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Title:

The influence of stable fly invasion on the behavior of captive black rhinoceros (*Diceros bicornis*)

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Abstract

To determine the effect of stable fly invasion on the state of captive animals, we investigated the fly abundance and related behavioral responses of captive black rhinoceroses before and after the use of pesticide. Two black rhinoceroses in the Kamine Zoo were used as the subjects. We compared the number of stable flies on their bodies, repelling behavior and maintenance behavior under normal husbandry conditions (control) and after pesticide application (debug). The number of stable flies decreased by nearly 80% under the debug condition compared with the control. Stable flies were observed most frequently on the bellies of the rhinoceroses, and the largest decrease in the number of flies after pesticide application was observed on the animals' backs. The frequency of the rhinoceroses' repelling responses also decreased, by an average of nearly 60%, after the debug treatment compared with the control. Time allocation to the maintenance behaviors of standing rest and lying rest increased significantly, by 5–10%, under the debug condition for both rhinoceroses. On the other hand, the time allocation to walking decreased significantly, by approximately 9–13%, in both rhinoceroses with pesticide application. These results suggest that fly invasion drastically disrupted rhinoceros behavior, and that it likely poses a severe burden in zoo animals during the fly season.

Keywords

animal welfare, defensive behavior, hematophagous insect, repelling

behavior, zoo

Introduction

The stable fly (*Stomoxys calcitrans*) is a hematophagous fly belonging to Diptera: Muscidae. The males and females of this species suck blood from a variety of animal hosts (Hafez and Gamal-Eddin, 1959). The damage caused by this fly is quite substantial and is associated with direct economic losses, such as decreased milk yield in dairy cattle (Bruce and Decker, 1958) and decreased body weight in beef cattle (Campbell et al., 1987). Taylor et al. (2012) estimated that losses in the United States reached $360 million for dairy cattle, $358 million for cow-calf herds, $1.3 billion for pastured cattle and $226 million for cattle on feed, for a total impact on the US cattle industry of $2.2 billion per year. Thus, this fly is regarded as a main pest of farm animals in many countries (Todd, 1963; Venkatesh and Morrison, 1980; Kunz et al., 1991; Aorigele et al., 2003).

Another crucial issue is that many blood-sucking insects serve as vectors for a variety of diseases. Wild free-ranging and grazing animals are highly exposed to such disease transmission by insects (Kamut and Jezierski, 2014). Equine infectious anaemia, habronemiasis, onchocerciasis, parafilariasis and thelaziasis are known to be transmitted by Muscidae insects. Muscidae are also intermediate hosts of parasites causing parafilariasis, thelaziasis and habronemiasis (Kamut and Jezierski, 2014). Therefore, invasion by stable flies is considered to be a major impediment to the hygiene control and welfare of captive-raised animals.

During invasions by stable flies, pain and itching associated with fly bites appear to cause substantial stress and affect various behaviors of animals. Dougherty et al. (1993) documented increased tail swinging and leg raising, and decreased feeding and resting, when they experimentally attached stable flies to the body surfaces of grazing cattle. King and Gurnell (2010) also reported that wild horses grazed less and moved more frequently with increasingly severe fly attacks. Furthermore, Duncan (1983) demonstrated that horses were forced to leave their preferred feeding areas when fly abundance was high.

Similar fly invasions of zoo animals have long been reported. However, these studies typically used traps to capture the flies (Rugg, 1982; Ose and Hogsette, 2014; Hogsette and Ose, 2017) and few studies have examined the direct effects of fly control measures on the behavior and state of animals. In the present study, we investigated differences in the behavior of captive rhinoceroses before and after the use of pesticides to determine the effect of stable fly invasion on the state of this zoo animal.

Materials and methods

Animals and management

The subjects for this study were two black rhinoceroses (*Diceros bicornis*) exhibited at the Kamine Zoo, Hitachi, Ibaraki, Japan. The two animals were a male (named Metro)–female (named Maki) pair. Metro was born on 10 November 1990 at the Miami Zoo, Florida, USA, and arrived at the Kamine Zoo on 20 August 1993. Maki was born at the Kamine Zoo on 8 June 1990.

The rhinoceros enclosure at the Kamine Zoo contains two indoor barns and an outdoor exhibit. The length of the outdoor exhibit space is approximately 97 m, and its area is almost 511 m^2. The exhibit is enclosed by a concrete wall and iron fence, and a sloped moat (1.5 m depth, 3.5 m width) is positioned on the audience side of the enclosure. A 36.5-m^2 pool inside the enclosure is used as a watering and bathing area. The centre of the exhibit includes a Japanese maple (*Acer palmatum Thunberg*) and several rocks and wallows. Generally, the rhinoceroses were exhibited from 8:30 to 17:00 and fed twice a day. The first feeding occurred during exhibition and the second feeding occurred after evening housing.

Behavioral observations

The experiment was conducted for 6 days (three pairs of days) from September to October 2015. To determine the effect of fly invasion, we established two conditions for 3 days each; the first day of each pair involved normal husbandry (control) conditions, and the second day involved pesticide application (debug). The active ingredient of the pesticide was 7.0%

etofenprox (bermitol aqueous emulsion, Aqua; Mitsui Chemicals Agro, Inc., Japan). The pesticide was sprayed evenly over the entire bodies of the rhinoceroses, except the face, using a special atomiser, between 10:00 and 10:30, while the animals were on exhibit. The weather conditions during the experiment did not differ significantly in terms of average temperature (control: 21.9 ± 4.6°C, debug: 21.0 ± 2.5°C; Welch two-sample *t*-test, $t = 0.29$, df = 3.10) or wind speed (control: 2.27 ± 0.60 m/s, debug: 1.67 ± 0.35 m/s; Welch two-sample *t*-test, $t = 1.49$, df = 3.22).

Observations were conducted for 8 h from 9:00 to 17:00 on each day. The rhinoceros body was divided into five parts (head, neck, belly, back and hip; **Figure 1**), and a 30 × 30-cm zone of each body part was photographed every 30 min for counting of the number of stable flies for estimation of the intensity of invasion. Grooming behaviors, such as rubbing and scratching, and self-defensive behaviors, such as flicking of the ear or tail, were defined as 'repelling responses', and the frequency of each behavior type was recorded using the continuous sampling method. To evaluate the rhinoceroses' quality of life, maintenance behaviors were divided into five categories – feeding, standing rest, lying rest, walking and others – and the frequency of each behavior type was recorded using a 2-min scan sampling method.

Statistical analysis

Only data from photographs in which the rhinoceroses' five body parts could be discriminated clearly were used for the analysis of the number of stable flies on the body surface (Metro: 126 photographs, Maki: 127 photographs). The average number of stable flies on each body part was measured in units of 100 cm^2 per hour and compared between the control and debug conditions for each rhinoceros using the paired t-test. The frequency of repelling responses during each 30 min after 10:00, when the pesticide was applied, were compared between the control and debug conditions for each rhinoceros using Student's t-test. The time allocation of each maintenance behavior under each treatment condition was assessed according to the frequency of each behavior in both animals for each of the 3 days using the χ^2 test. In addition, the difference in each maintenance behavior under the two experimental conditions was examined by residual analysis. All statistical analyses were performed with the free statistical software R (ver. 3.2.2; The R Foundation for Statistical Computing).

Results

The numbers of stable flies on the bodies of both rhinoceroses before and after pesticide application are shown in **Figure 2.** Under the control condition, the mean number of flies on Metro was 3.46/100 cm^2/h, which was slightly more than the number of flies on Maki (2.44/100 cm^2/h). Under the debug condition, the average numbers of flies decreased markedly on Metro

(0.87/100 cm^2/h; paired *t*-test, $t = 2.29$, df = 4; $P = 0.08$) and Maki (0.49/100 cm^2/h; paired *t*-test, $t = 3.70$, df = 4; $P < 0.01$). The percentage decreases in the number of flies on Metro ranged from 81.1% to 42.1% in order of belly, back, head, neck and hip, for an overall average 74.8% decline with pesticide application. On Maki, the percentage decreases ranged from 87.6% to 74.8% in order of belly, head, neck, back and hip, with an overall average decrease of 79.8%. Stable flies were observed most frequently on the bellies of both rhinoceroses (Metro: 8.15 flies/100 cm^2/h, Maki: 4.61 flies/100 cm^2/h). Under the debug treatment, the number of flies exhibited the largest decrease on the backs of both rhinoceroses (Metro: 81.1%, Maki: 87.6%).

Due to these substantial decreases in the extent of stable fly invasion, the frequency of the rhinoceroses' repelling responses also decreased drastically after pesticide application (**Figure 3**). The maximum number of Metro's repelling responses under the control condition was 164 times/h (2 September, 13:00), whereas this value declined to 61 times/h under the debug condition (3 September, 10:00). For all three pairs of days combined, the number of Metro's repelling responses differed significantly between conditions (control: 45.1 times/h, debug: 19.9 times/h; Student's *t*-test, $t = 2.93$, df = 12; $P < 0.05$); the overall mean percentage decrease was 56%. For Maki, the maximum number of repelling responses decreased from 119 times/h under the control condition (24 September, 12:00) to 54 times/h under the debug condition (20 October, 14:00). For all three pairs of days combined, the number of Maki's repelling responses differed significantly between conditions (control: 56.7 times/h, debug: 20.7 times/h; Student's t-test, t = 4.98, df = 12; $P < 0.0005$); the overall mean percentage decrease was 63.5%.

Time allocation to maintenance behaviors also differed significantly between conditions (Metro: $\chi^2 = 58.7$, df = 4, $P < 0.00001$; Maki: $\chi^2 = 27.2$, df = 4, $P < 0.00005$; **Figure 4**). The residual analysis indicated that the time allocation to standing rest and lying rest increased significantly (Metro) or tended to increase (Maki), by about 5–10%, from the control to the debug condition. By comparison, the time allocation to walking decreased significantly, by approximately 9–13%, in both rhinoceroses ($P < 0.0001$).

Discussion

The skin of the rhinoceros includes a well-keratinised epidermis and a thick, weighty and inelastic dermis (Cave and Allbrook, 1958). This type of skin is very well adapted for resisting attacks from the horns of conspecifics during aggressive behaviors (Shadwick et al., 1992). Due to the enhanced protective nature of this type of skin, one might assume that invasion by a parasite or blood-sucking insect might not pose a serious problem. However, rhinoceros skin is in fact extremely delicate, and damage caused by various hematophagous insects is quite common (Persons and Sheldrick, 1964;

Skinner and Smithers, 1990; Penzohn et al., 1994).

In the present study, the belly was the area of the body attacked most frequently by stable flies for both rhinoceroses. Previous studies have shown that the lower legs or torsos of farm animals were frequently attacked by flies (Lysyk, 1995; Eicher et al., 2001). In rhinoceroses, the belly skin, which included the flank and upper leg area in our study, is relatively thin and less stiff (Shadwick et al., 1992). Therefore, this area may have been vulnerable to concentrated attack compared with upper areas, such as the back and hip. In contrast, the largest decrease in the number of flies after pesticide application was seen on the back. Repelling responses are types of self-defensive behavior and often include stomping, kicking of the body, tail swishing, skin twitching and head or ear movement (Eicher et al., 2001). Rhinoceroses also defend their bodies from some insects by wallowing and coating their bodies with mud (Julia et al., 2001). However, the back is one of the most difficult places to reach for ungulates (Mooring et al., 2004, DeVries et al., 2006), and it cannot be covered well when wallowing. Thus, a comparatively large number of flies may remain on the back before pesticide application, and the effect of application would be large.

The number of repelling responses decreased significantly after pesticide application in this study. Rhinoceroses may spend approximately three times longer engaging in fly-repelling behavior during the fly season compared with the non-season. The primary repelling responses in both rhinoceroses, tail swishing and ear flipping, did not disappear completely under the debug condition in this study. Some flies remained on the animals' bodies (80%, not 100%, decline in the number of flies). In addition, the decrease in repelling behavior was not large compared with the decline in the number of stable flies due to pesticide application. Thus, these behavioral responses may have served other functions in addition to repelling insects. Kiely-Worthington (1978) reported that ear flicking is observed during some conflict or transitional situations between bouts of other on-going behaviors in some ungulates and cats, except in fly repulsion situations. She also reported that tail movement in animals, including rhinoceroses, serves a similar function to ear flicking, again except in fighting and fly repulsion situations (Kiely-Worthington, 1976; 1978). Therefore, a frequency of about 20 times/h for these behaviors may represent the normal condition.

After pesticide application, the resting behavior of both rhinoceroses increased, whereas walking decreased. One important function of resting, which includes sleeping, is energy conservation for metabolic recovery (Frazer and Broom, 1997; Olsson et al., 2011). Disruption of this behavior can lead to the deterioration of physical and mental health (Galindo and Broom, 2000, Misrani et al., 2019). Previous studies have documented decreased feeding and resting during extremely severe fly invasion, as animals must spend most of their time engaging in repelling behavior and escape movements (Dougherty et al., 1993; King and Gurnell, 2010). In contrast, the proportion of time spent in lying rest in their captive environments increased when animals were in comfortable conditions with few blood-sucking insects (Ito, 1971; Aorigere et al., 2003). In the present study, the reduction of fly invasion by pesticide application led to decreased walking from restlessness, and increased resting behavior. Therefore, the rhinoceroses likely felt more comfortable after pesticide application, as their ability to rest had been severely disrupted by stable fly invasion.

In conclusion, the impact of stable fly invasion on captive rhinoceroses was comparatively large. The resting behavior of the rhinoceroses was disrupted, and much of their time was spent engaging in fly-repelling behaviors under the control condition. To enable accurate assessment of the effects of such ectoparasite stress, researchers must establish the nature of normal behaviors as a baseline response level for each animal.

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Daisuke Kohari.

Ethical considerations

We read the policy of the journal on ethical consent and standard animal care, and our work was carried out in accordance with these policies. Furthermore, we limited animal use in accordance with "Three Rs" indicated in Directive 2010/63/EU.

Conflict of interest

The authors have no conflicts of interest directly relevant to the content of this article.

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Figure Legends

Figure 1

Body surface classification for the black rhinoceros.

Figure 2

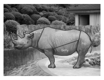
Numbers of stable flies before (control) and after (debug) pesticide application.

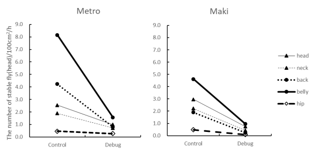
Figure 3

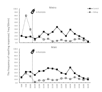
The frequency of repelling behaviors in both rhinoceroses before (control) and after (debug) pesticide application.

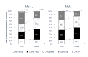
Figure 4

Time allocation to maintenance behaviors before (control) and after (debug) pesticide application. The numbers in each bar graph indicated percentages of each behavior (＊: $P<0.05$, †: $P<0.1$, Residual analysis).




Metro
Maki
The number of stable fly(head)/100cm²/h
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Control
Debug
head
neck
back
belly
hip




Metro
Maki
Control
Feeding
Stand rest
Lying rest
Walking
Others

Highlights

- ✓ Lower abdomen is easily attacked by flies also in rhinos.
- ✓ Rhinoceros are disturbed their rest for repelling blood-sucking flies.
- ✓ Behavior responses for fly repelling may include other normal behaviors.