Serum bile acids and the assessment of hepatic function in dogs and cats

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Abstract
Current literature in veterinary internal medicine regarding the clinical use of the serum bile acids test to assess hepatobiliary function in dogs and cats is reviewed. The test is best used in cases where clinical signs and routine laboratory tests are suggestive of liver disease. It is a highly sensitive and specific test of hepatic function, and is the best method of assessing liver function available to the private practitioner. Abnormal results do not determine etiology, severity, or prognosis of the disorder. They merely indicate the need for hepatic biopsy. The serum bile acids concentration should always be measured in both a fasting and a two-hour postprandial sample.

Résumé
Dosage des acides biliaires sériques et évaluation de la fonction hépatique chez le chien et le chat
Les auteurs présentent une étude bibliographique concernant l'utilisation clinique du dosage des acides biliaires sériques pour évaluer la fonction hépatobiliaire chez le chien et le chat. Cette épreuve de laboratoire est appropriée lorsque les symptômes cliniques et le bilan biochimique de base suggèrent une pathologie d'origine hépatique. Ce test est très sensible et spécifique, et représente pour le praticien la meilleure méthode de laboratoire pour évaluer la fonction hépatique. Les résultats de valeurs anormales ne permettent toutefois pas de déterminer l'étiologie, la sévérité et le pronostic de la maladie. Ils indiquent la nécessité d'effectuer une biopsie hépatique. La concentration sérique des acides biliaires devrait toujours être déterminée conjointement sur un échantillon prélevé chez l'animal à jeun puis 2 heures après l'ingestion d'un repas.

Can Vet J 1993; 34: 215-220

Introduction
Clinical signs of hepatic disease are often nonspecific. Patients may be presented for a variety of reasons, including neurological signs, polyuria, polydipsia, icterus, vomiting, lethargy, and ascites. Often, hepatic disease is not suspected until a serum biochemical profile is performed. The report may be accompanied by a suggestion from the clinical pathologist to measure serum bile acids. When used appropriately, measurement of the serum bile acids concentration is a sensitive and specific test of hepatobiliary function. However, in order to use this test effectively, the clinician must understand its theory, indications, and limitations. This paper will discuss criteria for evaluating diagnostic tests, and then, apply these criteria to the parameters of a standard serum biochemical profile that are suggestive of hepatobiliary pathology. It will review tests of hepatic function, and finally, discuss the physiology, theory, application, and interpretation of the serum bile acids test.

Method of literature review
Literature was searched using the CD-ROM systems Medline (U.S. National Library of Medicine, Silver Platter International, Boston, USA) and CAB Abstracts (Silver Platter International) for the publication years 1984 to 1992. The key words used were hepatic or liver function tests, bile acids, and dogs or cats. Commonly used veterinary textbooks were also consulted. However, because the emphasis of this paper was proper application of the serum bile acid test based upon clinical findings and clinical suspicion, textbooks in small animal internal medicine were used primarily.

Selecting a "diagnostic" test
To evaluate any diagnostic test, a clinician must consider its practicality (time, money, ease, safety), accuracy, sensitivity, and specificity. The accuracy of a positive test is represented by the positive predictive value. This is defined as the proportion of patients with a positive test who have the suspected disease. Conversely, the accuracy of a negative test, or the negative predictive value, is the proportion of patients with a negative test who do not have the suspected disease (1).

The positive and negative predictive values of a test are controlled by the sensitivity and the specificity of the test used, and the prevalence of the suspected disease in the population at risk. Sensitivity represents the proportion of patients with the suspected disease who have a positive test result. The greater the sen-
sitivity the smaller the number of false negatives. Specificity, on the other hand, is the proportion of patients without the disease who have a negative test result. The greater the specificity of a test, the smaller the number of false positives. Specificity and sensitivity influence whether a test will be valuable as a diagnostic test or a screening test. For example, if a test has very high sensitivity, a negative test result will permit the clinician to exclude the suspected disease as a differential diagnosis. However, if the sensitivity of the test is low, a negative test result does not exclude the suspected disease. Tests with high sensitivity are good screening tests; tests with high specificity are good confirmatory or diagnostic tests.

Liver enzymes
Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma glutamyl transferase (GGT) are the liver enzymes used clinically to evaluate the presence of hepatic disease. Measurement of increased serum concentrations of these substances may suggest hepatobiliary disease. Elevated serum concentrations are not, however, definitive evidence of significant liver pathology.

Both ALT and AST are contained within the cytosol of the hepatic cells. An increased serum concentration of either of these enzymes is suggestive of hepatocellular membrane damage. The liver is the predominant source of ALT in the dog and cat. Elevated serum concentrations can be observed in animals treated with corticosteroids or phenobarbital (2). In cats, hyperthyroidism may also increase serum ALT (3). These elevations usually do not represent significant hepatic pathology and can be viewed as false positives for liver disease. Muscle necrosis has also been shown to increase serum ALT (4). False negatives occur when there is significant hepatic damage but no elevation in the ALT concentration. The specificity of serum ALT concentrations for diagnosing hepatic disease has been shown to be high, while the sensitivity is only moderate (5,6). This means that the probability of false negatives is higher than the probability of false positives.

In dogs and cats, serum AST can originate from other tissues in the body, such as skeletal muscle, heart, kidney, and brain (2,7,8). The specificity of serum AST has been found to be higher than that of ALT for hepatic disease (5,6), but its sensitivity is lower (5). This lower sensitivity means more false negatives; therefore, serum AST is a poor screening test for hepatic disease. It has been speculated that AST is better in cats than in dogs for detecting liver disease (8).

Alkaline phosphatase and GGT are membrane bound enzymes found in high concentrations in the biliary system and on hepatocytes. Their serum concentrations are often elevated by cholestasis. A bone isoenzyme of ALP is also present and may be responsible for the elevated concentration commonly found in hyperthyroid cats (3). This bone isoenzyme may also be elevated in young growing animals. In dogs, production of ALP can be induced by treatment with corticosteroids, phenobarbital, or other microsomal enzyme inducers (7). A separate steroid-induced iso-

enzyme does not exist in the cat; consequently, in this species, any elevation in the serum ALP concentration comes from the liver or bone. In the diagnosis of liver disease in dogs, serum ALP has been found to have much lower specificity, but higher sensitivity than either ALT or AST (5,6). The higher sensitivity lowers the probability of false negatives; therefore, serum ALP may be better than ALT as a screening test for liver disease. However, it is subject to more false positives. Serum GGT has lower sensitivity than ALP for liver disease, but similar specificity (6).

Used in combination, the liver enzyme concentrations are helpful in detecting hepatic disease. However, the enzyme concentrations can not be used to assess liver function. In some cases of hepatic dysfunction, they may be normal. Conversely, the liver can be functioning normally even when marked liver enzyme abnormalities exist. Diagnosis of hepatic disease should not be dependent upon liver enzyme concentrations alone; the signalment, history, and physical examination of the patient must be considered. These factors help to determine the clinician’s “index of suspicion” (prevalence) for the disease (1). If the clinician is convinced that a patient suffers from hepatic disease, then the positive predictive value of the liver enzyme measured increases and the negative predictive value decreases. In this case, a negative test result would have a high probability of being a false negative. On the other hand, if one does not believe that a patient has hepatic disease, then the prevalence of disease is low, and a positive test result is probably a false positive. This applies regardless of the sensitivity and specificity of the test used. Unfortunately, the clinician is often uncertain if liver disease exists. When this happens, but the clinician is 30–70% confident (1) that hepatic disease is present, a liver function test is indicated.

Liver function tests
Serum concentrations of glucose, urea, albumin, and cholesterol may be used to assess hepatic function. These products are synthesized by the liver. However, more than 80% of the hepatic mass must be lost before any change in these parameters is detected (7). Further, serum glucose, albumin, cholesterol, and urea concentrations can be influenced by other factors, such as intestinal disease, metabolic disease, endocrine disease, renal disease, sepsis, or diet. These biochemical parameters are not very sensitive or specific indices of liver function.

Bilirubin concentration can also be used to assess liver function. Serum bilirubin is dependent upon the rate of heme pigment formation, albumin binding, hepatobiliary circulation and uptake, hepatic storage, conjugation, and elimination. Therefore, hyperbilirubinemia can result from increased production (prehepatic); decreased uptake, conjugation, and storage (hepatic); or decreased elimination (posthepatic). In one study of animals suspected of having liver disease, total bilirubin concentration was shown to have high specificity but very low sensitivity for liver disease. As well, the predictive value of a negative test was low (5).
Conjugated and unconjugated bilirubin can be measured by using van den Bergh reagents. Elevated concentrations of the unconjugated form may indicate increased heme pigment liberation or delayed hepatic uptake and processing (8). Acute hemolytic disorders are most commonly responsible for increased unconjugated bilirubin; however, this occurs early in the disease process (8). Usually, by the time a dog or cat is examined by a veterinarian, an equilibrium has been established between conjugated and unconjugated forms of bilirubin. Consequently, information from physical examination, history, and liver enzymes generally make differentiating forms of bilirubin unnecessary (8,9).

Sulphobromophthalein (BSP), indocyanine green (ICG), ammonia tolerance (ATT), and serum bile acids (SBA) are examples of liver function tests. Sulphobromophthalein is an exogenous indicator of hepatic function. After BSP has been injected, its serum concentrations are measured against time. The rate at which the drug is eliminated assesses a complex series of mechanisms, including albumin binding, portal circulation, hepatocyte uptake, cytosolic protein binding, conjugation, and biliary excretion (8). Unfortunately, BSP now has limited availability because it has been reported to cause anaphylactic and local reactions in man (10). In the cat, BSP retention is difficult to assess because of its extremely rapid excretion after injection (10). Indocyanine green has fewer side effects than BSP, but it is technically a more difficult assay to perform in the laboratory (2). Both tests are affected by obesity, edema, ascites, albumin concentration, and sampling techniques (2,7). False positive results will occur in an icteric animal because BSP and ICG compete with bilirubin for uptake, metabolism, and excretion (2).

Serum ammonia concentration can also be used to assess hepatic function, but a single baseline sample can be normal even in a patient with signs of hepatic encephalopathy. Products other than ammonia may be responsible for the clinical signs (11). The ATT is a provocative test of hepatic function. It is performed by measuring the fasting serum ammonia followed by measuring the serum ammonia after the oral administration of ammonium chloride. It is a very sensitive indicator of hepatic function and portal circulation; significant elevations were detected following 60% heptectomy in dogs, but not after 40% (12). Unfortunately, because ammonia samples are not stable, they must be analyzed immediately. Furthermore, there is a potential risk of inducing neurological signs with ammonium chloride administration in a patient with impaired liver function. The variability of results because of improper sample handling restricts the use of ATT to facilities that have the capability of performing the analysis.

Measurement of SBA is a relatively easy, safe, and rapid means of assessing hepatic function. Bilirubin is stable in serum for long periods of time and can be frozen. They are, consequently, ideal for private practitioners who send samples to regional laboratories. Bilirubin is equivalent to the ATT in detecting deficiencies in hepatic mass or circulation (8,13,14) and are less variable than BSP or ICG (10). The test does not involve the administration of any dye, chemicals, or other exogenous substances and, therefore, has no risk to the patient.

**Physiology of bile acids**

Bile acids are produced by hepatocytes and represent an end product of hepatocellular cholesterol metabolism (15). They are secreted in the bile and serve to generate osmotic pressure for biliary water and electrolyte secretion. Bile acids are amphipathic sterols (15) which incorporate hydrophobic molecules (cholesterol, monoglycerides, fatty acids) into micelles facilitating digestion and absorption of fat (7). As well, they promote the hepatic excretion of cholesterol, lecithin, steroids, bilirubin, BSP, and ICG (16).

The primary bile acids (cholic acid and chenodeoxycholic acid) are synthesized by the liver. In dogs and cats, they are conjugated primarily with taurine (17), secreted across the canalicular membrane into the bile ducts, and stored in the gall bladder. Eating triggers a series of neurohumoral stimuli (vagal, cholecystokinin) causing gall bladder contraction and bile flow into the duodenum. Most of the bile acids are then actively absorbed by the ileum (15) and extracted from the portal circulation by the liver. Bile acids reaching the colon are deconjugated and converted to secondary bile acids by bacteria. Some of these acids are lost in the feces. Fecal losses are 2%-5% of the circulating bile acid pool per day (2,10). Figure 1 shows bile acid metabolism.

Enterohepatic circulation of bile acids is extremely efficient, with 90%-95% of the bile acids extracted on their first pass through the liver (2,10). Normally, one would expect a very low concentration of bile acids in the systemic circulation. Normal SBA concentration is dependent upon normal hepatic mass, biliary system, portal circulation, and intestinal absorption. In virtually all forms of liver dysfunction, the liver maintains its capacity for the synthesis of bile acids, but it loses its ability to extract bile acids from circulation. Consequently hepatobiliary disease is detected by an increase in the concentration of circulating bile acids.

**Commonly used bile acid assays**

At the Western College of Veterinary Medicine (WCVM), a quantitative enzymatic method is used to
determine total SBA. This involves the oxidation of 3-α-hydroxy bile acids, which results in a chemical reaction that reduces a dye and causes a color change (18). The magnitude of the color change, measured using a spectrophotometer, is directly proportional to the concentration of SBA. The test has been validated in the dog and the cat (19-21). It effectively detected known quantities of cholic, taurocholic, glyccholic, deoxycholic, taurodeoxycholic, chenodeoxycholic, and glycochenodeoxycholic acid in feline and canine sera (21). It does not measure SBA that lack the 3-hydroxyl group or are esterified or sulfated in the number 3 position. The quantity of these individual SBA is not thought to be clinically significant (21).

The other commonly used assay is a radioimmunoassay. It has been validated in both the dog and cat using known concentrations of taurocholic acid (22). Some investigators feel it may be slightly more sensitive than the enzymatic method, but it can be more expensive and less convenient to perform (5,23,24). Further, the antibody used reacts selectively with only certain SBA and can underestimate the total concentration (21,25). The test does not measure total SBA, consequently, reference values for the radioimmunoassay are lower than those for the enzymatic method (23). Nevertheless, results of this assay and the enzymatic assay are reported to be comparable (19,23).

Applications of the serum bile acids test
Use of the SBA test is indicated whenever hepatobiliary disease is suspected. The test has high sensitivity and specificity. However, why one performs the SBA test and how one interprets it are also very important. The SBA test is excellent when one is uncertain whether a patient has hepatic disease. The test can detect the presence of occult liver disease, such as a portosystemic shunt or hepatic cirrhosis. Conversely, SBA concentration will determine whether an elevated liver enzyme is related to hepatic dysfunction. If SBA are abnormal, hepatic biopsy is indicated. If SBA are normal, significant functional hepatic disease is less likely.

Concentrations of SBA have been useful in dogs for the diagnosis of portosystemic vascular shunts (PSS) (26,27), hepatitis, cholestasis, cirrhosis, steroid hepatopathy (27-29), extrahepatic bile duct obstruction, and hepatic neoplasia (27,29). They have also been used to diagnose hepatobiliary disease in cats (19).

Concentrations of SBA have not been useful in differentiating between different forms of hepatic disease (19,28). They do not indicate the severity of the abnormality or its prognosis (5). Measurement of SBA will add little useful diagnostic information if the clinician is confident, based on clinical and laboratory findings, that liver disease exists. Hepatic biopsy is indicated under these circumstances.

The sensitivity of the SBA test is increased by submitting a fasting (FSBA) and a two-hour postprandial (PPSBA) sample (5,6,10,30). The prescription diets, c/dR for cats and p/dR for dogs (Hill’s Pet Products, Topeka, Kansas, USA) have been recommended as the test meal (10). The combination of the fasting and postprandial concentrations was found to have excellent sensitivity in dogs for the detection of cirrhosis, PSS, glucocorticoid hepatopathy, cholestasis, and chronic hepatitis (5). In one study of 170 dogs with suspected liver disease, the enzymatic assay was found to have 100% specificity for hepatic disease (no false positives) at FSBA values greater than 20 μmol/L and at PPSBA values greater than 25 μmol/L (5). Sensitivity, specificity, and positive and negative predictive values were highest when FSBA and PPSBA were combined. Further, when a dog with suspected hepatobiliary disease had a FSBA or a PPSBA greater than 25 μmol/L, the positive predictive value of the test was about 100%. A smaller study using 35 dogs found similar results; however, the study was flawed because it did not obtain liver biopsies from all of its control dogs. The specificity results with combination testing contrast those from a study of FSBA alone. In 150 dogs with suspected liver disease, 100% specificity was found at FSBA concentrations greater than 50 μmol/L (28). This was greater than the reference value for the laboratory used in the study. In cats on the other hand, FSBA concentrations greater than 15 μmol/L were found to be 100% specific for liver disease (19). Combination testing has also been recommended for cats (10), but clinical trials are lacking.

It must be stressed that these studies used a population of animals with presenting complaints that were suspicious of liver disease. The control patients were those members of the above population who did not have histological evidence of liver pathology. None of the control animals were healthy. Reference ranges are usually established using 95% confidence intervals. This means that in a normally distributed population of healthy animals, 2.5% of the animals can have a test value higher than the reference range. Increasing the reference range to include more of these healthy animals will increase the specificity of the test. Concurrently, it will decrease the sensitivity and increase the probability of false negatives. Reference values at the WCVM for FSBA and PPSBA are less than 10 μmol/L and 20 μmol/L, respectively. Another study establishing reference ranges for the enzymatic method found a value of 29 μmol/L to be the upper limit for PPSBA (23).

Combination testing was most effective in the detection of PSS. In this anomaly, the FSBA concentration is sometimes normal, but the PPSBA concentration is usually markedly abnormal (5,13,26). A single fasting sample would fail to detect some of these animals. Prolonged fasting may allow the liver time to extract bile acids from the circulation in some cases of PSS (26). One study demonstrated no advantage to combination testing over FSBA; however, this was not the purpose of the study and only a small number of animals were assessed (14).

Interpreting the results — What is significant?
The results of the SBA test are usually unequivocal when performed properly with a fasting and two-hour postprandial sample. Occasionally, confusion arises when the test is conducted on a patient with a healthy liver. For instance, one sometimes finds a higher concentration of bile acids in the fasting sample than in the postprandial sample. This may occur with a spon-
taneous contraction of the gall bladder during a prolonged fast (5). However, the FSBA concentration should not exceed the laboratory reference range for the PPSBA if the liver is healthy. Another source of variability can be attributed to individual differences in the response time of the gall bladder to feeding. Clinical studies are lacking to assess whether this is significant.

It is also possible for the SBA concentration of an animal with impaired liver function to be within the reference range (false negative). Transient decreases in bile flow may lower the concentration. The serum concentration of bile acids can also be lowered by impaired ileal function (5). These two conditions are generally rare.

Elevations in the SBA concentration occur when there is defective hepatoporal circulation, loss of functional hepatic mass, hepatobiliary obstruction, or laboratory error. If hepatic disease is suspected and the SBA concentration is elevated, hepatic biopsy is indicated. It has been speculated that minor elevations can occur in patients treated with glucocorticoids (27). Glucocorticoids may disrupt bile acid metabolism by altering canicular permeability and causing cholestasis. In dogs, this should be supported by an elevation in the concentration of serum ALP. A recent study found that topical glucocorticoids elevated ALP, but did not affect SBA (31).

Conclusion
The SBA test is a very sensitive and specific indicator of hepatic function. The sensitivity of the test should be increased by submitting both a fasting and a two-hour postprandial sample. The test is most useful in the diagnosis of hepatic disease when the clinician is suspicious, but not positive, that hepatic disease exists. The most valuable applications of the test are to identify impaired liver function, to confirm or deny the presence of significant hepatic disease, and to decide if hepatic biopsy is indicated. The test is noninvasive, easy to perform, convenient to submit to a regional laboratory, and when used for appropriate reasons, relatively simple to interpret.

Although hepatic biopsy is absolutely necessary to confirm the type of liver pathology present, certain patterns of liver enzyme and bile acid abnormalities can be predictive. An increased concentration of SBA in the absence of jaundice or hepatic enzyme elevations suggests occult liver disease. This is found with PSS and cirrhosis. Abnormal SBA in the presence of jaundice can distinguish between prehepatic (hemolysis) and hepatic jaundice (5). Clinically, however, hemolysis is usually obvious so this distinction is unnecessary. Abnormal serum concentrations of both hepatic enzymes and bile acids have been found with hepatic necrosis, lipidosis, hepatitis, neoplasia, bile duct obstruction, and cholestasis (5). Hepatic biopsy is always necessary for a definitive diagnosis.

References

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COMING EVENTS

ÉVÉNEMENTS À VENIR

CVMA Convention Corner/Le coin des congrès

1993—Edmonton July 7-10 juillet
1994—Quebec City July 6-9 juillet
1995—Victoria July 12-15 juillet

MAY/MAI 1993

Animal Health Week '93. May 2-8, 1993. Theme: Animal Health Week Affects Us All. The Canadian Veterinary Medical Association's 8th Animal Health Week is a national campaign which highlights the importance of animal health and the role that veterinarians play in our society. Contact: Canadian Veterinary Medical Association, 339 Booth Street, Ottawa, Ontario K1R 7K1; tel: (613) 236-1162; fax: (613) 236-9681.

The First International Workshop on Advanced Reproductive Technologies. May 3-7, 1993 at the Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan. Topics will include: traditional embryo transfer training; new information on the use of embryo transfer for genetic improvement and on disease control and import/export issues; new freezing techniques such as vitrification and the "one-step" straw; specific instructions on the thawing and transfer of frozen embryos; diagnostic spermatology; in vitro fertilization; and the use of real-time B-mode ultrasonography. Speakers include Drs. Reuben Mapletoft, Gregg Adams, Albert Barth, Marcelo Del Campo, Mark Jacobson, and Vladding Pavlyshyn. Courses offered in English and Spanish. Contact: Continuing Veterinary Education Section, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0; tel: (306) 966-7267; fax: (306) 966-8747.

Australian Veterinary Association Annual Conference. May 16-21, 1993 on the Gold Coast at Jupiter's Casino/The Hilton Hotel, Queensland, Australia. Program includes sessions on veterinary public health, sheep, acupuncture, small animals, embryo transfer and veterinary nursing. Four plenary symposia will also be conducted on veterinary education, immunology, vector-borne diseases and exotic diseases. Contact: Pamela Holsinger & Associates, P.O. Box 718, West End, Queensland 4101 Australia. tel: (07) 846-5858; fax: (07) 846-5859.

26th International Conference on the History of Veterinary Medicine. May 31 – June 4, 1993 in Amersfoort/Utrecht, The Netherlands. Sponsored by The World Association for the History of Veterinary Medicine. Contact: Secretariat, 26th International Congress on the History of Veterinary Medicine, Faculty Library, Faculty of Veterinary Medicine, P.O. Box 80.159, 3508 TD Utrecht, The Netherlands.

JUNE/JUIN 1993

Saskatchewan Animal Health Technologists Association. June 5, 1993 in Yorkton. Executive meeting at 2:00 p.m.; general meeting at 2:00 p.m. Location to be announced. Contact: Sue Thiessen, Saskatchewan Animal Health Technologists Association, Box 346, Sub P.O. #6, Saskatoon, Saskatchewan S7N 0W0.


International Symposium on Addictions '93. June 11-12, 1993 in Guelph, Ontario. Hosted by the Homewood Health Center and the Office of Continuing Education, University of Guelph. Theme: Addictions '93: Emerging Issues and the Health Care Professional. The symposium will focus on two main areas: the diagnosis and management of dual disorders — a combination of addictions and psychiatric illness; and the recognition and management of impairment in health care professionals. Contact: Dr. Graeme Cunningham, Director, Homewood Alcohol and Drug Services, Homewood Health Center, 150 Delhi Street, Guelph, Ontario N1E 6K9; tel: (519) 824-1010; fax: (519) 824-1827.

JULY/JUillet 1993

Surgical Forum — Australian College of Veterinary Scientists. July 4-9, 1993 at the Twin Waters Resort, Sunshine Coast, Queensland, Australia. Contact: Dr. Roger Clarke, Secretary, Surgery Chapter, Australian College of Veterinary Scientists, P.O. Box 234, Bunduurn, Australia 3083; tel: 03 467 2255; fax: 03 467 7369.

Canadian Association of Veterinary Ophthalmology Annual Meeting. July 6-7, 1993 in Edmonton, Alberta. Features an indeth presentation on the cornea, including the use and abuse of contact lenses in animals. Guest speaker is Dr. Paul Dice from the Animal Eye Clinic in Seattle. Contact: Richard Christmas, Canadian Association of Veterinary Ophthalmology, 233 17th Avenue SW, Calgary, Alberta T2S 0A4.

Fourth International Livestock Environment Symposium. July 6-9, 1993 at the University of Warwick Conference Park, Coventry, England. Contact: Eldridge Collins, VPI and SU, Agricultural Engineering Department, College of Agriculture and Life Sciences, Blacksburg, Virginia 24061; tel: (703) 231-7600; fax: (703) 231-3199.

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