Management of intraspecific aggression in two bull giraffes (Giraffa camelopardalis ssp. rothschildi)

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Abstract
Maintaining nonbreeding individuals in zoological collections may sometimes necessitate housing bachelor groups. In turn, intact cohabiting males may express increased intraspecific agonistic behaviors, and management intervention may be indicated. Where castration is deemed inappropriate (e.g., future breeding, or anesthesia and surgery-related risk), the immune contraceptive gonadotrophin-releasing hormone (GnRH) is increasingly used as an alternative. When intraspecific aggression (sparring) in two bull giraffes housed as a bachelor pair at Knowsley Safari, UK, escalated in frequency and intensity (despite management interventions), further mediation was warranted to moderate sparring behaviors. The Ex situ Program recommendation was for one giraffe, the (slightly) older, outwardly mature (darker, strong musth) individual, to be treated with the GnRH vaccine Improvac® (Zoetis). To gauge the efficacy of vaccination, behavioral observations were conducted during each vaccination phase to identify changes in the frequency of sparring behaviors. In addition, fecal samples were collected by keepers and sent to Chester Zoo’s Endocrine Diagnostic Laboratory for analysis to compare androgen levels between the pre- and postvaccination phases. Testicular atrophy was investigated using both visual inspection and photographic images. The GnRH vaccine Improvac® initially appeared to be associated with reduced aggressive behaviors in the two bull giraffes. Sparring behaviors decreased in frequency after each vaccination phase, although these did not significantly diminish until phase 4. Physiological markers were inconclusive as testosterone concentrations varied throughout the phases, although levels remained low after the fourth vaccination phase. Approximately 8 months following the initial vaccination with Improvac®, the unvaccinated bull exhibited heightened aggression, resulting in physical aggression and injury to the vaccinated bull. As a result, both bulls are now on an Improvac® vaccination schedule, which has enabled them to remain housed together as a bachelor pair.

KEYWORDS
aggression, bachelor pairs, Improvac, sparring

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1 | STATEMENT OF THE PROBLEM

The ex situ management of Giraffa camelopardalis has resulted in conservation success, with populations breeding well in zoos (Schwarzenberger et al., 2022). As ex situ populations are currently stable, maintaining nonbreeding individuals in zoological collections may sometimes necessitate housing bachelor groups. Currently, Knowsley Safari houses two male Rothschild’s giraffes (G. camelopardalis ssp. rothschildi), both 9 years old with an 8-month age difference and similar in size. There are no females on site. The pair have been housed together since birth and managed as a bachelor pair since 2017. Sparring behaviors initially occurred at a frequency typical of subadult bull giraffes and were infrequent and gentle, and the social relationship remained stable. However, in 2021, sparring sessions began to occur more frequently, with lining up, leg lifting, and mounting behaviors being observed. In 2022, sparring increased substantially in frequency, intensity, and duration, becoming a daily occurrence with significant body blows resulting in swelling and hair loss (Figure 1). The sparring sessions appeared to be exacerbated further by external stimuli (loud noises, keeper talks, high density of visitors), and the giraffes were becoming less responsive to keepers and more focused on each other.

Intraspecific aggression in giraffes usually presents itself through necking and sparring. It is thought to have two main functions: practice for subadult bulls to gain fighting skills (Bercovitch & Berry, 2015) and to establish dominance hierarchy and social cohesion (Leuthold, 1979; Wolf et al., 2018). Sparring is a natural behavior in bull giraffes that maintains a hierarchy of size and age. Increased aggression levels in giraffes, particularly in those belonging to bachelor groups, have been attributed to the establishment of these hierarchies (Wolf et al., 2018). When sparring, bull giraffes will position themselves side by side, often head-to-head or head-to-tail (lining-up), and proceed to swing their neck, striking their head and ossicones against the body of the sparring partner (Granweiler et al., 2021).

Aggression is a complex behavior thought to be influenced by social learning, environmental stimuli, social hierarchy, and physiological factors (Pandolfi et al., 2021), with evidence that certain forms of aggression are also positively correlated with testosterone levels (Harding, 1983; Knol & Egberink-Alink, 1989; Lincoln, 1972; Teichroeb & Sicotte, 2008). Interventions, including castration and administration of gonadotrophin-releasing hormone (GnRH), have been successfully used to reduce circulating androgens to moderate aggression in several species, such as felids and canids (Asa et al., 2012; DeCaluwe et al., 2016; Harley et al., 2019), equids (Line et al., 1985), goats (Bishop et al., 2016), bulls (Thompson, 2000), and Loxodonta africana (Bertschinger & Lueders, 2018). Two recent studies found that the GnRH vaccine effectively reduced testosterone levels in giraffes (Moresco et al., 2022; Schwarzenberger et al., 2022). However, neither studied the effects of behavioral changes nor whether vaccination effectively mitigated aggression (specifically sparring) in bull giraffes.

In response to increased aggression between the bulls at Knowsley Safari, the animal management team consulted with the park’s veterinary surgeon and the European Association of Zoos and Aquariums (EAZA) Ex situ Program (EEP) for Rothschild’s Giraffe. They decided to administer Improvac® (Zoetis) for gonadal suppression, aiming to reduce testosterone-driven aggression (sparring). This intervention targeted the more mature bull (exhibiting darker coloration and strong musth), who frequently initiated sparring despite less effective neck swinging and thrusting. To assess effectiveness, we observed social interactions and recorded all sparring behaviors during each vaccination phase. Additional markers for the reduction of testosterone included visual observations and photographs to assess testicular atrophy and the collection of fecal samples to measure fecal testosterone metabolites (FAMs).

2 | GENERAL HUSBANDRY AND MANAGEMENT INTERVENTIONS

The indoor house measured 14.4 m × 14.3 m and was split into multiple stalls with multiple bedded areas providing separate resting areas or communal areas based on the giraffes’ preferences. The exterior enclosure consisted of a sand area directly in front of the house, followed by a larger grass area, a feeding platform used for visitor experiences, along with several trees, scratching brushes, browse stations, and enrichment devices, such as barrel and puzzle feeders, to slow feeding behavior and increase the complexity of feeding. Giraffes were given paddock access when daytime temperatures exceeded 5°C and overnight access when night-time temperatures exceeded 12°C. Giraffes were kept together 24/7 but usually chose separate sleeping/resting areas overnight. The daily diet consisted of Waterhouse browser ruminant cubes and Dengie Alfa-A (molasses free), with alfalfa and salt licks. Leaf browsing was offered (ad libitum) throughout the spring, summer, and fall, and winter browsing was maintained with frozen leaves and browse silage.
In response to a significant increase in sparring behavior observed in 2021, the zoo implemented several management interventions to reduce its intensity and frequency. These efforts aimed to minimize potential stressors and encourage natural behaviors. Specifically, the zoo closed the house to visitors to provide a quiet area with minimal disturbance. The daily pellet feed was offered in smaller, more frequent feeds, interspersed with nettles alongside additional and more frequent enrichment and browse. Keepers ensured the browse was provided in multiple areas within the house and paddock, enabling the giraffe to choose where to feed and to engage in feeding behavior for longer. Despite these comprehensive efforts, anecdotal observations indicated that the interventions were not successful in diminishing the aggressive interactions and sparring behavior. In response to this finding, the park took action in 2022 to address the ongoing issue by administering the GnRH vaccine Improvac®.

3 | ADMINISTRATION OF GNRH VACCINATION IMPROVAC®

The EEP recommendation was for one giraffe, the slightly older male, to be treated with the GnRH Improvac® vaccine. The decision was based on known risk factors, for example, giraffes may have long-term reduction of androgen levels after the termination of Improvac® vaccinations (Schwarzenberger et al., 2022). The older bull was chosen because he outwardly exhibited signs of a mature bull giraffe (darker coat coloration, emitting a strong musth smell). However, both individuals had been observed to initiate sparring sessions and exhibited varying degrees of dominance toward one another. Vaccinations were administered intramuscularly using a dart gun by the zoo’s veterinarian. Training for hand vaccination was ongoing when the first vaccination was deemed necessary; therefore, hand injections were not immediately actioned. Per Improvac® guidelines, booster one (1) was provided no earlier than 3 weeks after the initial primer dose, and subsequent boosters were given no more than 10 weeks between doses, as evidenced in Table 1. Dosage was increased from 3 to 5 mL in the fourth vaccination phase due to continued sparring behavior observed by keepers and animal managers. The higher dose has previously been used in males for immunocontraception with positive results (Schwarzenberger et al., 2022) (Table 1).

4 | BEHAVIORAL MONITORING

The effectiveness of the GnRH vaccine was determined by recording the relative frequencies of selected sparring behaviors (Table 2) and examining changes in the frequency of these behaviors performed throughout the vaccination phases. Data were collected between 09:00 and 17:00 across each phase, using one-zero focal sampling (Lehner, 1992) by three observers. Observations were carried out when time allowed, ensuring a relatively even distribution across the days, avoiding feeding and cleaning periods or when keepers were
TABLE 2 Ethogram of observed agonistic behaviors.

<table>
<thead>
<tr>
<th>Sparring behaviors</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necking/rubbing</td>
<td>One giraffe rubs its head or neck against a conspecific’s body</td>
</tr>
<tr>
<td>Lining-up</td>
<td>The two giraffes stand parallel to each other; they may be head-to-head or head-to-tail</td>
</tr>
<tr>
<td>Sparring</td>
<td>The giraffe swings its neck and strikes its head against the body of the sparring partner; this may include striking with ossicones</td>
</tr>
</tbody>
</table>

with the giraffe. The number of observation days per week varied, with a minimum of one observation day and a maximum of four per week. The prevaccination phase, which coincided with fecal collection, was brief due to the change in husbandry practices (indoor housing) due to cold weather, and observations did not recommence (due to staffing) until administration of Improvac®. During observation, an interval timer was set to notify the observer of 15 discrete minutes from the start of an observation period. During each 1-min interval, the observer would record all the behaviors performed, identified using the ethogram in Table 2. The ethogram was created by observing the behaviors of the bulls at Knowsley Safari and modifying the ethogram of general interactions and bull–bull behavior by Seeber et al. (2012). The recorded behaviors were specific to the agonistic interactions observed between the bulls during ad libitum observations.

A total of 103, 15-min observations of the vaccinated bull (VB) were conducted (Table 1). A behavior was given a one for every minute interval when initiated and performed, and a zero if the behavior was absent during that minute. Before the observations commenced, interrater reliability testing was performed by each of the three observers watching the same 5-min videos and recording the behavior performed at minute intervals using Epicollect5 (Centre for Genomic Pathogen Surveillance, 2023).

Behavioral data were analyzed and plotted using GraphPad Prism version 10.0.2 (GraphPad Software). A Kruskal–Wallis statistical test was utilized to determine if the observed number of intervals the sparring behaviors occurred per 15-min observation significantly differed across the vaccination Phases. A Dunn's multiple comparison (DMC) test for pairwise comparison was used to determine between which phases differences in behavior were shown.

### TABLE 3 Pairwise comparisons of the vaccination phases using Dunn’s multiple comparison test.

<table>
<thead>
<tr>
<th>Phases compared</th>
<th>Standard test statistic</th>
<th>Adjusted p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 vs. 2</td>
<td>1.863</td>
<td>0.625</td>
</tr>
<tr>
<td>1 vs. 3</td>
<td>2.428</td>
<td>0.152</td>
</tr>
<tr>
<td>1 vs. 4</td>
<td>3.377</td>
<td>0.007**</td>
</tr>
<tr>
<td>1 vs. prevac</td>
<td>0.762</td>
<td>&gt;0.999</td>
</tr>
<tr>
<td>2 vs. 3</td>
<td>0.289</td>
<td>&gt;0.999</td>
</tr>
<tr>
<td>2 vs. 4</td>
<td>1.547</td>
<td>&gt;0.999</td>
</tr>
<tr>
<td>2 vs. prevac</td>
<td>0.995</td>
<td>&gt;0.999</td>
</tr>
<tr>
<td>3 vs. 4</td>
<td>1.427</td>
<td>&gt;0.999</td>
</tr>
<tr>
<td>3 vs. prevac</td>
<td>1.386</td>
<td>&gt;0.999</td>
</tr>
<tr>
<td>4 vs. prevac</td>
<td>2.460</td>
<td>&gt;0.999</td>
</tr>
</tbody>
</table>

**Significant difference between vaccination phases.

**Significant difference between vaccination phases.

collected opportunistically during the vaccination phases as he was not vaccinated. All samples were sent to Chester Zoo’s Wildlife Endocrinology Diagnostic Laboratory for analysis.

### 5.1 Testicular atrophy

Keepers conducted visual monitoring throughout each vaccination phase, and photographs of the VBs’ testicles were taken during phase 1 and after phase 4. Photos were taken by the same individual, from a similar distance, angle, and with the giraffe in a similar stance.

### 6 RESULTS

#### 6.1 Behavior

A Kruskal–Wallis test revealed a statistically significant difference in sparring behaviors across the vaccination phases, $H(4) = 13.74, p = .008$. Pairwise comparisons using DMC were used to pinpoint significant differences across the vaccination phases (Table 3). Sparring frequency remained similar between the prevaccination phase and phase 1 (Figure 2). However, phase 1 exhibited a significantly higher mean frequency of sparring per 15-min observation ($M = 5.92, SD = 6.83$) compared to phase 4 ($M = 0.14, SD = 0.53$). While no other pairwise
comparisons between phases revealed statistically significant differences, a general trend of decreasing sparring behavior was observed across the vaccination phases (Figure 2).

6.2 | Hormone assay

The Chester Zoo Wildlife Endocrinology Diagnostic Laboratory reported that the giraffe exhibited varying androgen (testosterone) concentrations throughout both collection periods (Figure 3). Both bulls showed similar concentrations, although peaks in testosterone occurred at different times across the phases. The VB exhibited peaks in testosterone on November 16, 2021 (1168.08 ng/g) and during the second vaccination phase on August 9, 2022 (854.63 ng/g). In late September, there was a peak in the UB concentration (1068.42 ng/g), but it had returned to its previous level when sampled on October 1, 2022, with the VB concentration of 146.31 ng/g and UB 180.03 ng/g.

6.3 | Testicular atrophy

We compared photographs taken in phase 1 and a photograph taken after the fourth vaccination phase (Figure 4).
7 | DISCUSSION

Improvac® vaccination in a single bull giraffe appeared to reduce intraspecific aggression levels initially. Sparring behaviors initiated by the VB decreased in frequency after each vaccination phase. Comparatively, other mammals immunized with Improvac®, specifically to reduce aggression, showed behavioral changes after the second and third vaccinations (Bishop et al., 2016; Brewster & Nevel, 2013; Lueders et al., 2014; Martínez-Giménez et al., 2021; Rydhmer et al., 2010). Our findings were similar, with a decrease in the frequency of sparring in phase 2 and phase 3 compared to phase 1. However, a statistically significant reduction did not occur until phase 4, where the mean incidences of sparring were less than one per 15-min observation. This aligns with the findings of Schwarzenberger et al. (2022), advising that male giraffes may require four or five vaccinations at 4-week intervals for contraceptive effect.

Monitoring testicular atrophy in male giraffes is another measure for determining Improvac® vaccination success (Schwarzenberger et al., 2022). Following the fourth vaccination phase, photographs revealed a significant reduction in scrotal and testicular size. While keepers observed a change in appearance near the end of phase 3, a consensus was lacking to the extent of reduction, with varying responses. This was likely due to slight variations in the giraffe’s stance (weight bearing) and the keeper’s angle of view. We recommend standardization for monitoring testicular atrophy, ideally using a crush, to ensure consistency in distance, angle, and height for direct comparison, particularly if used as a single measure of vaccination efficacy.

As reported by Chester Zoo, fecal androgen metabolite (FAM) concentrations exhibited significant variation throughout the four vaccination phases. While both bulls demonstrated similar overall FAM concentrations, the timing of peak levels differed significantly between individuals. This individual variation in peak timing makes it challenging to definitively attribute the observed decrease in FAM levels postvaccination solely to the Improvac® treatment. However, 2 weeks after the third dose and throughout the fourth vaccination phase, the VB FAM concentrations remained low, similar to findings by Schwarzenberger et al. (2022), which concluded that four doses at monthly intervals are sufficient to suppress androgen concentrations.

The mechanism underpinning the unexpected testosterone increase after initial vaccination remains unclear. This paradoxical effect contrasts with the expected action of GnRH vaccines, which block pituitary stimulation and suppress sex hormone production. Unlike GnRH agonists, which temporarily stimulate the pituitary gland and cause an initial surge in gonadotropins and gonadal activity, vaccines should theoretically prevent such hormonal increases. Interestingly, Moresco et al. (2022) discussed gonadal stimulation in some female giraffes and noted that collections observed estrus-like behavior in females after Improvac® vaccination. These findings warrant further investigation to understand this unexpected response.

A side effect of the vaccination protocol’s efficacy is that the pair’s social dynamics changed. Approximately 8 months following the initial vaccination with Improvac®, the UB exhibited heightened aggression and dominance behaviors toward the VB. The UB was observed vocalizing, resource guarding, and dominance posturing toward the VB and the keepers. These behaviors resulted in significant injury to the VB and subsequent avoidance behaviors. These behavioral changes, including heightened aggression, dominance displays, and possessive behavior (similar to mate guarding, e.g., increased proximity, defensive dominance display), toward the VB resulted in an animal management decision to separate the giraffes overnight. Doing so allowed the VB to rest and sleep undisturbed by the UB’s continual attention. However, management intervention alone was ineffective in mitigating the UB’s aggression, and in March 2023, the UB received the first monthly Improvac® injection.

8 | CONCLUSION

Our case study demonstrates that vaccination with Improvac® was associated with a reduction in sparring behavior between the giraffes, but only after the fourth dose. This coincided with low FAM and the
reduction in size of the scrotum and testes. However, it is important to note that pre- and postvaccination FAM concentrations showed similar overall levels between the bulls, although the timing of their peak levels differed. Furthermore, we found that 8 months after treating one of the two bull giraffes with Improvac®, there was a shift in social dynamics, requiring further management intervention and subsequent vaccination of both bulls. At the time of writing, the bulls remain housed together (separated at night), and the authors report that modest improvements in the social dynamics have been observed, including less avoidance and occasional social feeding at the browse station with no observed sparring behavior. In retrospect, vaccinating both bulls simultaneously might have circumvented injury and agonistic behavior. However, concerns regarding the potential for long-term reductions in androgen levels following the cessation of Improvac® treatments led us to exercise caution and initially vacinate only one bull.

ACKNOWLEDGMENTS
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CONFLICT OF INTEREST STATEMENT
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT
This husbandry report documents the practices used to manage the sparring behavior of two similarly sized/aged bull giraffes at Knowsley Safari. The administration of Improvac, as outlined in this paper, was not for research but for the practical management of two bull giraffes and, therefore, was not eligible for review by Knowsley’s research and ethical review panel. The observational study was approved by Knowsley Safari’s Ethics Committee before the study. All management practices conducted during this study were reviewed and approved by the Head of Living Collections, the animal managers, and the veterinary surgeon responsible for the giraffes and informed by recommendations from the EAZA Ex situ Program.

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