264

INFLUENCE OF MANAGEMENT STRESS ON THE IMMUNE PROFILE AND DISEASE RESISTANCE OF FARMED DEER

J.F.T. GRIFFIN, Deer Research Laboratory, Department of Microbiology, University of Otago, P. O. Box 56, Dunedin, New Zealand
C.G. MACKINTOSH, Invermay Agricultural Research Centre, Mosgiel, New Zealand.
J.P. CROSS, Deer Research Laboratory, Department of Microbiology, University of Otago, P. O. Box 56, Dunedin, New Zealand
G.S. BUCHAN, Deer Research Laboratory, Department of Microbiology, University of Otago, P.O. Box 56, Dunedin, New Zealand

Abstract: Cellular immunity as measured by mitogen transformation (ConA) was significantly reduced ($P < 0.01$) immediately following capture of thirteen red deer (*Cervus elaphus*) hinds. Transformation responses recovered gradually over the first five weeks in captivity to normal levels by seven weeks post capture. In a second experiment, immune reactivity and hematological changes were monitored in twenty-eight red deer fawns prior to and following weaning. There was a drop in blood leukocyte numbers in the weeks after weaning which became significant ($P < 0.01$) at seven and eight weeks post weaning. Hemoglobin levels dropped consistently for five weeks following weaning when there was significant reduction ($P < 0.01$) below weaning values. Lymphocyte transformation with Con A was unaffected in the first two weeks after weaning but dropped steadily for a further five weeks, when it became significant ($P < 0.01$) at six weeks. Specific immune reactivity to mycobacterial antigens as measured by lymphocyte transformation, was consistently low in fawns vaccinated at weaning and later exposed to extreme climatic conditions following weaning. Weaning appears to affect both immunocompetence and hematological parameters in red deer. Environmental stress associated with adverse climate and changed nutrition may have a greater direct impact on immunocompetence than weaning. The changes in laboratory parameters of hematology and immunity following capture and weaning mirror phases when there is increased susceptibility to infectious disease. Increased susceptibility to experimental infection with *Yersinia pseudotuberculosis* was also found in deer exposed to fasting, transport, and relocation.

Key Words: disease susceptibility, immunity, stress

Résumé: L'immunité céllulaire mesurée par la transformation mitogène (ConA) fut réduite significativement ($P < 0.01$) tout de suite après la capture de treize biches rouges (*Cervus elaphus*). Les réactions de transformation remontèrent progressivement pendant les cinq premières semaines de captivité jusqu'aux niveaux normaux dans la septième semaine après capture. Dans une deuxième expérience, les réactions immunitaires et les changements hématologiques furent observés chez vingt-huit faons rouges avant et après le sevrage. Il y eut un abaissement des chiffres leucocytes pendant les semaines après le sevrage, ce qui devint significatif ($P < 0.01$) sept et huit semaines après le sevrage. Les niveaux hématologiques baissèrent systématiquement pendant cinq semaines, ce qui devint significatif ($P < 0.01$) sous les valeurs au moment du sevrage. La transformation lymphocyte avec ConA ne changea pas pendant deux semaines mais elle baissa progressivement pendant cinq semaines de plus, lorsque cet abaissement devint significatif ($P < 0.01$) pendant la sixième semaine. La réactivité immunologique spécifique aux antigènes mycobactériels mesurée par la transformation lymphocyte fut invariablement bas pour les faons vaccinés au sevrage et exposés par la suite aux conditions climatiques extrêmes. Le sevrage semble influencer la compétence immunologique et les paramètres hématologiques du cerf rouge. Plus que le sevrage, le stress environnemental associé au climat adverse et aux changements d'alimentation impacte directement la compétence immunologique. Les changements des paramètres expérimentaux de l'hématologie et de l'immunité reflètent les phases où il y a une plus grande susceptibilité aux maladies infectueuses. On trouva aussi une plus grande susceptibilité à l'infaction expérimentale par *Yersinia pseudotuberculosis* dans les cerfs exposés à la jeûne, au transport, et au déplacement.

Mots-Clés: immunité, stress, susceptibilité aux maladies

Introduction of stress associated with management of wildlife is considered to be one of the most important factors which influence the success of a postcapture management program. The degree and rate of change within the environment impacts significantly on the health and well-being of animals within a habitat. Unless the animal can adapt to the changing environment, undue stress will result in the detriment of the animals well-being. Ranching or domestication of wildlife for intensive farming presents a unique challenge to the adaptive response of the host. The striking absence of new species which have been domesticated throughout the past 6,000 years infers that the adaptive responses required for domestication have mitigated against its success for many species of wildlife. Whereas there is significant indirect evidence that environmental stress causes an impairment of immune function, the specific mechanisms involved in these responses are incompletely described. The only conclusion which can be stated is that the endocrine factors which are produced during exposure to stress can have multifaceted effects within the immune system. The current series of experiments evaluate the effects of capture, adverse climate, and weaning on disease resistance, the hematological, and immune parameters in deer.

Experiment 1: Influence of Capture on Lymphocyte Mitogenesis

Methods

Lymphocyte transformation was carried out on blood samples obtained from a series of 13 mixed-age adult red deer (*Cervus elaphus*) hinds captured from the wild on the west coast of New Zealand. The animals were captured using projectile nets and transported 100 km by road in a covered truck. The animals were housed in enclosed pens and fed lucerne (*Medicago sativa*), hay and pellets *ad libitum*. Fresh water was available to the animals in close proximity to the feed. All animals were captured from a similar habitat with a high plane of nutrition and all were in good physical condition at capture. Blood samples were obtained from the animals one day after they had been translocated and placed in the enclosed sheds, and at weekly intervals thereafter for six weeks. Peripheral blood mononuclear cells were obtained by separation of the cells on ficoll/conray buoyancy gradients (Specific gravity 1.082) by carefully layering the blood, diluted 1 in 2 in culture medium (RPMI 1640), prior to centrifugation at 800 g for 20 min. Mononuclear cells at the ficoll interface were removed, washed twice in culture medium, adjusted to 2 x 10^6/ml in RPMI containing 10% normal stag serum. Cell suspensions were dispensed in 100 µl amounts into microtitre wells and cultured in triplicate with 50 µl of Concanavalin A (50 µg/ml) or in culture medium alone. Cells were incubated for 5 days at 37°C in a humidified atmosphere containing 5% CO_2 and air. Fifty µl of tritium labelled thymidine (3H-Tdr, specific activity 6 Ci/mmol) containing 10 µCi/ml was added to each well. The cells were incubated for a further 18 hr and harvested onto glass fibre filter discs prior to counting in a beta scintillation counter (LKB). Mean counts/min were obtained from triplicate cultures in Con A and the negative control cultures. Mitogen transformation activity was estimated as mean counts/min with ConA minus counts/min in control cultures.

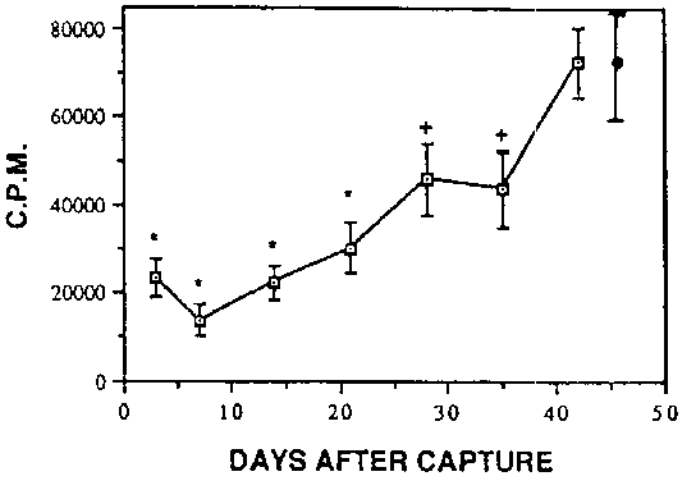

Fig. 1. ConA mitogen reactivity of cervine lymphocytes capture. An open box indicates mean CPM' s in captured hinds. ● is the reference range for normal adult hinds. * is significant reduction in lymphocyte reactivity ($P < 0.01$) at day 0-21 and ($P < 0.05$) at day 28-35 post-capture (+ represents the reduction).

Results

The results given in Fig. 1 show that there was a significant depression in lymphocyte reactivity in the animals in the first five weeks following capture. By camparison with aged matched normal farmed deer the lymphocyte function was dramatically depressed for the first three weeks following capture ($P < 0.01$), and was still significantly reduced ($P < 0.05$) at weeks four and five, but had recovered to normal values within six weeks following capture.

Experiment 2: Influence of Weaning on Hematology and Immunologic Reactivity in Red Deer Fawns

Methods

Blood samples were obtained from 28 female red deer at 14 weeks of age, prior to, and for eight weeks following weaning. The animals were segregated from their dams and placed on a separate pasture 2 km distance from the hinds. The animals were fed on conventional pasture, but severe change in the climate four weeks following weaning necessitated supplemen-

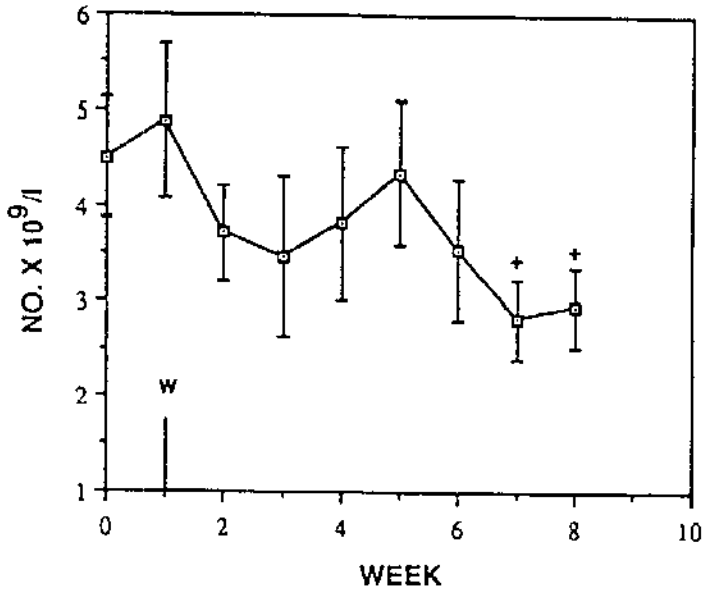

Fig. 2. Total white blood cell counts in deer following weaning. W = weaning. + = significant reduction ($P < 0.01$) by comparison with sample pre-weaning. Cell numbers were calculated using a Technicon H 6000/C analyser.

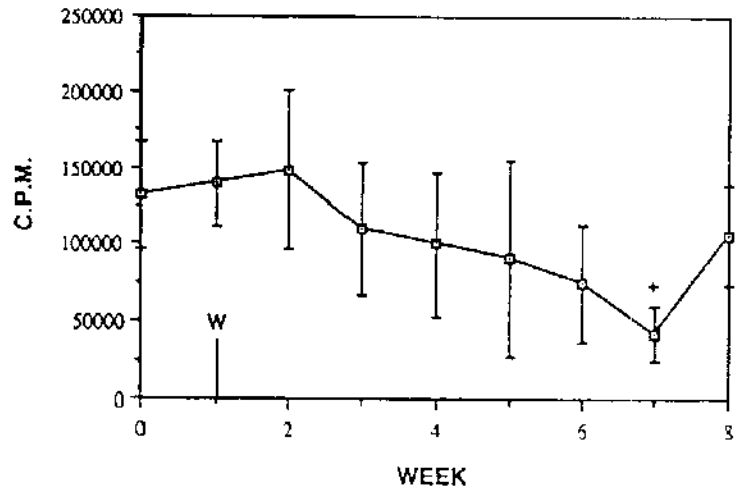

Fig. 4. ConA stimulated lymphocyte transformation in the weeks following weaning. Transformation of lymphocytes ($2x10^5$) measured after 6 days of culture with 2.5 µg Con A. Results are expressed as group mean cpms ± S.D. W = weaning. + = significant reduction ($P < 0.01$) by comparison with pre-weaning values.

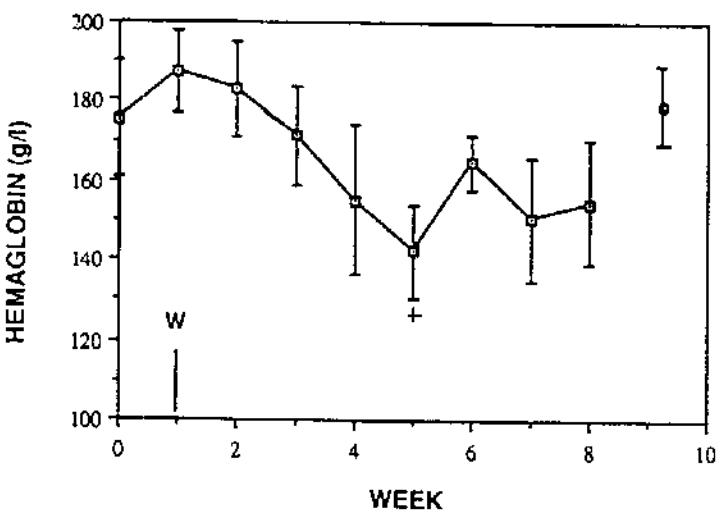

Fig. 3. Deer haemoglobin levels in the weeks following weaning. W = weaning. + = sample obtained four weeks post-weaning had a significant ($P < 0.05$) reduction in haemaglogin by comparison with pre-weaning values. Open circle is mean value for 230 normal 6 month old fawns.

tary hay be fed to the fawns. Hemoglobin levels, total white cell counts, and differential mononuclear and granulocyte counts were obtained using an automated cell counter (Technicon H6000C) calibrated for counting deer leukocytes (Cross et al., 1988). Lymphocyte transformations using Con A were carried out on peripheral blood mononuclear cells separated and cultured according to the method in Experiment 1.

Results

The data given in Fig. 2 show changes in total white blood cell counts in fawns in the week prior to and following weaning. There was a consistent though non-significant drop in total white cell numbers from one week following weaning onwards. Whereas there appeared to be a partial recovery in white cell counts at four weeks following weaning a significant ($P < 0.01$) reduction was found at week six and seven post weaning. Changes in total white cell counts were paralleled by changes in total lymphocyte numbers, while other cell populations appeared to be unaffected. The hemoglobin levels (Fig. 3) obtained from blood samples showed a consistent drop for five weeks post weaning. The only sample that showed a significant ($P < 0.05$) reduction in hemaglobin levels was the sample obtained at five weeks post weaning. Lymphocyte tranformation values with Con A are given in Fig. 4. These data show a gradual

decrease in lymphocyte reactivity from two to six weeks post weaning. A significant decrease was seen between the value obtained at weaning and the value obtained six weeks later ($P < 0.01$).

Experiment 3: Immunocompetence in Weaned Fawns

Methods

Specific cellular immunocompetence as measured by antigen specific lymphocyte tranformation was evaluated in a group of 21 fawns vaccinated with killed BCG vaccine (Connaught Laboratories). Animals were vaccinated with 0.3 mg heat killed BCG emulsified in 1 ml of Freunds Incomplete Adjuvant. Blood samples were obtained prior to vaccination and for the succeeding eight weeks. As a control, a group of eight six month old fawns were vaccinated two months after weaning and similar assays of immunocompetence were carried out for ten weeks post vaccination. Mononuclear cell reactivity was measured using cells separated and cultured according to the protocol defined in Experiment 1. Antigen specific reactivity was obtained by co-culturing cells with 2.5 µg bovine tuberculin (PPD-B, CSL Laboratories) for six days.

Results

The results given in Fig. 5 show that the weaned fawns had remarkably low levels of cellular reactivity specific for PPD following vaccination. A minimal response was seen only in the single sample obtained four weeks post vaccination. By contrast significant reactivity was evident in the older fawns. Significantly increased levels of immunocompetence to vaccine ($P < 0.05$) was seen at four weeks post vaccination in the older animals. The difference between the two groups became further amplified ($P < 0.01$) after four weeks. Interpretation of these results is complicated somewhat by a severe change in climatic conditions for the weaned animals from one week post weaning onwards.

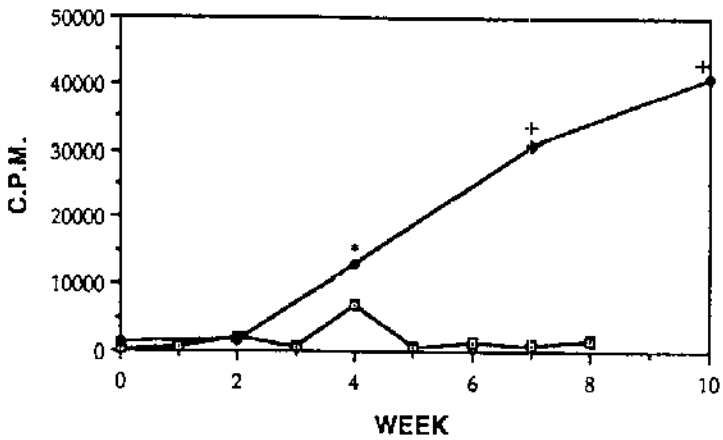

Fig. 5. Lymphocyte transformation responses to mycobacterial Antigens in deer vaccinated with *M.bovis* antigens. All animals vaccinated with 0.3 mg heat killed *M. bovis* (BCG) in Freunds Incomplete Adjuvant. Mononuclear cells (2 x 105) were stimulated with 2.5 µg *M. bovis* PPD in six day cultures. Activity was measured by uptake of ^{3}H TdR and results expressed as group mean cpms. Open box = fourteen week old fawns vaccinated at weaning. Closed circle = control six month old fawns vaccinated at day 0. * = $P < 0.05$;+ = $P < 0.01$.

Experiment 4: Management Stress and Susceptibility to Infection

Methods

In the course of a study designed to test the efficacy of vaccines for yersiniosis, a stress model was established to produce experimental infection which could evaluate protective efficacy of vaccines. Previous attempts to produce clinical disease in normal animals following oral challenge with different strains of *Yersinia pseudotuberculosis* had not been successful. In this experiment, 140 five-month-old red deer fawns of mixed sex were fasted for 24 hr, transported 5 km to a new location, and challenged with a virulent strain of *Y. pseudotuberculosis*. Animals were inoculated orally with 3 x 10^{10} virulent live organisms following relocation. After release into open paddocks, there was a snow storm which further compounded the stress to which these animals were exposed. For comparison, a non- stressed control group of 30 animals of similar age and mixed sex were left at the original site and were not exposed to fasting or transport. Both groups of animals were exposed to a similar oral challenge of *Y. pseudotuberculosis*.

Table 1. Susceptibility to experimental infection with *Y. pseudotuberculosis* in deer exposed to fasting, transport, and adverse climate.

Deer status	Clinical disease (%)	Fatality (%)
Stressed	60/140 (43)	21/140 (15)
Control	9/30 (30)	0/30 (0)

Results

Clinical disease was monitored in all animals in the days following challenge with live organisms. Any evidence of scour or fecal softening, inappetance, or lassitude were noted. Animals showing any clinical evidence of yersiniosis were treated with antibiotics and fluid therapy. Clinical disease was found in affected animals from one to ten days post challenge.

The results given in Table 1 show unexpectedly high levels of clinical disease and mortality in animals exposed to stress. By contrast, lower levels of clinical disease and no mortality were found in the non stressed control group.

following challenge with live organisms. Any evidence of scour or fecal softening, inappetance, or lassitude were noted. Animals showing any clinical evidence of yersiniosis were treated with antibiotics and fluid therapy. Clinical disease was found in affected animals from one to ten days post challenge.

The results given in Table 1 show unexpectedly high levels of clinical disease and mortality in animals exposed to stress. By contrast, lower levels of clinical disease and no mortality were found in the non stressed control group.

Discussion

Capture of animals from the wild represents an extreme form of physical and psychological stress. Experiments in deer capture have shown gross changes in physiology which produce post capture myopathy and fatalaties in red deer (McAllum, 1985). Associated changes in inflammatory cell activity (neutrophils) and protein (haptoglobin) are also found following capture. Mortality as high as 25% may be found in the weeks following capture. A significant increase in susceptibility to infectious disease such as foot abscess is also found in the period immediately following capture (van Reenen and Innes, 1985). The data presented in Fig. 1 shows marked impairment of cellular immune function in the period following capture which appears to coincide with the period of altered physiology and increased susceptibility to disease. Yersiniosis caused by *Y. pseudotuberculosis* is found most commonly in fawns following weaning (Mackintosh and Henderson, 1984). The disease occurs sporadically and is usually associated with adverse change in weather conditions or nutritional stress in weaned animals. The results given in Fig. 2 and 3 show that whereas weaning alone does not appear to assert a direct affect on leukocyte numbers or haemoglobin levels, it may produce a window of susceptibility which may be opened by post weaning stressors. Outbreaks of yersiniosis are classically found in animals purchased after weaning, which are subsequently transported and exposed to nutritional or climatic stress. The absence of significant change in haemotologic or immunologic parameters in the weaned fawns for the first month following weaning would infer that there is no direct link between weaning and altered immunocompetence or disease resistance. In the present study, unforseen adverse changes in weather conditions in the weeks following weaning could more readily be the cause of changes in immune physiology rather than weaning itself. The combination of weaning and adverse weather conditions is further highlighted in the results given in Fig. 5 where animals were vaccinated with bacterial antigens at weaning. Again, adverse weather conditions occurred in the weeks following weaning to compound the influence of weaning on host immunocompetence. The contrasting immunocompetence of slightly older deer not exposed to adverse weather conditions following vaccination points to the likely impact of adverse climatic conditions on immunocompetence. While it is recognised that six month old fawns do not represent an ideal control for 14 wk old fawns at weaning, other studies in our laboratory have shown (M. Hibma and F. Griffin, pers. commun.) that animals immunized with protein antigen (Keyhole limpet hemocyanin - KLH) appear to have similar levels of cellular reactivity and antibody to that found in unweaned fawns or older animals at six to eight months of age. While not conclusive, these preliminary results infer that altered nutrition and climatic conditions may assert a much greater affect on disease resistance and immunocompetence than weaning alone. Our experience suggests that well managed fawns show no significant change in behaviour, weight gain or well-being following weaning, provided that adequate levels of nutrition are maintained and the animals are not exposed to adverse climate. Prevailing autumn weather conditions in the South Island of New Zealand means that animals can often be exposed to adverse climatic conditions following weaning.

There has been a direct link established between stress, immunocompetence and increased susceptibility of animals to infectious disease (Kelley, 1980; Riley, 1981). Curtis (1974) has shown that perinatal stress causes an increased incidence of gastroenteritis in piglets. Shimizu et al. (1978) have established a direct link between the temperature at which pigs are held and their susceptibility to viral gastroenteritis. Their studies have also demonstrated that continuing exposure to a given temperature can result in adaptation and normal levels of disease resistance. Kelley et al. (1982) have demonstrated altered levels of immunocompetence in calves exposed to elevated or reduced temperatures.

Table 1. Results of postmortem examinations of deer at the Rural Veterinary Center, Camden (1980-1990).

Diagnosis	Fallow deer (%) n = 262	Chital deer (%) n = 113	Rusa deer (%) n = 30	Red deer (%) n = 18
Perinatal mortality	59.9	40.7	23.3	33.4
Chemical immobilization	1.1	8.0	6.7	-
Post-capture myopathy	2.3	16.8	3.3	5.5
Malnutrition/ starvation	2.3	0.9	-	-
Misadventure/ trauma	4.2	8.8	13.3	5.5
Infection	9.5	2.7	6.7	11.2
Toxicity	5.0	-	20.0	5.5
Parasitism	7.7	-	-	-
Malignant catarrahl fever	-	-	10.0	5.5
Neopasia	2.3	-	6.7	-
Exposure/ hypothermia	0.4	20.3	-	-
Dystocia	0.8	-	6.7	27.9
Lactic acidosis	2.3	-	-	-
Miscellaneous	1.1	0.9	3.3	-
Undiagnosed	1.1	0.9	-	5.5

"Shipping Fever" (Hoerlein, 1980) is a classic disease produced in cattle following transportation. Increased incidence of fatal infectious disease is found following transport and is subject to variables such as quality of transport, ambient temperature, stock density, novelty, and time involved in transportation. Experiences with farmed deer in New Zealand have shown that a number of infectious diseases may be precipitated or exacerbated by climate and management stress (Griffin, 1989). Such diseases range in severity from bacterial diseases causing foot abscesses, found in animals following capture (van Reenen and Innes, 1985; Griffin, 1987), to acutely lethal virus infections such as malignant catarrhal fever (MCF), in animals following transport or exposure to extreme climatic changes (McAllum, 1980). An increased incidence of severity of tuberculosis has also been found following exposure to adverse climate or during breeding (Griffin, 1988).

The stress paradigm used in this report involved fasting animals for 24 hr prior to transport and relocation in a new farm setting. By coincidence, an extreme change in climatic conditions occurred immediately following relocation of the animals and their challenge with experimental infection. While the combination of fasting, transport and adverse weather may appear unduly complex it is nonetheless typical of what can happen in animals following weaning and relocation onto a new farm. The data given in Table 1 shows high levels of clinical disease and some fatalaties following experimental challenge with *Yersinia pseudotuberculosis*. A lower incidence of clinical disease without any fatality was seen in a control group of animals not exposed to fasting or transport stress prior to challenge with virulent organisms. Clinical presentation of disease and the levels of mortality found in the stressed, experimentally infected animals were similar to the patterns found in normal farmed fawns undergoing sporadic outbreaks of yersiniosis (Mackintosh and Henderson, 1984).

The data presented here provide further evidence for stress associated changes in physiology and disease susceptibility in deer exposed to adverse conditions. Animals have significant alterations in immunocompetence in the weeks following capture, the period during which there appears to be increased susceptibility to infectious disease. Experiences gained in the capture of wild deer in New Zealand have defined that animals should be given prophylactic penicillen and selenium immediately following capture to prevent acutely fatal infection or myopathy. Animals appear to eat remarkably well following capture and it has been identified that extreme thirst with restricted access to water, rather than feeding, may be the most limiting factor in ensuring successful adaptation of deer to post capture farming conditions.

The combination of weaning, adverse weather and altered nutrition produces increased susceptibility to infectious disease, especially yersiniosis in young fawns. While it is not possible to control weather conditions, great care should be taken to ensure that adequate nutritional planes are maintained in animals in the weeks following weaning. Should it be necessary to feed supplements it is important that animals be allowed time to adapt to supplementary feed. When there is evidence of a yersinia outbreak all animals directly affected should be given fluid replacement therapy and antibiotics according to the regime as defined by Mackintosh and Henderson (1984). Prophylactic antibiotics may be administered to "in-contact" stock considered to be at-risk from disease.

Literature Cited

CROSS, J.P., C.G. MACKINTOSH, and J.F.T. GRIFFIN. 1988. The effect of physical restraint and xylazine sedation on haematological values in red deer (*Cervus elaphus*). Res. Vet. Sci. 45: 281-286.

CURTIS, S.E. 1974. Response of piglets to perinatal stressors. J. Anim. Sci. 38: 1031-1037.

GRIFFIN, J.F.T. 1987. Stress and disease in farmed deer. Pub. Vet. Deer Soc. 2: 3-9.

GRIFFIN, J.F.T. 1988. The aetiology and diagnosis of tuberculosis in farmed deer. Ir. Vet. J. 42: 23-26.

GRIFFIN J.F.T. 1989. Stress and Immunity: A Unifying Concept. Vet. Immunol. Immunopathol. 20: 263-312.

HOERLEIN, A.B. 1980. Shipping Fever. *In:* Bovine Medicine and Surgery. H.E. Amstutz, ed., Am. Vet. Publ., Santa Barbara p. 99 -106.

KELLEY, K.W. 1980. Stress and Immune Function: A bibliographic review. Ann. Rech. Vet. 11: 445-447.

KELLEY, K.W., E. RANDALL, B.S. GREENFIELD, J.F. EVERMANN, S.M. PARISH, and L.E. PERRYMAN.1982. Delayed type hypersensitivity, contact sensitivity, and phytohaemagglutinin skin-test responses of heat and cold stressed calves. Am. J. Vet. Res. 43: 775-779.

MCALLUM H J F. 1985. Stress and post capture myopathy in red deer. *In:* Biology of Deer Production. P.F. Fennessy and K.R. Drew, eds., Royal Soc. of New Zealand Bull. 22: 65-72.

MCALLUM, H.J.F. 1980. Common diseases in farmed red deer. Ag Link FPP 254. Min Agric and Fisheries, Wellington, New Zealand.

MACKINTOSH, C.G. and T.G. HENDERSON. 1984. The epidemiology of yersiniosis in deer. *In:* Proc. Deer Course for Veterinarians No. 1. P.R. Wilson, ed., Deer Branch NZVA p. 34-42.

RILEY, V., 1981. Psychoneuroendocrine influences on immunocompetence and neoplasia. Science. 212:1100-1109.

SHIMIZU M., Y. SHIMIZU, and Y. KODAMA . 1978. Effect of ambient temperatures on induction of transmissible gastroenteritis in feeder pigs. Infect Immun. 21: 747-752.

VAN REENEN, G.M. and J.I.S. INNES. 1985. Preventive veterinary medicine in a commercial red deer herd. *In:* Biology of Deer Production. P.F.Fennessey and K.R. Drew, eds., Royal Soc. of New Zealand Bull. 22: 87-92.

First printed December 1991

Manufactured in the United States of America

Library of Congress Card Number 91-77128

ISBN 0-87090-000-5

Cite Papers as:
Smith, K. and A. Peischel. 1991. Intensive Wapiti Production in the Tropics: Hawaii. In: *Wildlife Production: Conservation and Sustainable Development*. eds. L.A. Renecker and R.J. Hudson, p. 503-515. AFES misc. pub. 91-6. University of Alaska Fairbanks. Fairbanks, Alaska.

Cite Book as:
Renecker, L.A. and R.J. Hudson, eds. 1991. *Wildlife Production: Conservation and Sustainable Development*. AFES misc. pub. 91-6. University of Alaska Fairbanks. Fairbanks, Alaska 601 pp.

Proceedings from the Second International Wildlife Ranching Symposium. Edmonton, Alberta, Canada. June 1990

Copies of the proceedings may be obtained by writing to:
Dr. Lyle A. Renecker
309 O'Neill Bldg.
University of Alaska Fairbanks
Fairbanks, Alaska 99775
U.S.A.

Please make checks payable to:
UAF Reindeer Research Program Support Fund
U.S. $70.^00 **(includes postage and handling)**

Please allow two weeks for delivery in the U.S., and two or three months for delivery overseas.