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Serum Haptoglobin Concentrations in Ringed Seals (*Pusa hispida*) from Svalbard, Norway

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ABSTRACT: Haptoglobin (Hp) levels were measured in blood serum from 185 apparently healthy ringed seals (*Pusa hispida*) from Svalbard (age range, 1–30 yr) collected during the spring seasons of 2002 through 2004. The Hp concentration was 0.26±0.02 g/l (mean±SE; range, 0.0–1.5 g/l). Maturity status, body condition index (CI), interactions between CI and maturity status, and sex and maturity status all had significant influences on Hp levels. Mature males had significantly higher Hp concentrations compared with mature females (0.30±0.03 vs. 0.17±0.01 g/l, $F_{1,16}=14.9$, $P<0.01$). No differences were found between Hp levels in immature male and female seals (0.33±0.06 g/l). The Hp levels increased significantly in immature seals when the CI decreased. Despite the fact that mature males had a significantly lower CI than mature females, no direct correlation was found between Hp concentration and CI among adults. The elevated Hp values found in mature males might be related to injuries inflicted among males while fighting during the mating season or to high stress levels related to mating competition. The Hp concentrations of ringed seals measured in this study are useful baseline data for development of a diagnostic tool for future monitoring of the general health of this and other ringed seal populations.

Key words: Diseases, haptoglobin, health monitoring, *Pusa hispida*, ringed seal, stress.

Haptoglobin (Hp) belongs to a group of blood proteins called acute-phase proteins (APPs), which take part in the restoration of homeostasis when an animal is suffering from inflammation, toxicologic damage, exhaustion, starvation, or other stress or trauma (Eckersall, 2000). Haptoglobin is released from the liver, and its measurement in blood is used widely as a diagnostic tool to detect various diseases in humans and domestic production animals (Kent, 1992; Gruys et al., 1994; Delanghe and Langlois, 2001). A standardized method for measuring Hp has been developed, but because levels and the APP response profiles differ between species (Murata et al., 2004), it has been recommended that each species be examined separately (Eckersall et al., 1999; Skinner, 2001). The Hp levels in declining populations of Steller sea lions (*Eumetopias jubatus*) and harbor seals (*Phoca vitulina*) in Alaska (USA) differed significantly from those measured in animals from stable populations of the same species (Zenteno-Savin et al., 1997), suggesting that the declining populations might be experiencing an acute-phase stress response. These findings also suggest that Hp levels might be useful bio-indicators for monitoring the general health status of seal populations.

This study investigated how Hp levels in apparently healthy ringed seals (*Pusa hispida*) from Svalbard, Norway (~77–80°N, 10–20°E), vary among different age and sex-groups and animals in different condition states. This population is exposed to low levels of harvest (Lydersen, 1998) and low pollution levels (Wolkers et al., 1998). A total of 185 ringed seals (110 males and 75 females) were shot during the spring seasons (April and May) of 2002 through 2004 in fjords on the largest island in the Svalbard archipelago, Spitsbergen, Norway. Animal sampling included the measurement of body mass, standard length according to the method described by Scheffer (1967), and blubber thickness according to the method described by Ryg et al. (1988). Blood was sampled from the extradural intravertebral vein, and extracted blood was transferred into 10-ml vacutainers. Serum was separated by centrifugation within 4 hr at 3,000 rpm for 10 min and then kept frozen (~20 C) until analyses were performed. A lower canine tooth was taken for age determination, and reproductive organs were collected for evaluation of reproductive status (immature vs. mature based on the presence of mature follicles, corpora lutea, or corpora albicantia in the ovaries of females or the presence of spermatozoa in the epididymis or spermatogenesis in the seminiferous tubules in males, as described by Lydersen and Gjertz [1987]).

A multispecies Hp assay developed by Tridelta Development Limited (Maynooth, Kildare, Ireland) was selected for use in this study. Analyses were performed using an Advia® 1650 System (Bayer Corporation, Tarrytown, New York, USA). To compare the condition of animals of various body sizes, a body condition index (CI) was developed and calculated as follows: $CI=BlM/M^{0.75}$, where BlM is blubber mass and M is body mass. The BlM was calculated based on a formula from Ryg et al. (1990a): $B=4.44+5693(L/M)^{0.5}\times D$, where B is blubber content (%), M is body mass (kg), L is standard length (m), and D is dorsal blubber thickness (m). This formula estimates the blubber content of phocid seals with less than 3% error. Blubber content was multiplied by M/100 to get the animal's BlM. The term $M^{0.75}$ is the metabolic body mass according to Kleiber (1975). The statistical software SAS/STAT Version 6 (SAS Institute Inc., Minneapolis, Minnesota, USA) was used for the regression analyses. Normality was confirmed by Kolmogorov-Smirnov with the Lillefors option test, and differences between groups were tested using an analysis of variance (ANOVA; type III) with Statistica (Ver 6.1 for Windows®, StatSoft Inc., Tulsa, Oklahoma, USA). Values are presented as the mean±SE.

The overall mean serum Hp concentration for the ringed seals in this study was 0.26±0.02 g/l (range, 0–1.5 g/l; $n=185$) (Table 1). A regression model examining the effects on Hp levels of sex, age, and condition as well as their interactions was dominated by age, sex, and the interaction between age and sex; condition of the animals was not significant. When age was replaced in the model with maturity status, however, the maturity status ($F=7.50$, $P=0.007$), condition ($F=7.94$, $P=0.005$), and the interaction terms between condition and maturity status ($F=6.13$, $P=0.014$) and between sex and maturity status ($F=7.30$, $P=0.008$) were all significant. Mature males had significantly higher Hp levels compared with mature females (ANOVA: $F=17.00$, $P<0.001$) (Table 1) and significantly lower CIs (1.03±0.02) compared with mature females (1.23±0.03) (ANOVA: $F=35.56$, $P<0.001$), although CI and Hp level were not correlated among adults. Among immature seals, Hp levels in males and females did not differ (combined value, 0.33±0.06 g/l; ANOVA: $F=2.28$, $P=0.143$) (Table 1), and no interaction between CI and sex was found (ANOVA: $F=0.88$, $P=0.357$). However, Hp levels decreased with increasing CI, $F=5.24$, $P=0.03$).

The animals included in this study all appeared to be in generally good health. Twenty of the animals were surveyed for *Brucella* sp., with negative results (Tryland et al., 2005). Many animals in this study also underwent a general screening of their serum chemistry, but no abnormal values were found (Tryland et al., unpubl. data). Significant differences in Hp levels

TABLE 1. Serum haptoglobin (Hg) concentrations measured in different age and sex groups from 185 ringed seals collected during the spring seasons of 2002 through 2004 at Svalbard, Norway.

		Hp concentration (g/l)	
Age and sex	Sample size	Mean±SE	Range
Mature males	93	0.30±0.03	0.0–1.4
Immature males	17	0.26±0.05	0.1–0.7
Mature females	65	0.17±0.01	0.0–0.6
Immature females	10	0.46±0.15	0.1–1.5
Total	185	0.26±0.02	0.0–1.5

were found that were related to sex, maturity status, and condition. The seals in this study were collected late in the breeding season, which overlaps with commencement of the molting period. These annual events place energetic and physiologic demands on the seals (Ryg et al., 1990b; Lydersen, 1995). It was somewhat surprising that male condition was lower than female condition, because lactation is extremely energy demanding in phocid seals (Lydersen and Kovacs, 1999). However, ringed seal mothers do feed throughout lactation, and many females lose their pup to predation by polar bears (*Ursus maritimus*) and arctic foxes (*Alopex lagopus*) early in the breeding season and so do not complete lactation. The low Hp levels in the female ringed seals were similar to those in female cattle that had given birth to calves and were lactating (Saini et al., 1998; Chan, 2004). Adult male ringed seals must defend a territory that contains adult females if they are going to participate in mating. Virtually all males have bite wounds, particularly on the hind flippers, tail, and at the base of the foreflippers in May, as a result of male-male aggressive interactions (Smith and Hammill, 1981; present study). The area around these wounds is often swollen and occasionally infected. Stress associated with fighting, in addition to the wounds and their inflammation, might be responsible for the elevated Hp levels measured in adult males compared to those in adult females (Solter et al., 1991; Eckersall, 2000). The cold temperatures, clean pack-ice environment, and regular bathing of wounds in saltwater, however, likely reduces the risk of infection and the need for immune responses by ringed seals compared to those of animals in other circumstances. Eight males with particularly high Hp values did not have markedly worse wounds compared with those of other males in the study.

Immature seals do not participate in mating, so it was not surprising that the Hp levels among these younger animals were more uniform between the sexes. The CI was more tightly linked to Hp levels in the immature animals. Their small size might make them more vulnerable to the energy stress involved in molting if they go into this process in poor condition (with little blubber). Additionally, some young ringed seals bear heavy parasite infections, such as lungworms (Onderka, 1989), that might influence Hp levels. Such infections were not explored in our study. In addition, our sample sizes for immature animals were small, so firm conclusions regarding sex differences or Hp vs. CI should be made with reservation for this group.

Levels and response profiles of APPs like Hp have been shown to differ among species (Murata et al., 2004), but the mean Hp levels for ringed seals in this study fell within the ranges reported for Steller sea lions and harbor seals from stable populations in Alaska (Zenteno-Savin et al., 1997). Nursing harp seal pups (*Pagophilus groenlandicus*) from the White Sea have been reported to have a somewhat higher mean value (1.48 g/l; Erokhina, 2002); however, no confidence limits were presented around this estimate to enable more in-depth comparisons. This harp seal study also reported a surprising lack of correlation between Hp levels and extreme nutritional stress (starvation) in pups of this species.

Concentrations of Hp have been used as a health marker for monitoring various wildlife species (Duffy et al., 1993, 1994; Zenteno-Savin et al., 1997; Seiser et al., 2000). More studies that cover a range of health and condition values broader than those represented by the animals in this study are required to determine diagnostic Hp levels for use as health markers in ringed seals, but the results of this study suggest that Hp levels might be a useful tool in future monitoring of the health status of this species. The Hp levels varied with condition among juveniles and between adult males and females, perhaps because of higher trauma or stress levels in adult males during the breeding season.

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LITERATURE CITED

CHAN, J. P. W., C. C. CHU, H. P. FUNG, S. T. CHUANG, Y. C. LIN, R. M. CHU, AND S. L. LEE. 2004. Serum haptoglobin concentration in cattle. Journal of Veterinary Medical Science 66: 43–46.

DELANGHE, J. P., AND M. R. LANGLOIS. 2001. A review of biological aspects and the role in laboratory medicine. Clinica Chimica Acta 312: 13–23.

DUFFY, L. K., J. W. BOWYER, J. W. TESTA, AND J. B. FARO. 1993. Differences in blood haptoglobin and length-mass relationships in river otters (*Lutra canadensis*) from oiled and nonoiled areas in Prince William Sound, Alaska. Journal of Wildlife Diseases 29: 353–359.

———, ———, ———, AND ———. 1994. Evidence for recovery of body mass and haptoglobin values of river otters following the Exxon Valdez oil spill. Journal of Wildlife Diseases 30: 421–425.

ECKERSALL, P. D. 2000. Acute-phase proteins as markers of infection and inflammation: Monitoring animal health, animal welfare and food safety. Irish Veterinary Journal 53: 307–311.

———, S. DUTHIE, M. J. M. TOUSSAINT, E. GRUYS, P. HEEGAARD, M. ALAVA, C. LIPPERHEIDE, AND F. MADEC. 1999. Standardization of diagnostic assays for animal acute-phase proteins. Advances in Veterinary Medicine 41: 643–655.

EROKHINA, I. A. 2002. Effect of underfeeding at the period of breast-feeding on biochemical parameters of blood plasma in pups of the Greenland Seal *Phoca groenlandica*. Journal of Evolutionary Biochemistry and Physiology 38: 198–201.

GRUYS, E., M. J. OBWOLO, AND M. J. M. TOUSSAINT. 1994. Diagnostic significance of the major acute-phase proteins in veterinary clinical chemistry: A review. Veterinary Bulletin 64: 1009–1018.

KENT, J. E. 1992. Acute-phase proteins: Their use in veterinary diagnosis. British Veterinary Journal 148: 279–282.

KLEIBER, M. 1975. The fire of life: An introduction to animal energetics. Robert E. Krieger Publishing Company, Huntington, New York, New York, 453 pp.

LYDERSEN, C. 1995. Energetics of pregnancy, lactation, and neonatal development in ringed seals (*Phoca hispida*). *In* Whales, seals, fish and man, A. S. Blix, L. Walløe, and Ø. Ultano (eds.). Elsevier B.V., Amsterdam, The Netherlands, 734 pp.

———. 1998. Status and biology of ringed seals (*Phoca hispida*) in Svalbard. NAMMCO Scientific Publications 1: 46–62.

———, AND I. GJERTZ. 1987. Population parameters of ringed seals (*Phoca hispida* Schreber, 1775) in the Svalbard area. Canadian Journal of Zoology 65: 1021–1027.

———, AND K. M. KOVACS. 1999. Behavior and energetics of ice-breeding, North Atlantic phocid seals during the lactation period. Marine Ecology Progress Series 187: 265–281.

MURATA, H., N. SHIMADA, AND M. YOSHIOKA. 2004. Current research on acute-phase proteins in veterinary diagnosis: An overview. Veterinary Journal 168: 28–40.

ONDERKA, D. K. 1989. Prevalence and pathology of nematode infections in the lungs of ringed seals (*Phoca hispida*) of the western arctic of Canada. Journal of Wildlife Diseases 25: 218–224.

RYG, M., T. G. SMITH, AND N. A. ØRITSLAND. 1988. Thermal significance of the topographical distribution of blubber in ringed seals (*Phoca hispida*). Canadian Journal of Fisheries and Aquatic Sciences 45: 985–992.

———, C. LYDERSEN, N. H. MARKUSSEN, T. G. SMITH, AND N. A. ØRITSLAND. 1990a. Estimating the blubber content of phocid seals. Canadian Journal of Fisheries and Aquatic Sciences 47: 1223–1227.

———, T. G. SMITH, AND N. A. ØRITSLAND. 1990b. Seasonal-changes in body mass and body composition of ringed seals (*Phoca hispida*) on Svalbard. Canadian Journal of Zoology 68: 470–475.

SAINI, P. K., M. RIAZ, D. W. WEBERT, P. D. ECKERSALL, C. R. YOUNG, L. H. STANKER, E. CHAKRABARTI, AND J. JUDKINS. 1998. Development of a simple enzyme immunoassay for blood haptoglobin concentration in cattle and its application in improving food safety. American Journal of Veterinary Research 59: 1101–1107.

SCHEFFER, V. B. 1967. Standard measurements of seals. Journal of Mammalogy 48: 459–462.

SEISER, P. E., L. K. DUFFY, A. D. MCGUIRE, D. D. ROBY, G. H. GOLET, AND M. A. LITZOW. 2000.

Comparison of pigeon guillemots, *Cepphus columba*, blood parameters from oiled and unoiled areas of Alaska eight years after the *Exxon Valdez* oil spill. Marine Pollution Bulletin 40: 152–164.

SKINNER, J. G. 2001. International standardization of acute-phase proteins. Veterinary Clinical Pathology 30: 2–7.

SMITH, T. G., AND M. O. HAMMILL. 1981. Ecology of the ringed seal, *Phoca hispida*, in its fast ice breeding habitat. Canadian Journal of Zoology 59: 966–981.

SOLTER, P. F., W. E. HOFFMAN, L. L. HUNGERFORD, J. P. SIEGEL, S. H. STDENIS, AND J. L. DORNIER. 1991. Haptoglobin and ceruloplasmin as determinants of inflammation in dogs. American Journal of Veterinary Research 52: 1738–1742.

TRYLAND, M., K. K. SØRENSEN, AND J. GODFROID. 2005. Prevalence of *Brucella pinnipediae* in healthy hooded seals (*Cystophora cristata*) from the North Atlantic Ocean and ringed seals (*Phoca hispida*) from Svalbard. Veterinary Microbiology 105: 103–111.

WOLKERS, J., I. C. BURKOW, C. LYDERSEN, S. DAHLE, M. MONSHOUWER, AND R. F. WITKAMP. 1998. Congener-specific PCB and polychlorinated camphene (toxaphene) levels in Svalbard ringed seals (*Phoca hispida*) in relation to sex, age, condition, and cytochrome P450 enzyme activity. Science of the Total Environment 216: 1–11.

ZENTENO-SAVIN, T., M. A. CASTELLINI, L. D. REA, AND B. S. FADELY. 1997. Plasma haptoglobin levels in threatened Alaskan pinniped populations. Journal of Wildlife Diseases 33: 64–71.

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