30 The Impact of Domestication on Red Deer Immunity and Disease Resistance

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Abstract

Domestication of red deer (*Cervus elaphus*) in New Zealand has caused changes in patterns of infectious diseases such as tuberculosis (*M. bovis*), malignant catarrhal fever (MCF), and yersiniosis (*Y. pseudotuberculosis*). Among the factors known to influence disease susceptibility and immunity are: capture from the wild and adaptation to farm conditions, transport, handling, and weaning. A series of experiments has examined the influence of some of the above stressors on immune function in farmed deer. Hematologic parameters, plasma proteins, nonspecific lymphocyte mitogenesis (using concanavalin A), and specific humoral (using ELISA) and cellular immune lymphocyte transformation have been measured in stressed immunized deer. Transport, handling, and nutritional deprivation have an acute but transient impact on immune function, which may be expressed as enhancement or suppression of immunity. Transport and fasting increase the susceptibility of deer to experimental infection with *Y. pseudotuberculosis*. Behavioral responses may not give an indication of the negative impact of management stress on immunocompetence. The present data show that aggressive animals, which appear to be adversely affected by restraint, have normal or enhanced levels of immune reactivity.

Key words: Domestication, disease resistance, farmed deer, immunity, red deer

Introduction

For centuries it has been recognized that nature resists change. Claude Bernard (1878) identified the physiologic changes that occur in animals exposed to a changing environment. He identified a steady state within the animals' interior (milieu interieur), characterized by its constancy, and the external environment typified by its variability. Cannon (1929) described the "flight-fight" reflex of animals exposed to extreme changes in the environment (stress) and identified pathology associated with extreme levels of stress. Selye (1946) described the "general adaptation syndrome" (GAS) as the response of animals exposed to stress. These early workers considered the central physiological response to environmental change was directed through adrenal function with increased levels of corticosteroids causing altered physiologic responses within the host. More recent studies (Mason 1968; Dantzer and Mormede 1985) have demonstrated that more complex endocrine responses are produced following exposure to environmental stress, which may vary depending on the type of stress or species of host affected. Selye (1973) recognized that all forms of stress need not produce pathology within the host. Stress which had an adverse affect was defined as distress while it caused eustress when the animal was no adversely affected.

Domestication of wildlife represents a uniqu and extreme example of environmental stre During domestication, animals are exposed to a

idly changing environment which imposes ll-recognized stressors on the animal. Physical ssors include capture, restraint, transport, osure to novel sounds, odors, and climatic tremes. Psychological stressors may involve erarchical dislocation, overcrowding or isotion, and exposure to unfamiliar surroundings wbank 1985; Griffin 1989).

Materials and Methods

the present study, two stress paradigms have been used to evaluate the impact of management stress on immunologic and inflammatory paramters and disease susceptibility patterns in deer posed to typical stressors found in intensified management of farmed deer. In the first study, young deer (3 to 5 months) were fasted for 24 h, ansported over 30 km, and relocated in a new arm setting. Animals were then challenged orally ith 3×10^{10} live *Yersinia pseudotuberculosis* to valuate resistance to experimental infection. nimals were maintained on a limited nutrional plane for 24h following experimental challenge. All animals were monitored closely for inical evidence of yersiniosis as seen by scourng or changed behavior. When evidence of nical disease was present, individual animals ere treated with antibiotics and electrolytes. mmunologic function in this group of animals was evaluated by immunizing the animals with a novel antigen, keyhole limpet hemocyanin KLH), either 28 days prior to the imposition of ress or 1 day following completion of the stress gime. Blood samples were taken from the imals at weekly intervals from the outset. ecific antibody (Ab) titers were measured using n ELISA assay (Hibma and Griffin 1988) and llular immune function was measured using nspecific mitogens (concanavalin (Con) A) by vitro culture. In all, 30 animals were stressed, d 30 nonstressed control animals were chal- ed experimentally with infection. Specific im- ne function and Con A reactivity was mea- d using two groups of ten animals either nunized 28 days prior to stress or challenged 1 following stress.

n a second study, groups of four animals were lied to monitor the effect of restraint on ..mmatory cells and imunologic function. The animals were restrained in a standard crush, or exposed to differing doses of ACTH, to evoke levels of cortisol as a probe for steroid-related changes in hematological and immunologic function. Two control groups were used; one of which were well-adapted "placid" animals, the other of which were "aggressive" (needle shy) animals which had developed a fractious response to earlier handling. Circulating neutrophil levels in peripheral blood were assessed at 30 min, 1, 4 and 24 h following restraint or injection of ACTH. These were measured using automated (HC6000-Technicon) counting of leukocytes using techniques adapted for studies in deer (Cross et al. 1988). In addition, immune function, as measured by lymphocyte transformation using specific antigen (KLH), was evaluated over 6 weeks from the time of initial restraint or injection with ACTH. All animals were immunized with 500 μg of KLH in Freund's complete adjuvant (FCA) by subcutaneous injection.

Results

Deer showed increased susceptibility to experimental infection following exposure to transport and nutritional stress (Table 30.1). Control animals showed a 30% incidence of clinical disease and no fatalaties following challenge with these high doses of live *Y. pseudotuberculosis*. By contrast, 60% of stressed animals developed clinical disease, and 13% died.

Animals immunized 4 weeks prior to stress showed a classic antibody response, with significant and optimal antibody (Ab) titers developing 14 and 21 days following immunization (Fig. 30.1). These titers had waned by 35 days following

TABLE 30.1. Increased susceptibility to experimental infection[a] in deer exposed to transport and nutritional stress

Animal	Status Clinical disease	Death
Stressed	18/30 (60%)	4/30 (13%)
Normal control	9/30 (30%)	0/30 (0%)

[a]Animals were challenged orally with 3×10^{10} live Y. pseudotuberculosis organisms following transport, nutritional deprivation, and relocation.

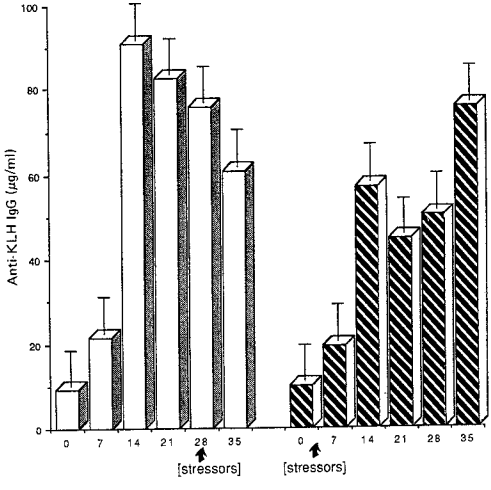

FIGURE 30.1. Levels of Immunoglobulin G specific for KLH in groups of red deer exposed to transport, nutritional, and climatic stress, immunized 28 days prior to or 1 day following exposure to stress. Results are expressed as mean + SE.

immunization. By contrast animals immunized 1 day after the imposition of transport and nutritional stress showed marked alteration in immunologic activity. Responses at 14 to 21 days following immunization were significantly lower than in the control group, and a bimodal response was seen with reduced antibody peaks evident at day 14 and day 35 following immunization.

Nonspecific cellular immunity in animals sampled at 7 days and 1 day prior to stress and at 1, 7, and 14 days following stress was evaluated. The results show a significant fall in lymphocyte reactivity 1 day following stress which decreased even further in the week following stress; there was only 30% of prestress reactivity at 7 days following stress, with recovery in the ensuing week (day 14; Fig. 30.2).

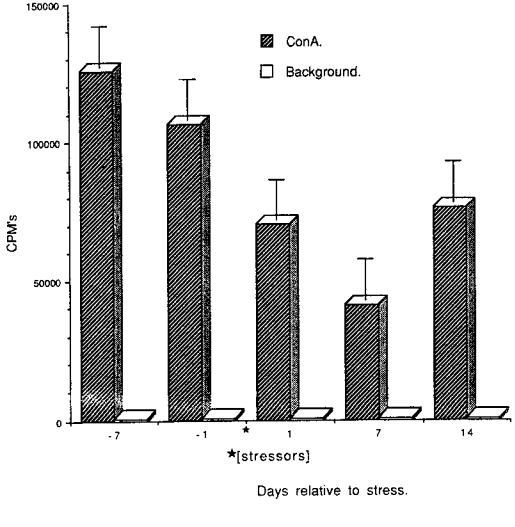

FIGURE 30.2. Concanavalin- (Con-) A induced lymphocyte transformation in a group of 50 red deer sampled prior to and following exposure to transport, nutritional, and climatic stress. Unstimulated background control values are also given. Results are expressed as mean + SE.

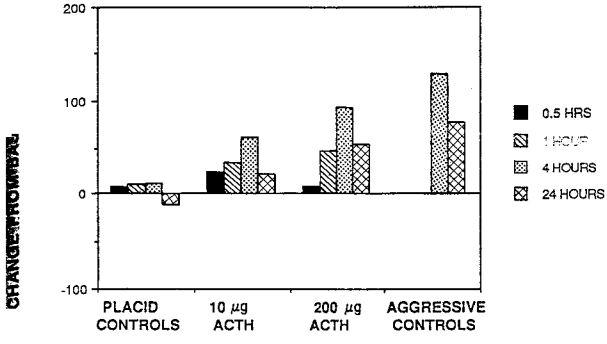

FIGURE 30.3. Neutrophil levels measured at 0.5 h, 1 h, 4 h, and 24 h after exposure to ACTH injection.

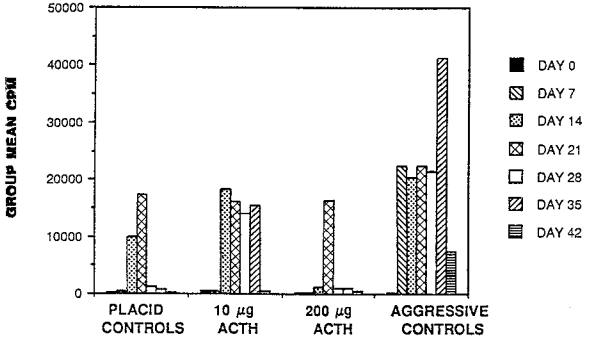

FIGURE 30.4. Lymphocyte transformation to KLH in animals exposed to ACTH simultaneously to immunization with KLH. Nonimmunized controls were segregated into "placid" or "aggressive" groups depending on their response to handling and restraint.

The results in Fig. 3 show that ACTH, used to induce steroid levels similar to those found in animals exposed to acute stress, had a significant impact on neutrophil levels compared with control animals subjected to physical restraint. "Placid" control animals had no change in neutrophil levels over a 24-h period following restraint, while "aggressive" animals showed a significant increase in neutrophil levels at 4 and 24 h following restraint. Animals treated with a low dose (10 μg) or a high dose (200 μg) of ACTH showed increasing levels of neutrophil leukocytosis with increasing dosage of ACTH. Animals treated with 200 μg of ACTH had steroid levels (80 to 120 ng/ml) similar to those found in the "aggressive" controls. Animals treated with 10 μg ACTH had steroid levels ranging from 20 to 60 ng/ml.

Antigen-specific lymphocyte transformation to KLH in the different groups shows that animals treated with 200 μg of ACTH had the lowest response to KLH, with moderate levels of cellular reactivity being evident only at 14 days following immunization. The "placid" control group showed moderate levels of activity at day 7 and day 14 following immunization. Animals treated with 10 μg ACTH had moderate levels of activity from 14 to 35 days following immunization. The response in the "aggressive" control group contrasted markedly with other groups studied. These animals showed significant specific cell reactions within 7 days of immunization, which remained unchanged for 21 days and peaked at 33 days, at very high levels.

Conclusions

The data presented here showed that management stress involving transport, fasting, or physical restraint can each impact on immunologic

function or inflammatory activity in farmed deer. Transport and nutritional deprivation caused a significant increase in susceptibility to clinical disease and death following experimental challenge with *Y. pseudotuberculosis*. Specific antibody production was also influenced by transport and nutrition where animals exposed to stress immediately prior to immunization showed reduced patterns of antibody production, by comparison with animals immunized 28 days prior to stress. The overall recovery of antibody production at 35 days following immunization would suggest that these stressors do not produce blanket impairment of antibody production but rather altered patterns of reactivity. Nonspecific mitogenic activity with Con A was also significantly influenced by transport and nutritional stress with a significant reduction in reactivity evident in the week following stress.

The response in the restraint experiment was interesting in that it demonstrated a reduction in immunologic activity in animals exposed to high doses of ACTH, sufficient to induce high levels of plasma steroids. The pattern of reactivity in these animals was marginally lower than that found in normal "placid" controls. By contrast low dose ACTH, which induced intermediate levels of plasma steroids, appeared to potentiate the immune response with significant reactivity being evident from 7 to 35 days following immunization and injection with low dose (10 μg) ACTH. These data would infer that moderate levels of steroids do not inhibit immunologic reactivity and may cause some level of enhancement. The striking observation from this experiment was the extremely high levels of immunologic reactivity in "aggressive" animals subjected to restraint prior to immunization. Such animals showed significantly elevated immune cell reactivity from 7 to 42 days following immunization. The time required to develop immune reactivity was shorter and the overall levels of immune reactivity were significantly higher in this group than in "placid" controls. These results suggest that the physiologic response in "aggressive" animals under restraint, though producing high levels of steroids and neutrophil leukocytosis, had no inhibitory effect on immunologic function, and may in fact have caused enhancement. This argues against steroids per se being the key factor in immunosuppression when animals are subjected to acute stress.

The results infer that acute stress may have little impact on immunologic function and even cause enhancement of function in animals adapted to cope with the acute stress. By contrast, transport and nutritional stress applied over a 48-h period cause a significant reduction in immunocompetence. This form of severe acute stress also increased susceptibility of animals to infection following oral challenge with live *Y. pseudotuberculosis*.

These findings suggest that care must be taken in the design of experiments to elucidate the relationship between stress and behavior with immunocompetence or disease susceptibility. One could argue that in fact the only normal response being produced was the response of the "aggressive" animal exposed to restraint, while 'placid" animals, though appearing behaviorly normal, may, in fact, have produced a response to restraint which impaired their immunocompetence. It further highlights the need for design or sampling and experimental protocols to ensure that true control values can be obtained in studies involving deer. Data from our laborator (Cross et al. 1988) demonstrate that animal handling can cause acute and precipitous change in the inflammatory profile and produce inaccurate values in studies designed to establish reference data for wild animals or normal farme animals. Splenic contraction and production o steroids which affect margination and redistr bution of circulating leukocytes dramaticall alter the hematologic profile of animals expos to adversive stimuli such as transport o restraint.

Independent studies have evaluated th impact of capture, weaning and nutrition deprivation on hematological values and im munity in red deer hinds and weaners (Grif et al. 1990). Capture causes a significant c pression in Con A induced lymphocyte rea vity, which is greatest at 7 days postcapture a which recovers gradually to normal levels o a 6-week period. Simultaneously, there is significant neutrophil leukocytosis, which is solved by 1 month postcapture.

Weaning appears to have little direct imp on hematological or immunological reacti

owever, a gradual decrease in Con A mito-
nesis and lymphocyte transformation with
tigen (tuberculin) was seen in animals sub-
ted to nutritional deprivation in the month
llowing weaning. This was paralleled by a
kopenia and anemia in nutritionally deprived
aned animals (Griffin et al. 1990).

From these studies it is evident that transport,
straint, and nutritional stress all can impact
irectly on immunocompetence, while weaning
ppears to have little impact on immunity or
ematology.

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