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Chairperson: Prof. K. Perk

Founder and honorary director, Koret School of Veterinary Medicine

CHRONIC VAGAL STIMULATION FOR VENTRICULAR RATE CONTROL IN A DOG WITH ATRIAL FIBRILLATION

Ohad D.[1], Sinay Y.,[2], Carmi B.,[2], Zaretsky A.,[3] and Shofti R.[3]

[1]Koret School of Veterinary Medicine, Hebrew University of Jerusalem, Israel,
[2]Biocontrol Medical Ltd., Yehud,
[3] Technion Faculty of Medicine, Haifa

Chronic atrial fibrillation (AF) is among the most common canine tachy-arrhythmias requiring therapy. While not immediately life threatening, it does reduce life quality and increases morbidity, and over time contributes to the development of congestive heart failure (CHF) and an abbreviated life expectancy. Frequently, AF recognition comes too late to allow conversion to normal sinus rhythm. Therefore, rate control is often preferred over attempted rhythm control. Chronic pharmacotherapy may fail to provide optimal or even adequate rate control, trigger intolerable side effects or toxicity, be impractical due to low owner compliance, or be contraindicated, however,.

Our goal was to empirically utilize a novel, non-pharmacological approach using a vagus nerve stimulating system to constantly prolong the atrio-ventricular refractory period in a patient with chronic AF.

A four-year old intact male Dogue de Bordeaux presenting with right-sided CHF was diagnosed with severe congenital pulmonic stenosis and tricuspid dysplasia, along with secondary, chronic AF. Presenting heart rate (HR) was 250/min despite pharmacotherapy including digoxin, enalapril and furosemide. Although balloon valvuloplasty was able to decrease the systolic pressure gradient across the pulmonary valve by 58% and although atenolol was added to the chronic therapeutic regimen, lack of owner compliance resulted in recurrent CHF and a high HR persisted at 200/min. It was therefore decided to implant a product in development: a vagus nerve stimulator (CardioFit™, Model 5000, BioControl Medical Ltd., Yehud). The device was implanted under a cervical muscle (along with a rate-sensing intra-ventricular electrode, implanted using fluoroscopic guidance) to chronically stimulate the left vagus nerve. Stimulation current was 1-10 mAmp, pulse width was 1 ms, and maximal stimulation frequency was 15 Hz. Minor adverse effects included a self-limiting cough during the first post-operative day. The ventricular response rate (VRT) was programmed at 140/min and digoxin therapy was discontinued. Heart rate was constantly documented by the implant. In addition, Holter monitoring (x24-45h) was performed 7 times through 9.5 months.

Throughout 291 days the VRT was tightly maintained at 142 ± 21 (range: 83 - 178/min), that differed only by ±14-15% from the programmed target HR.

This is the first attempted clinical use of a minimally-invasive device to constantly control HR during chronic, spontaneously occurring AF, in a client-owned dog. The device was well tolerated over 291 days, and while CHF was not totally eliminated, VRT was tightly maintained within the programmed range. Long term clinical benefits should be tested comparatively in a population-based study. If proven safe and effective, antegrade vagus stimulation may benefit veterinary patients with chronic, symptomatic supra-ventricular tachy-arrhythmia where pharmacotherapy is intolerable, ineffective, impractical, or contraindicated.

CHEMOTHERAPEUTIC TREATMENT OF XENOGRAFT *SPIROCERCA LUPI-ASSOCIATED* SARCOMA IN A MURINE MODEL

Stettner N.[1], Dank G.[2], Ranen E.[2], Lavy E.[2], Aroch I.[2], Harrus S.[2], Brenner O.[1] and Harmelin A[1].

[1] Department of Veterinary Resources, Weizmann Institute of Science, Rehovot, Israel.

[2] Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Israel.

The nematode *Spirocerca lupi* is primarily a parasite of dogs, but other carnivores may be infected. Esophageal granulomas, aortic scars and aneurysms are the most frequent lesions associated with this parasite. Neoplastic transformation of esophageal granulomas to osteosarcomas or fibrosarcomas has been noted in infected dogs.

The therapeutic options of esophageal tumors include surgical excision, chemotherapy and radiation. Esophageal surgery is difficult and is associated with frequent perioperative and postoperative complications. Often the procedure is not feasible due to extensive and/or multiple lesions. Chemotherapy is used as an adjunctive palliative treatment of soft tissue sarcomas following surgical removal, combined with platinum and doxorubicin-based protocols. To date, there was no available data on the effectiveness of chemotherapy in the treatment of spirocercosis-associated esophageal sarcomas, however.

In order to facilitate further studies of its unique pathogenesis and to allow research into improved diagnosis and treatment modalities, we have established a xenograft murine model of *Spirocerca lupi*-associated sarcoma.

Tumor samples were obtained during esophageal surgery from three different dogs admitted to the Hebrew University Veterinary Teaching Hospital, and diagnosed with *S. lupi*-associated sarcoma by detecting typical esophageal masses using endoscopy. Diagnosis was confirmed by histologic examination in all three cases. Three groups of NOD/SCID mice were inoculated with a different source of *S. lupi*-associated sarcoma by subcutaneous transplantation creating three lines of the tumor.

The three tumor lines showed differences in tumor appearance, growth rate, histology and degree of malignancy.

This model presents several advantages for the study of *S. lupi* -associated sarcoma: 1) Esophageal tumors are rare in dogs. A tumor model facilitates further studies by providing a readily available source. 2) Diagnosis of *S. lupi*-associated sarcoma based on endoscopic biopsies is tentative as differentiation between tumor and granuloma may be impossible. In the NOD/SCID model, the lack of the host immune response against the tumor yields a relatively pure population of sarcoma cells that can be used for cytological and cytogenetic analysis. 3) One of the most fascinating aspects of S. *lupi* infection is the induction of neoplastic transformation. The current mouse model reproduces the main features of the natural behavior of canine *S. lupi*-associated sarcoma. Thus it may allow studies on tumor transformation and on differences between this tumor and other sarcoma types. 4) The murine model can be use for further studies of alternative treatments including chemotherapy, radiotherapy and their combination with surgical excision.

In order to define the optimal chemotherapeutic treatment against *S. lupi*-associated sarcoma, we have investigated the effectiveness of four chemotherapeutic drugs, doxil, doxorubicin, carboplatin and cisplatin, using one line of the NOD/SCID xenograft mouse model.

Doxorubicin is the current treatment used against *S. lupi*-associated sarcoma.

Pegylated liposomal doxorubicin (Doxil®) is a liposomal formulation of doxorubicin, believed to act via identical molecular mechanisms. The differences in its efficacy and toxicity are related to altered pharmacokinetics. Cisplatin has shown activity against osteosarcoma in dogs and humans. Cisplatin is nephrotoxic and requires intensive saline diuresis. Carboplatin is a second-generation platinum compound that differs from cisplatin in its pharmacological and toxicity profile but has a similar efficacy.

Samples of xenografted osteosarcoma were inoculated SC into five groups of ten NOD/SCID mice. Tumor-bearing mice were divided into treatment and control groups. The treatment groups were injected with one of the drugs. The control group was injected with buffered saline. Tumor size was determined by caliper measurements twice a week. The mice were observed until tumor size reached an average diameter of 1.5cm.

Compared with the control group, significant inhibition of tumor growth was observed in the pegylated liposomal doxorubicin and the doxorubicin groups but not in the carboplatin and cisplatin groups ($P<0.05$).

Our results indicate that doxorubicin-based drugs are more effective against *S. lupi*-associated sarcomas in a mouse xenograft model. Despite slower growth in the doxil group as compared to the doxorubicin group, this was not statistically significant. The absence of statistical significance may be due to early termination of the experiment because of rapid tumor growth in the control group and the smaller sample size in the doxorubicin group, causing the SD to be relatively high.

Although currently more expensive, Doxil can probably provide a safer and more effective alternative to doxorubicin. Studies in dogs are needed to validate these findings on spontaneously developed tumors and to determine the optimal protocol. Combination of these drugs with surgical excision may improve the prognosis of dogs with this condition.

FECAL HORMONE ANALYSIS TO STUDY BEHAVIOR IN IBEXES, ELANDS AND RHINOCEROSES.

Shore L.

Kimron Veterinary Institute, Bet Dagan, Israel

Fecal hormone analysis, a non-invasive technique, was used to determine its value in behavioral studies of free moving animals. In the ibex, measurement of testosterone concentration was used to demonstrate that testosterone increases the level of aggressive interaction in femaie ibexes in early iactation but decreases aggressive interaction in males during pre-rut. Older females had higher testosterone levels and predominance of male births (1). In male elands, both corticosterone and testosterone increase during pre-rut and the dominant male showed "dominance stress" (2). Corticosterone and testosterone concentrations were measured in male rhinoceroses before and after they were placed in the same sleeping enclosure. The levels of the hormones rose briefly but returned to basal levels within a week indicating there was no long term stress (3).

Fecal steroid analysis can therefore be used to (a) indicate the level of aggressiveness; (b) determine dominance in a herd, and (c) determine the level of animal welfare.

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NEOSPOROSIS IN ISRAELI HORSES

Steinman A.[1], Kligler E. B. [1], Shkap V.[2], Mildenberg Z.[2] and Baneth G[1]

[1]Koret School of Veterinary Medicine, Faculty of Agricultural,
Food and Environmental Quality Sciences, Hebrew University of Jerusalem, Israel,

[2]Kimron Veterinary Institute, Bet Dagan, Israel

Neospora caninum was first identified as a cause of neurological disease in dogs in Norway in the early 1980s. Since then *N. caninum* has been reported as a major cause of disease mainly in cattle, and as a cause of sporadic disease in horses, goats, sheep and dogs. In Israel, *N. caninum* was first detected in aborted bovine fetuses in the mid 1990s. *N. caninum* was already suspected as a cause of equine disease in the early 1990s, however, unlike in cattle, the literature is scarce. Two species, *N. caninum* and *N. hughesi*, have been identified as infecting the horse and were associated with neurological disease and fetal loss, they can not be differentiated serologically, however . The purpose of the study was to determine the prevalence of anti-*Neospora* antibodies in tsraeii horses and to compare it to the prevalence in aborted mares and horses exhibiting neurological disease. Sera were collected from 800 borses and the presence of antibodies to *N. caninum* was determined by immunofluorescence antibody test (IFAT). Sera were also collected from 52 aborted mares and from 40 horses exhibiting neurological signs. A total of 95 (11.9%) of the 800 samples tested were antibody positive for *Neospora*. Significantly higher seropositivity was displayed by horses with neurological signs (21.2%) and from aborted mares (37.5%). There was a significant linear-by-linear association between age and seropositivity.

This is the first study to describe the presence of antibodies to *Neospora* spp. in horses in this region. The prevalence of antibodies to *Neospora* spp. in aborted mares was considerably higher than in asymptomatic horses further supporting the role of *Neospora* spp. in equine abortions. We showed for the first time that the prevalence of antibodies to *Neospora* spp. in a group of horses presenting with neurological signs was significantly higher than its prevalence in a group of asymptomatic horses.

ORAL MICROBIOLOGY IN DOGS AND NOVEL THERAPEUTIC APPROACH

Ezroni G. [1,2], Steinberg D.[2], Gati I.[3], Friedman M.[3] and Lavy E.[1]

[1] Koret School of Veterinary Medicine, the Hebrew University of Jerusalem, Israel

[2] Faculty of Dental Medicine, the Hebrew University of Jerusalem, Israel

[3] School of Pharmacy, the Hebrew University of Jerusalem, Israel

Oral diseases are common bacterial infections found in dogs, resulting as tooth decay, gingivitis, periodontal diseases and bad breath. Those diseases are associated with the presence of salivary and biofilm (plaque) bacteria. Preventive treatments such as tooth brushing would decrease the prevalence of the development of dental diseases in dogs, however compliance to such treatment is very low. A sustained release varnish for companion animals (VSRV), which allows a prolonged release of the active agent, is being developed.

A VSRV composed of an antibacterial agent embedded in a polymeric matrix was prepared. In-vitro antibacterial efficacy of the VSRV was tested in bioassays using *S. mutans* ATCC 27351. In-vivo efficacy of the VSRV, with chlorhexidine (CHX) as an active agent, was tested on a group of 10 Dackel dogs (ages from 2-5 years). VSRV was applied to the buccal areas of their teeth with a swab. Microbial samples from the oral cavity were taken before and after the application. The quantity of oral bacteria was determined by plating the saliva samples on selective agar media (streptococci; anaerobes; volatile sulfur compounds (VSCs) - producing bacteria; total bacterial counts) and enumerating the colonies semi-quantatively.

Preliminary results: VSRV with 2% (w/v) CHX as an antibacterial agent exhibited the best prolonged antimicrobial activity in vitro compared to 2% (w/v) cetylpyridinium-chloride and a placebo formulation. An application of the VSRV with 4% (w/v) CHX on canine dentition significantly reduced oral bacteria: general bacteria, streptococci and anaerobes bacteria during time ($\Delta_{\text{before-after 2 days application}}$=39%, 18%, 9% respectively). However, this VSRV had no effect on VSCs-producing bacteria, an indicator for halitosis.

In conclusion, canine health care by owners is a substantial issue nowadays. A VSRV containing an antibacterial agent (CHX) is a novel and simple method to maintain dental hygiene and prevent the development of caries, gingivitis and canine periodontal diseases.

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CLEAVAGE OF AKABANE VIRUS (AKAV) S SEGMENT GENOME IN THE BRAINS OF INFECTED FETUSES

Stram Y.[1], Levin A.[2], L. Kuznetzova[1], Brenner J.[1], Braverman Y.[1], Yadin H.[1], and Rubinstein-Guini M.[1]

[1] Kimron Veterinary Institute, P.O. Box 12 Beit Dagan, 50250

[2] Molecular Virology Department, Faculty of Medicine, Hebrew University of Jerusalem

Since 2002 there has been an increase in arthrogryposis/ hydranencephaly (AGH) incidence in Israel, caused by Akabane (AKA) and possibly by Aino (AIN) viruses. In response to the outbreak, serological, molecular-diagnostic and research tools were developed. AKAV sequences were detected by real time RT PCR in the brain tissue of 2 out of 20 tested calves and lambs that suffered from hydranencephaly.

When the S segments from the two infected calves were characterized it was concluded that the S genome were cleaved. To localize the cleavage site, the 3' segment of the S genome was cloned, sequenced and shown to be 430 bases long, which indicated a cleavage site between nucleotides 430 and 431 of the S segment in the antigenome.

This cleavage site was found to be specific and not a result of any degradation processes. Analysis of the S segment RNA secondary structure revealed that the cleavage site was located on a loop structure. Furthermore, flanking the cleavage site were stretches of 7 or 8 bases that were part of a stem with low free energy. This could stabilize the loop making it accessible to an, as yet, uncharacterized cleavage mechanism.

MOLECULAR SURVEY OF HEMOTROPHIC MYCOPLASMA SPECIES IN ISRAELI CATS

Witman O.[1], Harrus S.[1], Eyal O.[1], Bar Gal G.[1], Tasker S.[2] and Baneth G.[1]

[1] Koret School of Veterinary Medicine, Hebrew University of Jerusalem, Rehovot 76100, Israel.

[2] Department of Clinical Veterinary Science, University of Bristol, Langford, Bristol BS40, 5DU, U.K

Hemotrophic mycoplasmas (previously *Haemobartonella*) are the causal agents of feline infectious anemia. They have recently been reclassified as *Mycoplasma* spp. based on genetic analyses. The use of PCR and nucleotide sequencing has led to the definition of new hemotrophic *Mycoplasma* candidate spp. and to the finding of significant differences in their genetic structure. Three spp. or candidate spp. have been recognized in cats: *Mycoplasma haemofelis,* Candidatus *Mycoplasma haemominutum* and Candidatus *Mycoplasma turicensis.*

Several studies have attempted to distinguish the clinical and epidemiological characteristics of each infection in cats. This is the first survey of feline hemotrophic *Mycoplasma* spp. conducted in Israel using PCR. The goals of this study were to evaluate the prevalence and identity of the hemotrophic *Mycoplasma* in Israeli cats and to compare their prevalence and spp. distribution in different cat populations.

Blood samples were collected from 55 cats originating from different sources including the Veterinary Teaching Hospital in Bet Dagan, a blood bank and stray cats. PCR was performed using universal *Mycoplasma* primers. Feline immunodeficiency virus (FIV) detection was also performed by PCR. All PCR products (*Mycoplasma* and FIV) were sequenced to achieve identification at the species level.

A 54.5% prevalence rate of hemotrophic mycoplasmas was found among all samples evaluated (n=55). The three internationally known hemotrophic *Mycoplasma* spp. were found and Candidatus *Mycoplasma turicensis* was identified for the first time in Israel. The samples were divided into distinct sub-groups according to their origin, gender, age and their FIV status.

Significant differences ($p<0.005$) were found among the hemotrophic *Mycoplasma* spp. prevalent in cats from different origins. In cats from the veterinary hospital, blood bank and in FIV positive cats, the most prevalent sp. was Candidatus *Mycoplasma haemominutum*, while in stray cats and FIV-negative cats, Candidatus *Mycoplasma turicensis* was the most prevalent sp.

We conclude that the prevalence of hemotrophic *Mycoplasma* infection in Israeli cats appears to be high. The three known hemotrophic *Mycoplasma* spp. that infect cats are present in Israel and their prevalence apparently differs within distinct populations of cats.

BACTERIAL SYMBIONT IN *SPIROCERCA LUPI*

Gottlieb Y.[1], Kaufman M.[2], Markovics A.[3], Samish M.[3], Aroch I.[2], Ghanim M.[1], and Lavy E.[2].

[1] Department of Entomology, Agricultural Research Organization, Volcani Center,

[2]Koret School of Veterinary Medicine, Hebrew University of Jerusalem,

[3]Department of Parasitology, Kimron Veterinary Institute.

Symbiotic bacteria of arthropods and nematodes significantly influence various biological aspects of their hosts. For example, the intracellular bacteria of the genus *Wolbachia* is essential for its filarial nematode host's reproduction and may be involved in filarial diseases. Spirocercosis disease agent is the canine esophageal worm. This spirurid nematode, *Spirocerca lupi*, is transmitted by a coleopteran intermediate host, *Onthophagus sellatus*, to the final canine host. Neither *S. lupi* nor *O. Sellatus* has ever been tested for presence of bacterial symbionts. In order to find a better means to diagnose and control the canine spirocercosis, the presence of symbionts in the nematode and the beetle was assessed. Using molecular methods a novel symbiont has been detected in *S. lupi* which is closely related to *Comamonas* spp. (Brukholderiales: Comamonadaceae) of the β-proteobacteria. This bacterium appeared to be located in the gut epithelial cells of the nematode larvae. No other specific symbionts were found either in *O. sellatus* or in *S. lupi*.

Diagnosis of spirocercosis in the early stages can be challenging and most animals are diagnosed only in the advanced stage of the disease, once granulomas are already present in the esophagus. Finding a stable infection of symbiont in *S. lupi* has implications in further understanding the pathogenesis of spircocercosis. Moreover, resolution of the complex interactions among the different organisms involved in this system may eventually lead to novel and simple methods for diagnosis, prevention and treatment of this disease.

AVIAN REOVIRUS AS THE CAUSE OF "TENDON CROSS SYNDROME"

Lublin A.[1], Rosenbluth E.[1], Meir R.[1], Perk S.[1] and Perl S.[2]

[1] Division of Avian and Fish Diseases,

[2] Division of Pathology, Kimron Veterinary Institute, POB 12, Bet Dagan

Reovirus is the cause of viral arthritis of chickens especially the meat-type breeds. The disease appears between 4-16 weeks of age as swollen leg joints (especially the hock joint) and lameness.

This experiment is a part of a research project intended to clarify the association between reovirus and "tendon cross syndrome" that sometimes appears at or about the time of slaughter. We have attempted to follow the distribution of reovirus in the tissues after experimental inoculation of 1-day-old broiler chicks.

The tested hypothesis: The virus infects the chick at age of 1-day either by vertical transmission from the mother or by horizontal infection in the poultry house. It then becomes "hidden" somewhere in the body (in the lymphoid or other organs) during most of the growing period. Its levels increase with the disappearance of maternal antibodies as the birds mature, resulting in arthritis by the age of 4-5 weeks of age or as the tendon cross syndrome at slaughter at 6-7 weeks.

First, virulence for embryos and virus titers were determined for 5 isolates by Reed and Muench. Each isolate (allantoic fluid) was injected in 25 SPF fertile chicken eggs (5-7 days, in yolk-sac) in 10^{-1} - 10^{-5} virus dilutions, with daily examination of embryo mortality up to 10 days post-inoculation. No mortality was recorded in eggs inoculated with control allantoic fluids free of virus. The virus titers obtained were between $10^{4.4}ELD_{50}$/ml and $10^{7}ELD_{50}$/ml.

The inoculation experiment: was performed in 1-day-old broiler chicks (about 50 per group) with the isolate giving the highest titer: A). Foot-pad inoculation of 0.1 ml (FOOT-PAD MODEL). B). Direct insertion into crop (0.2 ml). C). Control group – allantoic fluid free of virus. The birds were examined daily for clinical signs. Starting at 2 days following administration and at another five time-points up to slaughter at 7 weeks, the birds were weighed, monitored for cloacal temperature, and venous blood was taken for virus titer (using ELISA) and fresh droppings collected for shedding of virus. In addition, organs were assayed for virus by inoculating fertile eggs and also by PCR of the allantoic fluids of dead and live embryos, and by histopathology. The organs sampled were: tendon cross, hock joint, foot-pad, bone marrow, spleen, liver, bursa of Fabricius, thymus, heart, lungs, kidneys, crop, stomach, duodenum, jejunum, colon, pancreas, cecal tonsils, testes/ovary. In addition, cloacal swabs and whole blood with anticoagulant EDTA were taken to determine whether the virus was shed in the droppings and viremia. The success of infection was confirmed by a significant increase of antibody titer in the infected groups compared with the control group in parallel with the disappearance of maternal antibodies at 2-3 weeks towards a peak at 6 weeks of age. In the foot-pad inoculation group, an incidence of 87% of the birds presented a tear in the tendons of one or both legs at age of 7 weeks, in comparison with 32% in the crop-inoculated and only 5% in the control groups

The PCR system included use of primers to conserved regions of the viral genome. In the foot-pad inoculated group the virus was diagnosed by PCR (following embryo isolation) within two days post-inoculation in the spleen and cross tendon. Thereafter, other organs became virus positive: lungs and kidneys at 5 days, thymus and bursa at 8 days, bone marrow and cecal tonsils at 13 days, and hock joint at 29 days post-inoculation. In the crop-inoculated group, the virus was detected during the whole period of the trial (between 2 days and 49 days) in the cross - tendon but not in the other organs. In the control group the virus could not be found in organs. Histopathological examination revealed tendinitis and tenosynovitis in the foot-pad inoculated group commencing at 2 days after inoculation, and in the crop inoculated group at 3 weeks post- inoculation.

Conclusions:

1. Reovirus is the cause of cross tendon syndrome in broilers.
2. In chicks that become infected by reovirus at hatching or already hatch carrying the virus, it can be found throughout the entire growth cycle (until the age of 7 weeks).
3. Reovirus populates the cross tendon at the time of infection and apparently persists there throughout the growth cycle.
4. Reovirus has affinity for lymphatic organs including the spleen, bone marrow, thymus, cecal tonsils, and also for lungs and kidneys.

RABIES DIAGNOSIS IN A 5 YEAR 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.

THE GRAY WOLF

The wolf is the direct ancestor of the domestic dog. Usually, packs of wolves hunt cooperatively. The hierarchy in the pack is very clear, and the alpha couple is usually the sole couple which breeds in the pack. Females in any given wolf population typically weigh less than their male counterparts. The mating ritual is repeated many times throughout the female's brief ovulation period, which occurs once per year, and differs from female dogs, with estrus usually occuring twice a year. The gestation period lasts about 63 days and the pups are born blind, deaf, and completely dependent on their mother. Wolves reach sexual maturity after two years. They live between 6-9 years in nature and can reach 16 years old in captivity. In Israel a few hundred wolves live in the wild, mostly on the Golan Heights or in the desert in the south of the country. The wolves of southern Israel are smaller than those in the north. The photographs show young females from the Negev desert (southern Israel).