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INTERACTIVE EFFECTS OF TURKEYPOX VIRUS AND *PLASMODIUM HERMANI* ON TURKEY POULTS

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ABSTRACT: Two experiments were conducted to examine the interactive effects of two disease agents of wild turkeys (*Meleagris gallopavo*), turkeypox virus and the malarial organism, *Plasmodium hermani,* on the health of turkey poults. Groups of domestic broad-breasted white turkey poults of 1 and 10 wk of age were infected with either turkeypox virus, *P. hermani,* both turkeypox virus and *P. hermani,* or were maintained as uninfected controls. The strains of turkeypox virus and *P. hermani* had been isolated from wild turkeys in southern Florida (USA). The goals of these experiments were two-fold and included both an examination of age differences in response to infections, and an examination of the effects of dual versus singular infections with the two agents. Both singular and concomitant infections of turkeypox virus and *P. hermani* were more detrimental to poults infected at 1 wk of age than to those infected at 10 wk, based on mortality, weight gain, and parasitemia. Dual infections of turkeypox virus and *P. hermani* were found to be slightly more harmful to 1-wk-old poults than were singular infections. No such interactive effects were noted in the poults infected at 10 wk of age.

Key words: Domestic turkeys, interactive effects, malaria, *Plasmodium hermani,* turkeypox virus.

INTRODUCTION

The wild turkey (*Meleagris gallopavo*) is one of the most important game species in Florida and has been the subject of considerable research (Williams and Austin, 1988; Forrester and Spalding, 2003). One facet of this research has involved a determination of the prevalence and significance of various diseases in a wild turkey population at Fisheating Creek Wildlife Management Area and Refuge in southern Florida (USA) from which 89 infectious and parasitic disease agents have been identified (Forrester and Spalding, 2003). A major focus of interest in that study was the health and disease status of wild turkey poults during their first several months of life after hatching, during which time mortality can reach 70% (Williams and Austin, 1988). Two of the most common and potentially devastating diseases are turkeypox and malaria (*Plasmodium hermani*; Forrester, 1991, 1992). In sentinel studies at Fisheating Creek using domestic turkey poults, it was found that prevalences of *P. hermani* and turkeypox sometimes reached as high as 40% and 100%, respectively, during the mosquito season in late summer and early autumn (Forrester and Spalding, 2003). Many of these were concurrent infections. Either of these diseases alone can be detrimental to turkey poults, but when combined the impact can be more severe. Akey (1981), for instance, found that less than 1-wk-old poults simultaneously exposed to *P. hermani* and turkeypox virus had a higher mortality rate, depressed weight gains, depressed packed cell volume, and an increased magnitude and duration of patent parasitemia than did singularly infected or uninfected poults. The present study was designed to explore more fully the effects of dual infections of turkeypox virus and *P. hermani* on turkey poults of various ages.

MATERIALS AND METHODS

Experimental animals

Domestic broad-breasted white (BBW) turkeys were purchased from Thaxton's Turkeys (Watkinsville, Georgia, USA) and used as an experimental model for the wild turkey. One-day-old poults were housed in vector-proof facilities

for the duration of the study. Poults were kept in heated brooders maintained at 39 C for 14 days before being moved to unheated facilities. At 10 days of age all poults were banded for identification and debeaked to reduce the prevalence of cannibalism. Water and 22% protein ration consisting of corn and soybean meal with trace mineral and vitamin supplements were provided ad libitum.

Experimental design

In Experiment I, domestic poults that were 1 wk old at the beginning of the experiment were used and in Experiment II, 10-wk-old domestic poults were used. Both age classes were subdivided into four groups of 11 birds each. Due to the failure of some poults to become infected with turkeypox virus, two of the experimental groups were reduced slightly in number later in the experiment. The first group served as uninfected controls, the second group was infected with poxvirus only, the third group with *P. hermani* only, and the fourth group with both poxvirus and *P. hermani.*

Malaria infections

Poults were infected with *P. hermani* (Strain P-41) via exposure to infected mosquitoes. This strain of malaria had been obtained from a wild turkey at Fisheating Creek Wildlife Management Area in Glades County, Florida and passaged from poult to poult six times by blood inoculation prior to being used in this study. Domestic BBW turkey poults with malaria parasitemias were used as malaria donor birds to establish infections in the natural mosquito vector, *Culex nigripalpus.* The methods used for infecting mosquitoes and allowing mosquitoes to infect poults followed Forrester et al. (1980) and Nayar and Forrester (1985). Ten to 17 days after being infected with *P. hermani,* the donor poults were exposed to female mosquitoes. Six batches of 300 to 500 mosquitoes each were allowed to feed to repletion on the donor poults. Ten to 12 days post blood meal, a random sample of 20 female mosquitoes from each of the six batches were dissected to determine the numbers of oocysts in their midguts. On day 16 the salivary glands of a sample of these mosquitoes were examined for the presence of sporozoites. This allowed for an estimation of the percentage of mosquitoes that were infected and the level of infection per mosquito. Eighty-three percent of the mosquitoes were infected with an average of 12.8 oocysts per female. Three to 4 wk after mosquitoes were blood-fed on infected donor birds, they were allowed to feed to repletion on restrained experimental poults. Each poult was exposed to 35 infected mosquitoes, the majority of which fed on the poults.

Turkeypox virus infections

Turkeypox virus infections were established via the bite of mosquitoes that had fed on turkeys with active pox lesions. *Culex nigripalpus* mosquitoes were used because they had been shown to be mechanical vectors of turkeypox virus by Akey et al. (1981). Eight donor birds were infected with poxvirus by scarifying an area on the top of the head and then rubbing the clean-cut surface of a pox lesion over the scarified area. The pox lesions were obtained originally from sentinel domestic turkeys exposed to mosquitoes at Fisheating Creek where turkeypox virus infected birds are known to occur (Akey et al., 1981). Thirty to 40 mosquitoes were allowed to feed only to partial repletion on donor turkeys, removed, and then allowed to feed to repletion on each experimental poult. The poults were restrained in a manner such that only their heads and faces were exposed to mosquitoes. Representative turkeypox lesions were fixed and preserved in 10% buffered formalin and examined histologically using standard techniques to verify the presence of inclusion bodies.

Uninfected controls

Poults from the two control groups were treated in the same manner as experimental birds, but were exposed to the bites of uninfected mosquitoes.

Parameters monitored

The parameters monitored included mortality, body weight, parasitemia of *P. hermani,* packed cell volume, and the ratio of immature to mature red blood cells. These values were measured for 7 wk post-infection (PI). All birds were weighed once per wk and on that same day a blood sample was drawn in a heparinized microhematocrit capillary tube, centrifuged for 5 min, and measured to determine the packed cell volume (PCV). Thin blood films were made three times each wk from each poult. These were air dried, fixed in 100% methanol, and stained with Giemsa stain at a 1:9 dilution for 1 hr. A total of 10,000 red blood cells was examined using an oil immersion lens at 1,000× magnification. Poults were examined three times each wk for evidence of pox lesions. All birds that died in the course of the study were examined at necropsy at which time the weight, age, sex, number and location of pox lesions, and the general body condition of each bird was recorded. When possible, blood smears

were made from heart blood. Tissue samples of heart, lung, liver, kidney, brain, bone marrow, spleen, and pox lesions were preserved in 10% buffered formalin, sectioned at 5–6 μm, and stained with hematoxylin and eosin for histopathologic analyses.

Statistical analyses

Data were analyzed using the Statistical Analysis System (SAS) general linear models procedure (SAS Institute, 1988). A square root transformation was used to normalize the data. For each individual parameter a treatment, age, treatment×age, sex, treatment×sex, age×sex, and treatment×age×sex interaction was tested for using a Type III Sum of Squares Test. Differences were considered to be significant at $P \leq 0.05$. Unless otherwise noted, P values are 0.05. When interactions were found to be significant, a Duncan's Multiple Range Test was applied to the data to determine the source of variability.

RESULTS

Mortality

Five of the 1-wk-old poults died between day 22 and 39 PI, but there was no mortality in any of the 10-wk-old poults. Three of the poults that died were infected with turkeypox virus and two with both turkeypox virus and *P. hermani.*

Poult #1 was a male infected with pox and malaria that died 22 days PI. There were nine pox lesions, three on the eyelids located such that one eye was completely closed and the other partially closed. Four lesions were on the nares and resulted in full closure of both. One was on the beak extending inside the mouth and one was under the beak. The poult weighed 382 g compared to the average of 533 g for the group and 671 g for the controls. The PCV was 32% compared to 30% for the group and 36% for the controls. Parasitemia was 416 per 10,000 red blood cells compared to a mean of 99 for the group on the same date.

Poult #2 was a male infected with pox and malaria that died 25 days PI. There was only one pox lesion; this was located on the beak and extended into the oral cavity. The poult weighed 592 g compared to an average of 533 g for the group and 671 g for the controls. The PCV was 29% compared to 30% for the group and 36% for the controls. The parasitemia was 283 per 10,000 red blood cells compared to a mean of 85 for the group on the same date.

Poult #3 was a male infected only with pox that died 25 days PI. There were five pox lesions, one on each eyelid such that both were totally obstructed. Another was on the left nare and completely occluded it, one was under the beak, and one large lesion was in the oral cavity. The poult weighed 388 g compared to an average of 604 g for the group and 670 g for the controls.

Poult #4 was a female infected only with pox and died 26 days PI. There were five pox lesions on its head. One was over one eye, two were obstructing the nares, and two large ones extended from the top of the beak to the inside of the mouth. In addition four small lesions were located on the legs. The poult weighed 415 g compared to an average of 604 g for the group and 670 g for the controls.

Poult #5 was a male infected only with pox and died 39 days PI. There were six pox lesions on the head, three located over one eye, two on the bottom of the beak, one of which extended into the oral cavity, and one that extended across the entire top of the beak and occluded both nares. In addition there was one lesion on the bird's leg and one on its wing. The poult weighed 547 g compared to an average of 1,153 g for the group and 1,395 g for the controls.

All the skin lesions from poults that died had microscopic changes compatible with avian pox infection, including cytoplasmic inclusion bodies (Bollinger bodies) in numbers that correlated well with the degree of epidermal hyperplasia. All lesions had superficial bacterial colonies intermixed with the surface exudate. In the remainder of the tissues, only mild lesions were detected consisting primarily of lymphoid aggregates or foci of subacute inflammation. Poult #4 had bacteria present

in the lung and a mild pneumonia. Poults 1 and 2 had mild to moderate amounts of granular brown pigment in reticuloendothelial cells that was compatible with malarial pigment. No exoerythrocytic stages of *Plasmodium* were detected in the tissues examined.

Weight gain

Age, sex, and age×sex interactions were significant. There were no significant treatment or treatment×age interactions. No significant differences were detected in the 1-wk-old poults until 5 wk PI at which time the turkeypox/malaria poults were lighter in weight than the control poults (Fig. 1a). At wk 6 the turkeypox/malaria group was significantly lighter than both the control and malaria groups. By wk 7, control, turkeypox, and malaria-infected poults were not significantly different from each other in weight. There were no significant differences detected throughout the experiment between the weight gains of any of the treatment groups of birds infected at 10 wk of age (Fig. 1b).

Packed cell volume

Significant treatment effects on packed cell volumes were detected for wk 2 through 7 PI. Age was a significant factor on wk 2, 3, 4, 5, and 7. Treatment×age

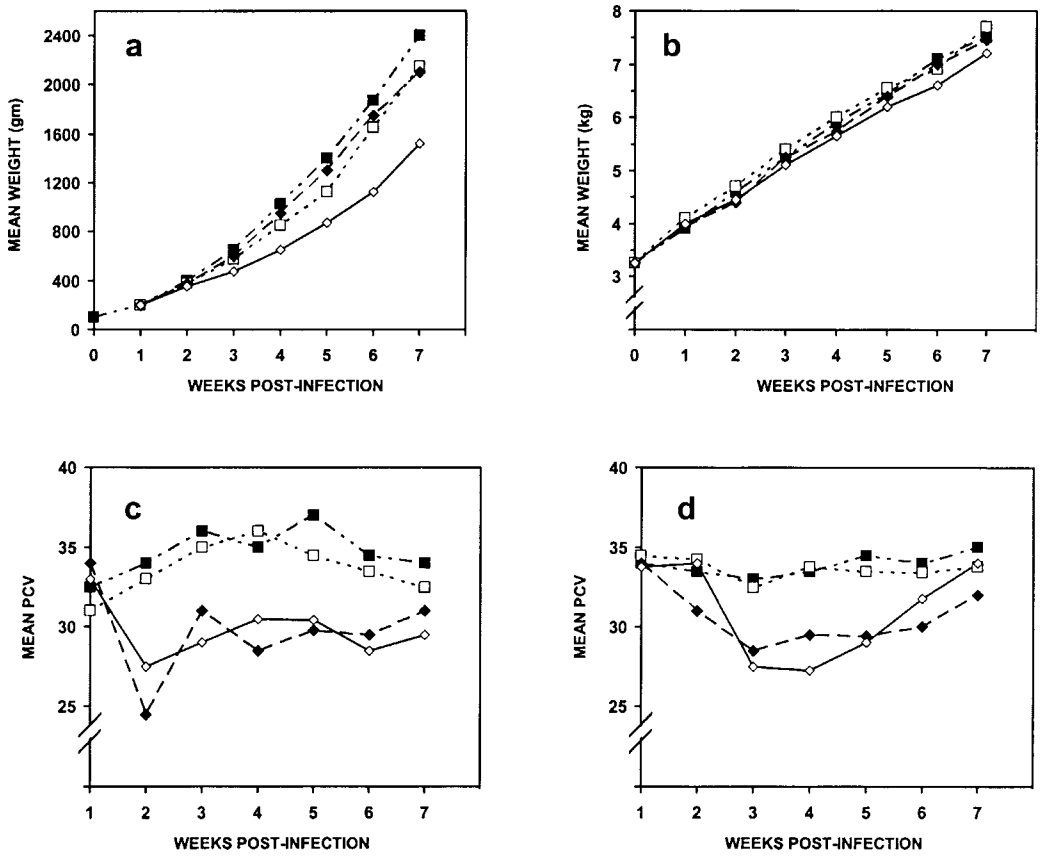

FIGURE 1. Mean weight gains and mean packed cell volumes of domestic turkey poults infected with turkeypox virus (open squares), *Plasmodium hermani* (solid diamonds), or both poxvirus and *P. hermani* (open diamonds) compared with uninfected controls (solid squares) over a 7-wk period. a. Mean weight gains of poults that were 1 wk old when infected (Experiment I). b. Mean weight gains of poults that were 10 wk old when infected (Experiment II). c. Mean packed cell volumes of poults that were 1 wk old when infected (Experiment I). d. Mean packed cell volumes of poults that were 10 wk old when infected (Experiment II).

interactions were significant only on wk 2 of the study. On wk 1 PI there were no significant effects detected among the 1-wk-old poults (Fig. 1c). On wk 2 through 6 the control and turkeypox-only groups had significantly higher values than both the malaria only and turkeypox/malaria groups. On wk 7 PI the control poults again had significantly higher packed cell volumes than the malaria-only and turkeypox/malaria group. The packed cell volumes of the pox-only poults were higher than the turkeypox/malaria birds, but were not significantly above that of the malaria-only poults. For the 10-wk poults, controls and turkeypox-only groups had significantly higher values than the malaria-only and turkeypox/malaria birds (Fig. 1d). On wk 6 and 7 the only significant difference detected was that the control poults had higher packed cell volumes than the malaria-only group.

Ratios of immature to mature red blood cells

There were significant treatment and age effects on the ratio of immature to mature red blood cells from wk 3 to the end of the experiment in the 1-wk-old poults. Significant treatment×sex interactions were detected on wk 2 and 6. Three wk PI the pox/malaria and malaria-only groups had more immature red blood cells than both the turkeypox-only and controls (Fig. 2a). On wk 4 the controls had significantly lower ratios than did the three treatment groups. By the 5th wk PI, the turkeypox/malaria group and the malaria-only group had significantly higher values than the controls and the turkeypox-only group. A similar pattern was observed throughout the study in the 10-wk-old poults (Fig. 2b). From wk 2 on the turkeypox/malaria and malaria-only groups had significantly higher ratios than did the controls and turkeypox-only group.

Parasitemia

The average day PI on which initial parasitemias were detected was 10.8, 8.7, 12.6, and 13.5 in the 1-wk-old turkeypox/malaria group, young malaria-only group, 10-wk-old turkeypox/malaria group, and 10-wk-old malaria-only groups, respectively (Figs. 2c and 2d). There were significant age effects from the fourth day after the initial parasitemia to the end of the study. Significant treatment effects were noted on days 23, 28, 30, and 44. Treatment× age interactions were identified on days 23, 25, 30, and 44. On days 23, 25, and 30 PI, the turkeypox/malaria and malaria only groups differed significantly from each other in the 1-wk-old but not in the 10-wk-old groups.

Turkeypox lesions

Turkeypox lesions were apparent 7 days PI and gradually began to slough off from 21 days PI to the end of the study in both 1-wk-old and 10-wk-old poults. No significant differences were found in the number of turkeypox lesions per poult. The mean numbers of pox lesions per poult (3.1 to 4.6) was very similar within three of the four groups that were exposed to poxvirus. The exception was the 10-wk-old pox only group which had a mean of 7.6 lesions per poult. This is misleading, however, as this higher value was largely attributable to one poult that had 51 lesions. These lesions were small and none was located on vital areas. If this one bird were to be excluded the mean number of lesions for this group would have been 2.1. All of the skin lesion samples from poults exposed to turkeypox virus in the course of the study had changes compatible with avian poxvirus infection, including cytoplasmic inclusion bodies in numbers that correlated with the degree of epidermal hyperplasia. All lesions had superficial bacterial colonies intermixed with the surface exudate.

DISCUSSION

Both singular and concomitant infections of turkeypox virus and *P. hermani* were more detrimental when poults were infected at 1 wk of age than when the infections occurred at 10 wk of age. Weight

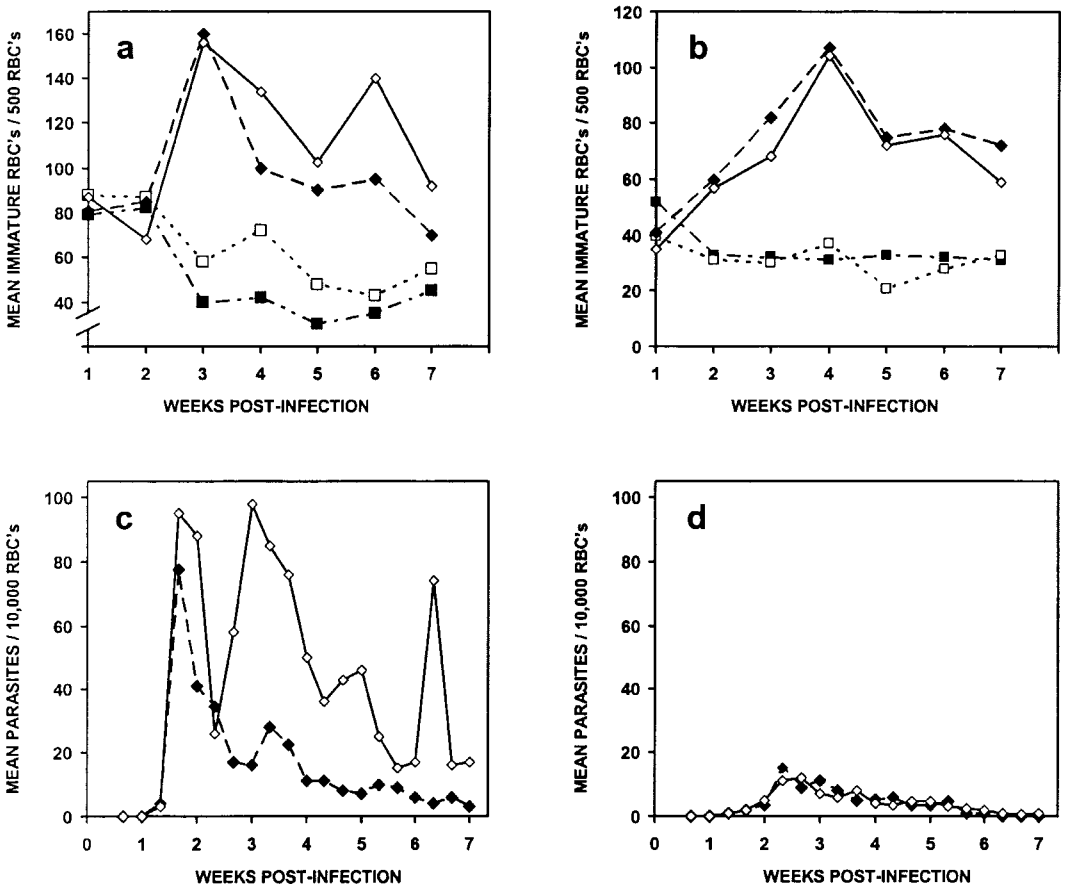

FIGURE 2. Mean numbers of immature red cells and parasitemias of *Plasmodium hermani* in domestic turkey poults infected with turkeypox virus (open squares), *P. hermani* (solid diamonds), or both poxvirus and *P. hermani* (open diamonds) compared with uninfected controls (solid squares) over a 7-wk period. a. Mean numbers of immature red blood cells in poults that were 1 wk old when infected (Experiment I). b. Mean numbers of immature red blood cells in poults that were 10 wk old when infected (Experiment II). c. Mean numbers of parasites (*P. hermani*) per 10,000 red blood cells in poults that were 1 wk old when infected (Experiment I). d. Mean numbers of parasites (*P. hermani*) per 10,000 red blood cells in poults that were 10 wk old when infected (Experiment II).

gains were not depressed in diseased 10-wk-old poults as they were in 1-wk-old poults and no mortalities occurred in any of the 10-wk-old birds, whereas five of the 1-wk-old poults died. Parasitemias of *P. hermani* were substantially higher in the 1-wk-old than in 10-wk-old poults. Also, there was a lower number of immature cells present in poults infected with *P. hermani* at 10-wk of age than 1-wk-old poults and packed cell volumes of the 10-wk-old poults were not as severely depressed and returned to nearly normal level by the end of the study than was the case with the 1-wk-old group. We conclude that when young turkey poults about 1 wk of age are infected with either *P. hermani* or with turkeypox virus and *P. hermani,* they are more prone to severe infection and mortality than older birds that are between 8 and 10 wk old.

Akey (1981) conducted a study similar to ours. He used domestic BBW turkey poults obtained from the same turkey farm as our birds and infected them in the same manner with the same strains of *P. hermani* and turkeypox virus at 5 days of age. The results were different from our find-

ings, especially in higher mortality rates. He reported no deaths in a group of 20 control poults, one death (5%) in another group infected only with malaria, four deaths (21%) in a group infected only with turkeypox virus, and 14 deaths (78%) in poults infected with both turkeypox virus and *P. hermani.* These higher mortality rates might have been caused either by using poults that were younger than 1-wk, or that had a nutritional deficiency or infection with extraneous disease agents. Many of Akey's birds had chondrodystrophy, a trait believed to be caused by deficiencies in manganese or certain vitamins (Austic and Scott, 1997). In addition there were histopathologic indications that many of his birds had some type of bacterial infection during the course of his experiments.

Our findings might be significant in understanding patterns of mortality of wild turkey poults in southern Florida. In this area hens begin laying eggs in March and hatching occurs primarily from mid-April to the first week in July with the peak in May (Williams and Austin, 1988). Normally this is the dry time of year and the rainy season doesn't occur until midsummer and early fall at which time there is a significant peak in mosquito breeding (Forrester, 1991). This pulse of mosquito vectors leads to increased transmission of pox and malaria (Forrester and Spalding, 2003), but would occur when most poults are several months of age and could handle infections without experiencing high mortality. If the rainy season (and hence the mosquito season) comes earlier in the spring when the majority of poults are less than 1 mo of age, the resulting pox and malaria infections could cause abnormally high amounts of mortality, because younger poults are susceptible to the harmful effects of these diseases. Forrester (1991) and Forrester and Spalding (2003) have reported that there was a correlation between above normal amounts of rainfall during 1964 and 1966 and a decline in the numbers of wild turkeys harvested by hunters during those and subsequent years. This decline might have been caused by the unusual early occurrence of the rainy season coupled with an early increase in breeding mosquitoes and subsequent transmission of pox and malaria in the young poult population, which would be at risk at that time of year. The result would be the depression in recruitment in the wild turkey population.

Additional research needs to be conducted to fully understand the impact of disease on wild turkey populations in Florida. This should include examination of the interactive effects of other infectious and parasitic agents on poults of various ages and field studies aimed at determining the impact of disease on reproductive efficiency and susceptibility to predation and hunting.

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