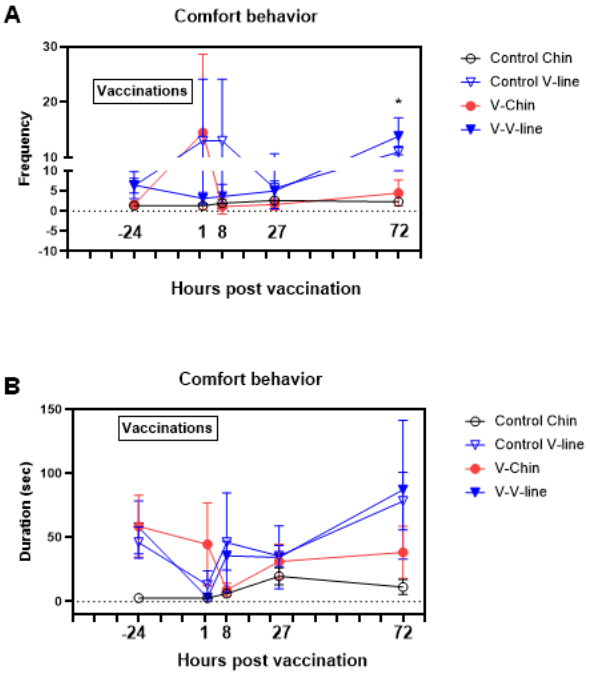


*Fig. 2. The effect of RHDV vaccination on comfort behavior of Chinchilla and V-line mature rabbits at various time points (-24, 1-, 8-, 27-, and 72-hours post-vaccination). The comfort behavioral responses in terms of frequency (A) and duration (B) in control and vaccine-injected rabbits (V-V-line and V-Chin) were assessed. Data were analyzed using a two-way ANOVA in GraphPad Prism software, followed by Tukey's post hoc test for group comparisons. Values represent the mean ± SD for each time point. Significant differences between groups are indicated by *P < 0.05.*

Ingestive behavior was measured by recording feeding and drinking duration (Fig. 5). Vaccinated Chinchilla rabbits showed a reduced feeding duration at 8 hours post-vaccination compared to the other groups (Fig. 5B, $P < 0.05$). Conversely, drinking duration significantly increased in the vaccinated rabbits at the same time point compared to non-vaccinated rabbits of either breed (Fig. 5D, $P < 0.05$).

For physical activity, both jumping and hopping behaviors were recorded. Jumping was not observed. Concerning hopping behavior (Fig. 6) vaccinated and non-vaccinated V-line rabbits showed reduced duration at 8 hours post-vaccination compared to Chinchilla rabbits (Fig. 6B, $P < 0.05$), suggesting that breed differences, rather than vaccination, influenced hopping duration.

These findings highlight the impact of vaccination on the behavioral patterns of Chinchilla and V-line rabbits, revealing distinct breed-specific responses that warrant further investigation into their implications for animal welfare and management practices.

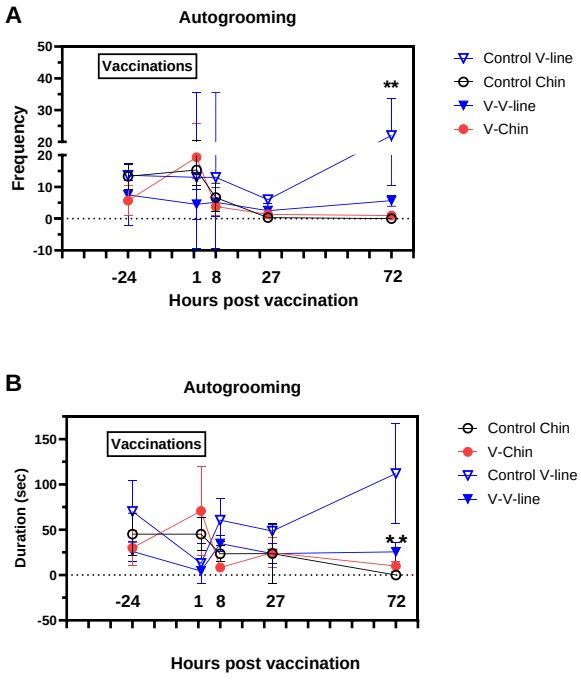


*Fig. 3. The effect of RHDV vaccination on body care behavior of Chinchilla and V-line mature rabbits at various time points (-24, 1-, 8-, 27-, and 72-hours post-vaccination). Autogrooming behavioral responses in terms of frequency (A) and duration (B) in control and vaccine-injected rabbits (V-V-line and V-Chin) were assessed. Data were analyzed using a two-way ANOVA in GraphPad Prism software, followed by Tukey's post hoc test for group comparisons. Values represent the mean ± SD for each time point. Significant differences between groups are indicated by *P < 0.05.*

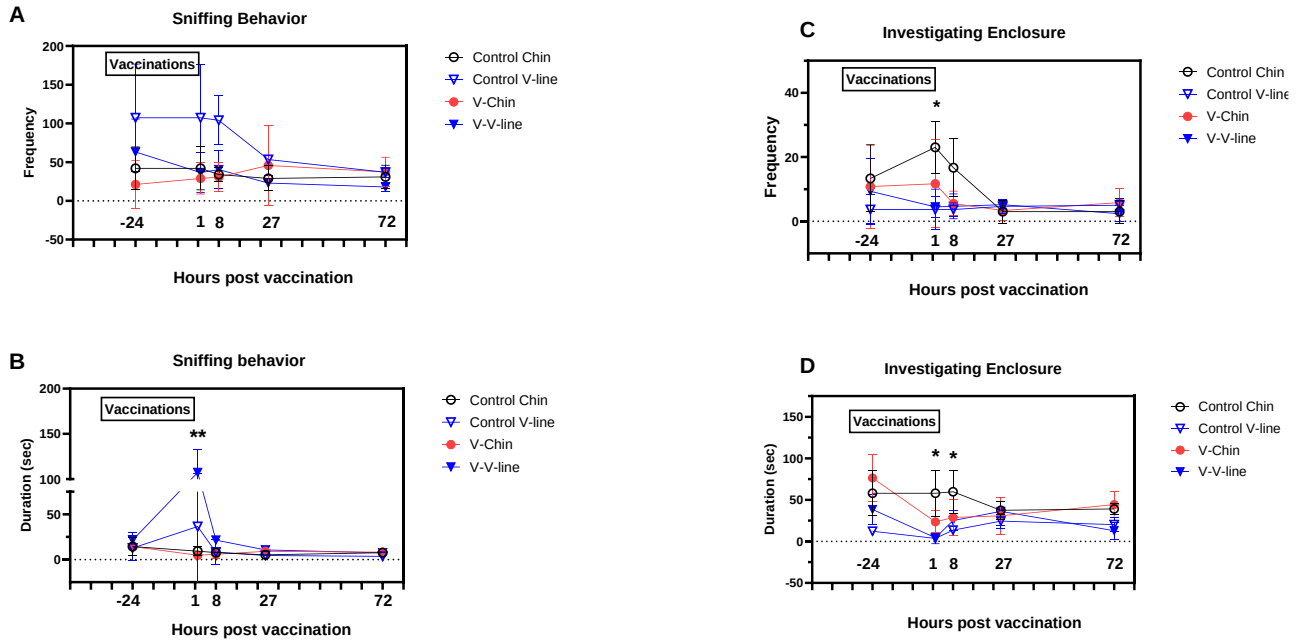


*Fig. 4. The effect of RHDV vaccination on exploratory behavior of Chinchilla and V-line mature rabbits at various time points (-24, 1-, 8-, 27-, and 72-hours post-vaccination). The data represent the mean ± SD for each time point of sniffing frequency (A), and duration (B), and investigating enclosure frequency (C) and duration (D). Behavioral responses in control and vaccine-injected rabbits (V-V-line and V-Chin) were assessed. Data were analyzed using a two-way ANOVA in GraphPad Prism software, followed by Tukey's post hoc test for group comparisons. Significant differences between groups are indicated by *P < 0.05.*

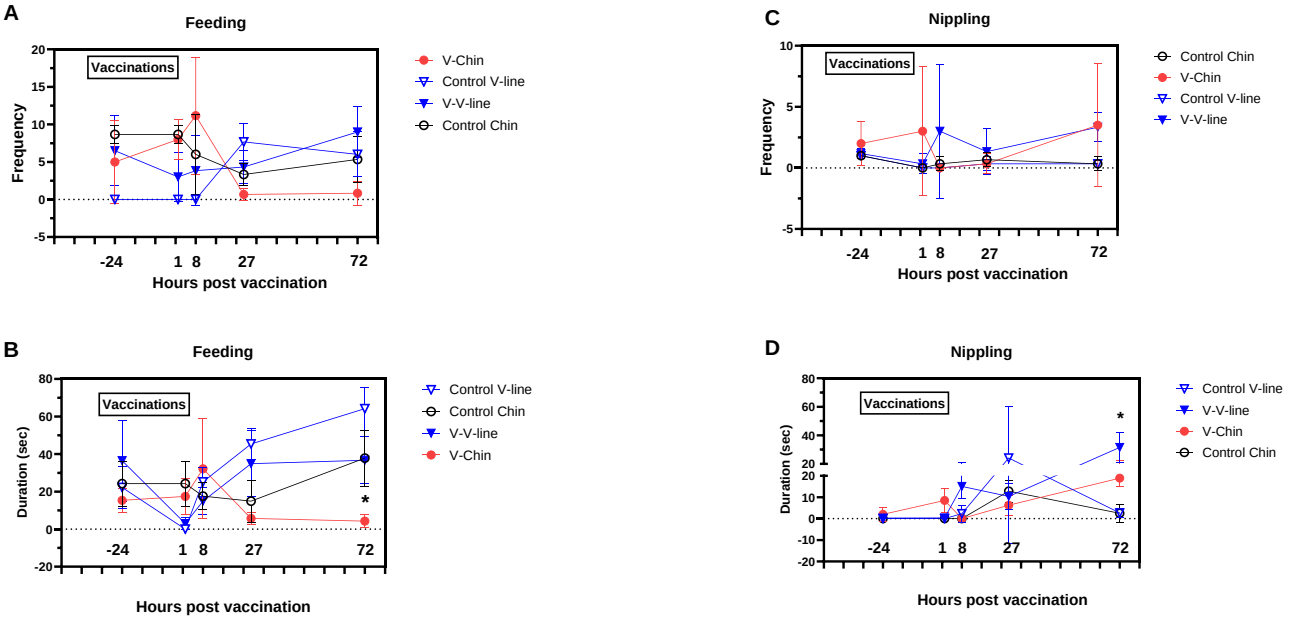


*Fig. 5. The effect of RHDV vaccination on ingestive behavior of Chinchilla and V-line mature rabbits at various time points (-24, 1-, 8-, 27-, and 72-hours post-vaccination). The data represent the mean ± SD for each time point of feeding frequency (A), and duration (B), nippling frequency (C) and duration (D). Behavioral responses in control and vaccine-injected rabbits (V-V-line and V-Chin) were assessed. Data were analyzed using a two-way ANOVA in GraphPad Prism software, followed by Tukey's post hoc test for group comparisons. Significant differences between groups are indicated by *P < 0.05.*

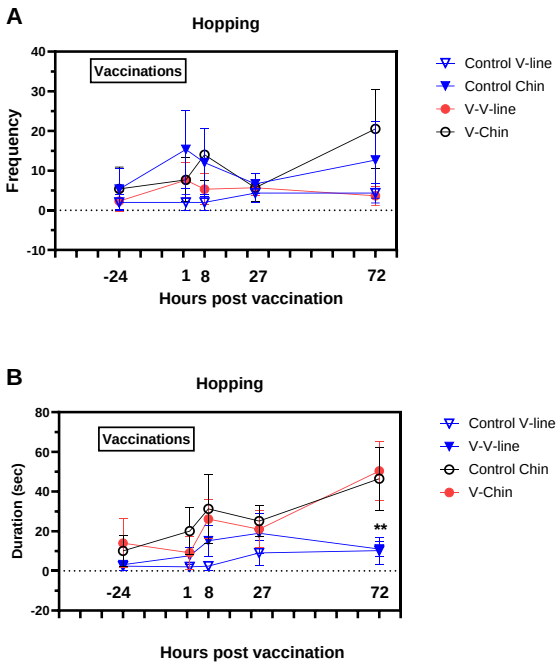


*Fig. 6. The effect of RHDV vaccination on the physical activity of Chinchilla and V-line mature rabbits at various time points (-24, 1-, 8-, 27-, and 72-hours post-vaccination). Hopping behavioral responses in terms of frequency (A) and duration (B) in control and vaccine-injected rabbits (V-V-line and V-Chin) were assessed. Data were analyzed using a two-way ANOVA in GraphPad Prism software, followed by Tukey's post hoc test for group comparisons. Values represent the mean ± SD for each time point. Significant differences between groups are indicated by *P < 0.05.*

Discussion

The present study aimed to assess the behavioral responses of two rabbit breeds—Chinchilla and V-line—after being vaccinated against rabbit hemorrhagic disease virus (RHDV). Our findings revealed significant breed-specific differences in comfort, exploratory, ingestive, and physical activities post-vaccination, suggesting that the immune response to RHDV may impact behavior in distinct ways across breeds. Notably, vaccinated V-line rabbits demonstrated reduced sitting frequency and hopping duration compared to Chinchilla rabbits, highlighting possible breed-related differences in activity levels and rest patterns. These results align with previous research, such as Baratelli et al., (2020) [19], indicating that immunological and physiological factors likely contribute to these behavioral changes. Additionally, reductions in self-grooming and feeding behaviors, accompanied by increased drinking, suggest that vaccination may induce temporary discomfort or stress, further modulating behavioral patterns. These findings emphasize the need for further investigation about the interplay between immune responses and behavioral adaptations in rabbits, particularly across different breeds.

In terms of comfort behavior (Fig. 2), neither lying nor stretching behaviors were observed in the vaccinated or control rabbits from either breed throughout the recording sessions. However, the frequency of sitting (Fig. 2A) was significantly lower in both vaccinated and non-vaccinated V-line rabbits compared to their Chinchilla counterparts ($P < 0.05$), while the duration of sitting (Fig. 2B) showed no significant differences between the breeds. These behavioral variations may reflect breed-specific immune responses to RHDV. Our results are consistent with the findings of other previous studies [19].

Since the rabbits were individually housed in cages, allo-grooming behavior was not included in this study. The analysis of self-grooming (auto-grooming) behavior revealed a significant reduction in both frequency and duration across all groups at 8 hours post-vaccination, compared to the control V-line rabbits (Fig. 3; $P < 0.01$). Similar reductions in self-grooming behavior after vaccination have been reported by other studies [20]. These findings suggest that vaccination may temporarily suppress grooming behaviors, potentially due to discomfort or stress induced by the immune responses.

Exploratory behavior was assessed through sniffing and investigating the enclosure (Fig. 4). The data revealed an increase in the duration of sniffing at 1-hour post-vaccination in V-line rabbits compared to the control and Chinchilla rabbits (Fig. 4B, $P < 0.1$). Conversely, all groups exhibited a reduced frequency of investigating the enclosure at 1- and 8-hours post-vaccination compared to the control Chinchilla rabbits (Fig. 4C, $P < 0.5$). The reduction in investigative behavior following RHDV vaccination has been similarly reported by another study [21]. However, the P-values reported here ($P < 0.1$ and $P < 0.5$) are likely to reflect the trends rather than statistically significant differences and should be interpreted with caution.

Ingestive behavior was assessed by recording the frequency and duration of feeding and drinking from nipples (nippling), as shown in Fig. 5. Vaccinated Chinchilla rabbits displayed a reduced duration of feeding at 8 hours post-vaccination compared to other groups (Fig. 5B, $P < 0.05$). Conversely, at the same time point, the duration of nippling was significantly increased in vaccinated rabbits compared to non-vaccinated rabbits of either breed (Fig. 5D, $P < 0.05$). These changes in ingestive behavior, characterized by reduced feeding and increased drinking following vaccination, are consistent with previous findings [22]. These alterations may reflect a physiological response to the vaccination, possibly related to changes in hydration needs or discomfort.

In terms of physical activity, we evaluated both jumping and hopping behaviors. Jumping was not observed during the recording sessions. The effect of vaccination on hopping behavior was not particularly distinct, as both vaccinated and non-vaccinated V-line rabbits exhibited a reduced duration of hopping at 8 hours post-vaccination compared to the Chinchilla breed (Fig. 6B, $P < 0.05$). The findings of this study provide critical insights into the behavioral responses of Chinchilla and V-line rabbits following vaccination against RHDV. The significant breed-specific differences observed in comfort, exploratory, ingestive, and physical activities highlight the complex interplay between immune responses and behavior in these two breeds. The reduced sitting frequency and hopping duration in vaccinated V-line rabbits, alongside the notable changes in self-grooming, feeding, and drinking behaviors, suggest that vaccination may induce temporary discomfort or stress, which can manifest in altered activity levels and behavioral patterns [23].

Moreover, the distinct patterns of exploratory behavior indicate that vaccination impacts how rabbits interact with their environment, with V-line rabbits exhibiting increased sniffing duration while showing decreased investigative behaviors. These results reinforce the necessity for further research to elucidate the mechanisms underlying these behavioral adaptations and their potential implications for animal welfare [24].

Conclusion

In conclusion, the observed dissimilarities in behavior following vaccination underscore the importance of considering breed-specific responses in the development of vaccination protocols and highlight the need for an ongoing investigation into how immune responses may affect behavioral adaptations in rabbits. Such knowledge could inform management practices aimed at optimizing health and welfare outcomes in both Chinchilla and V-line rabbit populations.

Authors' contribution

The work was equally distributed between authors. All authors have read and approved the final version of the manuscript.

Conflict of interest

There is no conflict of interest.

Acknowledgment

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