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RABIES DIAGNOSIS IN A 5 YEARS OLD MALE FROM EQUATORIAL GUINEA, AFRICA

David, D[1], Dvers, N[1]., Yakobson, B[1]., Cohen, M[1]. and Rubin, Y[2].
[1]Rabies Laboratory, Kimron Veterinary Institute, Bet Dagan, Israel.
[2]Israeli Medical Center, "LA PAZ" Bata, Guinea Equatorial.

After Asia, Africa is the continent most affected by rabies. Africa has about 24.000 (44 %) of the 55.000 worldwide rabies deaths annually. We describe a human rabies case, which occurred in a 5-year-old boy from the Republic of Equatorial Guinea, Africa, who was bitten on his neck by a stray dog 5 weeks prior to hospitalization. The boy received treatment against tetanus but not for rabies post-exposure prophylaxis. On December 17, the boy complained of a headache, general pain and weakness. The following day, the boy refused to eat and drink and was incoordinated. On December 19 (HD-1; hospital day-1), the boy was hospitalized at the Israeli Medical Center "La Paz", Bata City with encephalitis and hydrophobia. Rabies was suspected, and on 21 December 2007, ante-mortem specimens of cerebrospinal fluid (CSF), sera, saliva, and a skin biopsy were collected and sent to the Rabies Laboratory at the Kimron Veterinary Institute, Bet Dagan, Israel for diagnosis. By using the hemi-nested RT-PCR assay we detected rabies virus RNA in saliva and the skin biopsy. Rabies was confirmed and the Wisconsin protocol was applied. The sera and the CSF were found negative for antibodies in the Rapid Focus Fluorescence Inhibition Test (RFFIT) and in the indirect immunoflourescence assay for IgG and IgM until the HD 16. For virus isolation, suspensions of saliva CSF and skin biopsy were injected into tissue culture and suckling mice. The virus was isolated from the skin biopsy of the injected mice. Viral antigen was detected by direct immunoflourescence of frozen sections of the nuchal skin biopsy. Molecular analysis of the viral nucleoprotein was also performed, and a phylogenetic tree showed 99 percent identity with canine rabies virus sequences from Gabon. The child died on 19 HD due to renal insufficiency. Based on WHO data this is the first case of rabies reported from the Republic of Equatorial Guinea, Africa.

PLASMA ANTITHROMBIN ACTIVITY AS A DIAGNOSTIC AND PROGNOSTIC INDICATOR IN DOGS: A RETROSPECTIVE STUDY OF 149 DOGS

Segev, G. Kuzi, S. Haruvi, E. and Aroch, I.
Koret School of Veterinary Medicine, Hebrew University of Jerusalem, Israel, P.O. Box 12, Rehovot, 76100, Israel

Antithrombin (AT) is produced by the liver and is the major inhibitor of coagulation. It accounts for 80% of the total inhibitory effect of plasma on coagulation and irreversibly binds and inactivates several serine proteases, including thrombin and factor X. Decreased AT activity (ATA) is thought to deflect the hemostatic balance towards hypercoagulability, predisposing patients to thrombotic events, organ failure and death. It has been shown in people that hypoantithrombinemia is a marker of hypercoagulability, thrombosis and poor prognosis. Veterinary studies, however, did not demonstrate similar prognostic significance, thus, canine AT activity is currently interpreted based on human medicine guidelines. We hypothesized that ATA can serve as a prognostic marker in dogs, as has been shown in people. The objectives of this study were a) to describe the clinical and clinicopathologic signs, diagnoses and outcome of dogs presenting decreased vs. normal ATA, b) to identify diseases and mechanisms associated with hypoantithrombinemia, and c) to assess ATA as a prognostic indicator. This is a retrospective study of 149 dogs in which ATA was measured during their disease course. Dogs with normal and decreased ATA were compared. Hypoantithrombinemic dogs had a higher prevalence of leukocytosis, haemostatic abnormalities, hypoalbuminemia and hyperbilirubinemia compared to dogs with normal ATA. Hypoantithrombinemia was commonly present in immune-mediated hemolytic anemia (IMHA), pancreatitis, hepatopathy and neoplasia. It was associated with higher risk of mortality in the entire study population and with specific diseases (e.g., IMHA, neoplasia). The odds ratio for mortality significantly and progressively increased when ATA was <60% and <30% (10.33, 14.66 respectively). A receiver operating

characteristics analysis of ATA as a predictor of mortality showed an area under the curve of 0.7, and an optimal cutoff point of 60% yielding sensitivity and specificity of 58% and 85%, respectively.

In conclusions, canine ATA <60% indicates increased risk for mortality, similarly to human patients, but ATA has a limited value as a discriminating factor of the outcome.

BACTERIAL ETIOLOGIES OF LATE EMBRYONIC MORTALITY IN LANNER FALCONS AND OTHER *FALCONIFORMES* DOGS: A RETROSPECTIVE STUDY OF 149 DOGS

Lublin, A[1]., Gerchman, I[1]., Mechani, S[1]., Horowitz, I[2]., Hatzofe, O[3]. and Miller, Y[3].
[1]Division of Avian & Fish Diseases, Kimron Veterinary Institute, Bet Dagan,
[2]Zoological Center Tel Aviv Ramat-Gan Ltd. SAFARI, National Park, Ramat-Gan, Isael,
[3]Israel Nature and National Park Authority, Jerusalem, Israel

The goal of this study was to determine bacterial causes of pre-hatching mortality of captive raptors in Israel, and especially to clarify possible association with Mycoplasmas. *Mycoplasma* is known as one of the significant causes to pre-hatching mortality in birds. In recurrent mortalities in a breeding population of *Falco biarmicus* (Lanner falcon) in the raptor rehabilitation center in Tel Aviv University (4 pairs), *Mycoplasma falconis* was isolated from the tracheas of two females during two successive years. Treatment before the breeding season by feeding with dead mice enrofloxacin-administered, greatly reduced the embryonic mortality.

During 1995-1998 fertility rates of the Lanner falcons were quite high and ranged between 75 and 94%, however, the rate of hatching and rearing the chicks decreased from 83% in 1995 to 20-50% in 1996-1998. Mortality of embryos *in shell* occurred usually a few days before the end of the incubation period or on the day of hatching.

The main bacterial agents that had been diagnosed and could contribute to infection and mortality of embryos or young chicks were *Staphylococci, E. coli* and other coliforms, *Pseudomonas, Salmonella* and *Chlamydophila psittaci*. In January 1999 the research group commenced a continuing survey of the breeders, dead embryos, and young chicks, for the presence of *Mycoplasma* and other bacterial pathogens. The diagnostic methods for *Mycoplasma* included: cultivation of allantoic or yolk fluid from dead embryos or of tracheal/cloacal swabs from the breeders, direct immunofluorescence with monospecific antibodies to *M. gallisepticum* and *M. synoviae*, and for the four essential species of raptors, *M. falconis, M. buteonis, M. gypis,* and *M. corogypsi*, and PCR.

Each year before the breeding period a treatment regime was applied to the breeders. Between 1999 and 2007, each pair of falcons was fed during 7-10 days with 1 or 2 dead mice that had been injected with a single dose of enrofloxacin (20 mg/kg Lanner falcon body weight, IP). The total numbers of eggs laid each year during 1995-2007 ranged from 15 and 28, and the percentage of fertile eggs, between 47 and 95%. The fertility rate was not affected by the antibiotic treatment and averaged 70-76%, however, hatching percentage increased significantly ($p<0.05$), from 47% without treatment to 70% following treatment. In domesticated birds, Mycoplasmas are well known possible causes of embryonic mortality prior to or around hatching, especially the turkey species, *M. meleagridis* and *M. iowa*, but both species have no affect on egg production and early embryonic mortality.

Two species of *Mycoplasma* were identified, *M. falconis* and a second unidentified species. *M. falconis* is one of the main raptor Mycoplasmas, which may be found in healthy and also in diseased raptors in association with air sac pathologies. Other pathogens that were identified were *Staphylococci, E. coli* or other coliforms, *Salmonella*, and *Chlamydophila psittaci*. Fungi were not identified in association with any of the mortalities.

In a survey for infective agents causing embryonic mortality in four other species of raptors (*Gyps fulvus, Falco naumann, Hieraetus fasiatus* and *Torgos tracheliotus*), *Salmonella, E. coli, Pseudomonas, Chlamydophila psittaci* and *Aspergillus fumigatus* were identified. Only one case of *Mycoplasma* (unidentified species) was detected, in a dead Griffon vulture (*Gyps fulvus*) embryo.

FIRST CASE OF CANINE *BABESIA GIBSONI* INFECTION IN ISRAEL

Baneth, G[1]., Molad, T[2]., Yas, E[1]., Segev, G[1]., Yisaschar-Mekuzas, Y[1]., Fleiderovich, L[2]. and Shkap, V[2].
[1]School of Veterinary Medicine, Hebrew University of Jerusalem
[2]Division of Parasitology, Kimron Veterinary Institute

Canine babesiosis is a tick-borne infection caused by a spectrum of piroplasm species and sub-species. Reports of babesiosis from Israel have to date included documentation only of large *Babesia* forms in dogs while genetic characterization have identified only *Babesia canis vogeli* in dogs in Israel. *Babesia gibsoni* is a small piroplasm described in dogs from Southeast Asia, USA, South America, Australia and as a sporadic infection in Europe. *B. gibsoni* infection can be severe, and fatal, sub-clinical infections have been described especially in Pit Bull terriers and related breeds.

A fourteen year old male mongrel dog was referred to the Hebrew University Veterinary Teaching Hospital (HUVTH) and diagnosed with anal sac adenocarcinoma and perianal gland adenoma. It underwent surgery to excise these tumors but two months later was detected with stage II renal disease and a splenic mass for which splenectomy was performed. A month later the dog was admitted to the HUVTH again with lethargy and was found to have anemia with abundant spherocytes and thrombocytopenia. Following a tentative diagnosis of immune-mediated anemia, thrombocytopenia treatment with prednisone was initiated at immunosuppressive doses. An improvement was noted in the erythrocyte and thrombocyte counts a week after therapy was initiated, however, after a further week the dog presented with acute collapse and severe hemolytic crisis and fever. Three days later, forms of a small *Babesia* species were detected in Giemsa-stained blood smears. The dog was treated with imidocarb dipropionate, but subsequently when *Babesia* parasitemia and fever did not subside, atovaquone and azithromycin, recommended for treatment of *B. gibsoni*, were administered. The dog did not improve clinically and died four days after the diagnosis of babesiosis, despite the anti-protozoal and supportive treatments.

PCR targeting a fragment of the *Babesia18S* rRNA gene produced a 560 base pair amplicon that was sequenced and found 100% similar to a B. gibsoni sequence deposited in GenBank. Additional verification of the *Babesia* species' was obtained by PCR and sequencing of a 358 base pair segment of the ribosomal internal transcribed spacer (ITS) that was also 100% similar to a *B. gibsoni* Genbank deposit. These genetic analyses confirmed that the dog had been infected with *B. gibsoni.*

Further investigation of the dog's history revealed that it was brought to Israel 12 years earlier from Hong Kong. The dog received blood transfusions on 3 different occasions prior to diagnosis with babesiosis. Blood smears from all transfusions were negative for *Babesia*. Only the second transfusion was available retrospectively for additional testing and it was found negative for *Babesia* by PCR.

The origin of *B. gibsoni* infection in Israel, whether autochthonous or imported, remains unknown. *Rhipicephalus sanguineus*, a potential tick vector of *B. gibsoni* is widely spread in Israel. The dog resided in Israel for 12 years and received blood transfusions that could have transmitted autochthonous infection. However, the fact that this is the only documented case of *B. gibsoni* in Israel in a dog imported from an endemic area in Southeast Asia, that was splencetomized and treated with immunosuppressive therapy, may suggest an imported infection. These treatments may have reactivated a previously harbored cryptic infection.

A NEW FORM OF ANTIBIOTIC THERAPY FOR PETS USING A SINGLE DOSE SWELLING TABLET

Lavy, E[1]., Chamisha, Y[1]., Friedman, M[2]. and Hoffman, A[2].
[1] Koret School of Veterinary Medicine, Hebrew University of Jerusalem, and
[2] School of Pharmacy, Hebrew University of Jerusalem, Israel

The main advantage of using controlled-release oral medications is the reduction of dosage intervals, leading to improved patient and owner compliance.

Antibiotics are the most commonly prescribed drugs in small animal medicine for a variety of microbial infections, including pyoderma, ,ear infections, respiratory, gastrointestinal, urinary tract and wound infections. The most common veterinary treatment protocol is performed on an 'outpatient' basis and involves oral treatment with beta-lactam antibiotics, such as ampicillin, amoxicillin, amoxicillin-clavulanic acid, cephalexin and cefuroxime. The major drawback of many antimicrobial treatment protocols is related to the short biological half-life of beta-lactam antibiotics and to their pharmacodynamic properties that necessitate prolonged exposure of the pathogen to effective drug concentrations, by multiple

daily dosing throughout the treatment course that usually lasts for 5-7 days. As a result, one of the common reasons for failure of antimicrobial therapy is low pet owner compliance. Primary reasons for such poor compliance include being away from home throughout most of the day, difficulties with restraining the animal, and lack of experience and/or confidence.

As a potentially effective solution for this problem we propose an oral dosage form for beta-lactam antibiotics when a single administration should provide effective drug concentrations throughout the treatment course.

It is well known that for beta-lactam antibiotics, single administration treatment strategies cannot be based on the conventional oral 'slow release' formulations because they have a narrow 'absorption window' confined to the small intestine, and the drug effect would terminate shortly after the formulation reaches the colon. In contrast, a single dose controlled release antibiotic therapy (SCRAT) is designed to utilize the major pharmacokinetic and pharmacodynamic advantages of controlled release dosage forms for beta-lactam antibiotics, and may enable continuous input of beta-lactam drugs to absorption sites in the upper gastrointestinal tract over several consecutive days. The pharmaceutical basis of the SCRAT concept is an expandable swelling matrix tablet with prolonged retention in the patient's stomach.

INCOMPLETE ATRIOVENTRICULAR CANAL COMPLICATED BY CARDIAC TAMPONADE AND BIDIRECTIONAL SHUNTING IN AN ADULT DOG

Ohad, D.G[1]., Baruch, S[1]., Rubin, G[1]. and Perl, S[2].
[1] Department of Clinical Sciences (Ohad, Baruch, Rubin), Koret School of Veterinary Medicine, Hebrew University of Jerusalem, P.O. Box 12, Rehovot, 76100, Israel and
[2] Division of Pathology (Perl), Kimron Veterinary Institute (KVI), P.O. Box 12, Beit Dagan, 50250, Israel.

A 6-year old, 33 kg spayed female Weimranner dog was presented with a 3-day long history of partial anorexia, gradual depression, heavy breathing, and progressive abdominal distention. Mucous membranes were pale and gray-to-cyanotic with muffled heart sounds and no pulse deficit. Ascites (modified transudate), dyspnea and tachypnea (50/min) were noted and pleural effusion (modified transudate) was demonstrated. The packed cell volume was 44%, suggesting that hypoxia has unlikely been chronic by that point in time. Arterial blood gas and pH were consistent with mild respiratory alkalosis and hypoxia with a partial oxygen pressure of 48.8mmHg (normal range: 90-100 mmHg) and an oxygen saturation of 78.8% (97-100%). Electrocardiography revealed sinus tachycardia with a first degree atrio-ventricular block, a moderate right axis deviation of the mean electrical axis, and a right bundle branch block. Despite morphine administration and direct oxygen supplementation cyanosis remained, and a right-to-left shunting cardiac anomaly was therefore suspected. Echocardiography revealed no evidence of an inter-atrial septum from any of 5 standard views. Cardiac tamponade, right ventricular enlargement due to volume overload, and diastolic inter-ventricular septal flattening were demonstrated also. Pericardiocentesis, thoracocentesis and abdominocentesis, as well as oral and intravenous medications relieved cyanosis and other symptoms for only 8 hours, and euthanasia was then opted. Necropsy findings included severe right-sided cardiomegaly with no division between the two atria except for an endocardial fold 4 mm in width, extending from the superior aspect of the common atrium. It was thought to be the dorsal aspect of the primitive septum primum or septum secundum, remaining from the point in time when early development of the embryonic septum has ceased. There was a cleft in the mitral valve, consistent with the final diagnosis of an "incomplete atrioventricular canal". Slowly progressive right-sided congestive heart failure due to the left-to-right atrial septal defect likely culminated in an elevated right ventricular (RV) filling pressure. This gradually decreased right ventricular compliance enough to challenge right atrial drainage into the right ventricle. Blood flow from the right atrium has been likely "directed" towards the path of least resistance, i.e. through the left-sided portion of the common atrium and into the left ventricle. Hence, progressive intra-atrial right-to-left shunting developed, along with pulmonary hypertension (PHT) and sinus tachycardia. The parallel gradual development of pericardial effusion, likely secondary to right-sided congestive heart failure, culminated in cardiac tamponade, which eventually increased intra-atrial pressure even further. This could have further exacerbated the already developing right-to-left shunt within the common atrium, eventually leading to systemic cyanosis which exacerbated PHT. This positive feedback loop or "vicious" cycle was triggered by a congenital malformation but led only to terminal disease well into the patient's adulthood.

PREVALENCE OF EHRLICHIA, BABESIA AND HEPATOZOON SPECIES IN IXODID TICKS (RHIPICEPHALUS SANGUINEUS, RHIPICEPHALUS TURANICUS AND HYALOMMA SPP.) IN ISRAEL

Perlman-Avrahami, A[1]., Baneth, G[1]., Mumcuoglu, k.y[2]., Eyal, O[1]., and Harrus, S[1].
[1] Koret School of Veterinary Medicine, Hebrew University of Jerusalem, and
[2] Department of Parasitology, Hadassah Medical School, Hebrew University of Jerusalem, Israel

Tick-borne bacteria and protozoa are of major importance in veterinary medicine and are of public health significance. In this study we focus on pathogens that cause diseases in dogs. The objectives were to evaluate infection with Ehrlichia, Babesia and Hepatozoon spp. in ixodid ticks (*Rhipicephalus sanguineus, Rhipicephalus turanicus* and *Hyalomma* spp.), and examine the relationship between the prevalence of each pathogen and its geographic distribution in Israel. It was hypothesized that no differences would be found between the infection prevalence of *E. canis* and *B. canis*, while a low prevalence of *H. canis* was expected. Higher prevalence rates of the pathogens were expected in R. sanguineus compared to other tick spp. while no difference between the prevalence of the pathogens in the different geographical regions was expected.
A total of 1183 unengorged adult ticks were collected during the years 2002-2008 from three geographical regions in Israel (North, South and Center). *R. sanguineus, R. turanicus* and *Hyalomma* spp. ticks were collected in the field using the "Berlese funnel method", and the white flannel flag technique". Two to ten ticks of the same species were pooled, and 13 *Hyalomma* ticks were placed in individual tubes. DNA was extracted from the ticks and PCR assays were performed using specific primers. All positive samples were sequenced and compared to the deposited sequences in GenBank.
A high prevalence (10.7%) *of E. canis* was detected in this study. It was detected in *R. turanicus* and in *Hyalomma* spp. To the author's knowledge, this is the first report of E. canis detection in these 2 tick species. A low prevalence (3.1%) of *B. canis* infection while no evidence of *H. canis* infection was found. In addition, *Candidatus* Midichloria mitochondrii and *Anaplasma bovis* were detected for the first time in ticks in Israel.

INFECTIOUS LARYNGOTRACHEITIS VIRUS (ILTV): THE FIRST DIFFERENTIAL ASSAY FOR VACCINE AND FIELD VIRUSES IN CHICKEN ORGANS

Davidson, A., Nagar, S., Shkoda, I., Raibstein, I. and Perk, S.
Division of Avian Diseases, Kimron Veterinary Institute, Bet Dagan, P.O.Box 12, Israel 50250

Infectious laryngotracheitis virus (ILTV) causes a respiratory disease of poultry of variable severity, from a mild to an acute form with mortality rates up to 70%. Milder forms of ILT are manifested by nasal discharges, conjunctivitis, and reduced egg production, whereas in severe forms, the clinical signs range from gasping with efforts to inhale, coughing, excretion of bloody mucus, dysnea up to suffocation and rapid mortality. Development of differential diagnosis assays is important in cases of mild ILT, where the clinical signs might overlap with other respiratory pathogens.
Protection against ILT is achieved by vaccination, mostly with live vaccines. The cloacal vent application, developed by Dr.Y.Zamberg, has been effective in Israel when administered to 8 weeks-old chickens. However, in the last years, 4 clinical cases have appeared which served to develop the differential assay. Paradoxically, vaccines might have undesirable features, and their in vivo passages might result in virus transition to virulence, production of latently-infected carriers, disease in neighbouring, unvaccinated chickens and latent virus reactivation by stress factors and other pathogens.
The likelihood that both wild-type virulent ILTV strains and vaccine strains can potentially cause ILT, motivated us, (a) To find effective methods for rapid demonstration of virulent field ILTV directly from organs of affected birds, and to support diagnosis, where embryonated egg inoculation and change of embryo morphology is presently used, and (b) to develop a differential assay for wild type and vaccine strains. The present study approach is original in two aspects: (a) The direct use of chicken organs, without further replication, was intended to avoid genomic changes of the virus genome that might occur during replication, and (b) Feather sampling is valuable, as bird killing and necropsy are avoided, while repeated multiple sampling are feasible from same birds.
We evaluated 5 PCR methods to amplify ILTV from clinical samples, vaccine strains and healthy birds after commercial vaccination. Feather shafts, trachea homogenates or swabs of the same clinical case, were of similar efficacy to detect viral DNA by PCR by all assays; however in vaccinated birds fewer viruses were

detected. As the nested PCR detected the vaccine virus for 30 days post-vaccination, this method can be adopted for monitoring the vaccination efficacy, and also for the differentiation between vaccine and wild type ILTVs. The digestion of the nested PCR product with restriction enzymes could be done only with vaccine viruses but not with wild type isolates and this finding provided a differential assay.

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A PROSPECTIVE ASSESSMENT OF NEUTROPHIL CYTOPLASMIC TOXICITY IN HORSES WITH COLIC AS A DIAGNOSTIC AND PROGNOSTIC TOOL

Sarusy B., Levy O. and Aroch I.
Koret School of Veterinary Medicine, Hebrew University of Jerusalem

Horses with colic often present quantitative and morphological changes of neutrophils. The latter include cytoplasmic, nuclear and cell size toxic changes. Neutrophil toxic changes in horses have been described only generally in the literature, and include only subjective observations. There is a lack of prospective studies or data in specific disease conditions, notably colic. The present study prospectively examined the morphological changes occurring in circulating neutrophils of horses presented with colic, from admission through discharge or death at the Hebrew University Teaching Hospital. Data retrieved from medical records included signalment, history, physical examination findings, ancillary test results, diagnosis, presence and type of surgery, hospitalization time and outcome (alive or dead at discharge). CBC, differential leukocyte counts and morphological assessment of neutrophils in modified Wright's stained blood smears were performed periodically from arrival to discharge or death.

Horses with colic for > 12 hours that underwent surgery showed significantly ($p<0.022$) more severe neutrophilic cytoplasmic toxicity than those with colic signs of < 12 hours duration. When all horses with prolonged (>12 hours) colic were assessed neutrophil toxicity was significantly ($p<0.022$) more severe in those that eventually underwent surgery than those that did not, with a sensitivity of 70% and a positive predictive value of 92%. Significantly more severe neutrophilic cytoplasmic toxicity was observed in non-survivor horses vs. survivors on arrival, and at 12 and 72 hours post-admission ($p<0.0002$, $p<0.002$, $p<0.017$ respectively). The most severe neutrophilic cytoplasmic toxicity in this study was observed in colitis ($p<0.02$) than in other colic types. These horses were clinically and hematologically more ill than the other horses.

This is the first prospective study to examine consecutive morphological changes in equine neutrophils during the course of an illness in general, and specifically in equine colic. The results show that the nature and intensity of neutrophilic cytoplasmic toxicity depend on the nature and course of the colic, and is a useful diagnostic and prognostic aid in the assessment of horses with colic.