

## INVITED LECTURES

### Canine Leishmaniasis in Israel

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Canine leishmaniasis is a zoonotic vector-borne disease caused by protozoa of the genus *Leishmania* and transmitted by phlebotomine sand flies, which affects dogs from all continents except Oceania. The most important aetiological agent of canine leishmaniasis is *Leishmania infantum*, which also causes visceral and cutaneous disease in humans in some countries of Europe, the Middle East, Central Asia, Africa, and South America. Canine leishmaniasis was reported in central Israel in 1994. Since then, infection has also been detected in wild canines including jackals and foxes. Furthermore, infection with *Leishmania tropica*, a causative agent of human cutaneous leishmaniasis, has been detected in 2 dogs and also in jackals and foxes in Israel. A database of the canine cases of visceral leishmaniasis in Israel tested at the Koret School of Veterinary Medicine from 1994 to 2015 includes 391 dogs. These include dogs from central and northern Israel, but none from the arid Southern Israel, which is apparently not endemic for this disease. The number of infected dogs is probably underestimated, since not all dogs with clinical signs compatible with leishmaniasis are brought for diagnosis, and because dogs can carry infection sub-clinically. A clear overlap can be found between the regions in which canine and human visceral leishmaniasis are found, however, no clinical human cases have been found in some of the most active rural canine foci of the disease such as Nataf in central Israel and Klil in northern Israel.

### Updates in Small Animal Cardiopulmonary Resuscitation

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The goals of cardiopulmonary resuscitation are to preserve oxygen delivery to the heart and brain, while attempting to restore spontaneous cardiac function. Unfortunately, the survival of cardiopulmonary resuscitation (CPR) survival in veterinary medicine remains poor. Formerly, veterinary CPR was adopted from human medicine recommendations, and was influenced by the individual personal preference of different institutions, with no guidelines. However, in 2012 the Reassessment Campaign on Veterinary Resuscitation (RECOVER) guidelines were published as an official statement of the American College of Small Animal Emergency and Critical Care. This consensus statement is based on clinical and experimental studies conducted in animals, retrospective studies, and extrapolation of relevant recommendations from human CPR, while trying, as much as possible, to rely on evidence-based data. The goals of RECOVER guidelines and consensus statement are to improve veterinary CPR quality, identify existing knowledge gaps and create uniformity in CPR performance. This assembly of information emphasizes the importance of quick recognition of apneic, unresponsive animals, and immediate initiation of high-quality cardiac compressions. Other main guidelines include using uninterrupted

resuscitation cycles, avoiding hyperventilation, updated usage of medications and defibrillation, in particular, adrenaline and vasopressin, monitoring CPR by capnography, and recognition of post-cardiac arrest care as an integral phase of resuscitation, by using intensive systemic support after restoration of spontaneous cardiac function. Most of these guidelines are summarized by two algorithms, one for basic and advanced life support, and the other for post-arrest care.

## ABSTRACTS: SMALL ANIMALS AND WILD ANIMALS

### The Effect of Naturally Occurring Chronic Kidney Disease on the Micro-Structural and Mechanical Properties of Bone in Dogs

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Chronic kidney disease (CKD) is common in dogs. One of its inevitable consequences is secondary renal hyperparathyroidism, resulting in persistently high parathyroid hormone (PTH) concentration, which promotes bone resorption by activating osteoclasts in presence of osteoblasts. In humans, bone resorption, osteopenia, and increased risk of fractures are well documented, but the effect of CKD on bone quality has not been studied in dogs. The aim of this study was to investigate the effect of naturally occurring CKD on bone morphometry and mechanical properties. Femora of nine dogs with CKD and nine age, sex and body weight matched dogs were harvested after death or euthanasia. Morphometric properties were assessed using light microscopy and Micro CT. Young's modulus was assessed using the 4-point bending test. Lacunae were significantly smaller in the CKD group, with no group difference in Haversian canal and resorption cavity size. Resorption cavity density [10 (8-14) vs. 7 (4-9) mm<sup>-2</sup> in the CKD and control, respectively,  $P=0.001$ ] and overall porosity (2.3-fold higher in the CKD) were significantly higher in the CKD group. There was no group difference in Young's moduli. In conclusion, CKD in dogs is associated with deterioration in bone quality, which is not manifested clinically.

### Therapeutic Soft Contact Lenses May Alter the Efficacy of Topically Administered Ocular Drugs

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Therapeutic soft contact lenses (TSCLs) are often used in veterinary medicine to protect and support the cornea in cases of corneal trauma and disease. However, TSCLs are usually used as adjunct therapy, and are not meant to replace medical treatment, as the diseased eyes still need to be medicated topically. Yet the effectiveness of topical ophthalmic medications administered to a TSCL-covered eye, rather than directly to the bare eye, has never been studied. This led us to examine the efficacy of two topical ocular drugs with a quantifiable effect, 0.5% tropicamide (mydramide) and 0.005% latanoprost (glautan), in TSCLs covered and uncovered eyes of six Labrador retriever dogs. Pupil diameter (PD) and intra-ocular pressure (IOP) values were measured at time 0 and later hourly for 8 h, and the mydriasis or intraocular hypotension caused by tropicamide and

latanoprost respectively were examined, first with medication delivered to uncovered eyes, and after a one week interval, to eyes fitted with a TSCL. Results were statistically analyzed using the Wilcoxon paired signed ranks non-parametric test, as well as with repeated measures ANOVA, with the Greenhouse-Geisser correction, for simultaneous assessment of the effect of time, TSCL and drug. Tropicamide treatment resulted in significant mydriasis compared to baseline values ( $P = 0.005$ ) in both the TSCL-covered and -uncovered eyes. There were no significant differences in PD between experimental groups ( $P = 0.73$ ), though a slower onset of mydriasis, with a milder decline in tropicamide effect, was observed in TSCL-covered eyes ( $P = 0.018$ ). However latanoprost did not produce a hypotensive effect when applied to TSCL-covered eyes, compared to the IOP drop observed when administered to uncovered eyes ( $P = 0.015$ ). Nor was there a difference in IOP of TSCL-covered eyes with and without latanoprost treatment ( $P = 0.7$ ). Thus, it seems that the presence of TSCLs blocked the therapeutic effect of latanoprost, but not of tropicamide. We can conclude from this study that TSCLs have the potential to act as a selective barrier to topical ophthalmic drugs, allowing or preventing their absorption into the eye, as well as retaining it, and hence affecting their therapeutic properties. These findings may influence topical ocular treatment given to dogs fitted with TSCLs.

## Multidrug-Resistant *Staphylococcus pseudintermedius* in Israel

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*Staphylococcus pseudintermedius* is a major opportunistic pathogen of dogs and cats. It may be found colonizing various parts of the skin, such as the perineal area, nose, mouth and groin, occasionally causing infections of these sites and other ones. Over the last decade, methicillin-resistant *S. pseudintermedius* strains, shown also to be multidrug resistant (MDR) to a number of other antibacterial drugs, have been increasingly reported worldwide. Here, we describe the emergence of MDR *S. pseudintermedius* (MDRSP) in Israel, which was phenotypically characterized by a resistance profile to ten or eleven of eleven antibacterial drugs routinely tested in the Laboratory of Clinical Bacteriology and Mycology, the Kimron Veterinary Institute. Following the first MDRSP isolation in 2004, rates of its isolation increased to 29% in 2012 and 25% in 2013, and have been decreasing since. Bimodal distribution of resistance rates indicates the evolution of two distinct *S. pseudintermedius* populations over the last decade. Isolation of MDRSP from imported puppies upon arrival and molecular typing of representative imported and Israeli isolates suggest a route of introduction of MDRSP in Israel.

## Nuisances and Welfare of Free-Roaming Cats in Urban Settings and Their Association with Cat Reproduction in Israel

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Free roaming cats (FRC) are abundant in cities worldwide. Increasing populations of such cats might result in impairment of cat welfare, and cause nuisance and public health risks. In order to study the seasonal dynamics of FRC populations and its association with events of cat welfare impairment and nuisance, we analyzed a database of FRC-associated citizens' telephone complaint events, which were registered in five cities in Israel (total human

population of 1.42 million residents) during years 2007 to 2011. These complaint events were classified to the following six categories: cat carcasses, kittens, parturition, aggressive behavior toward people, invasion to human facilities, and cat injuries and distress. Overall, 87,764 complaint events associated with these categories were registered in the five cities during the study period (123.2 complaint events per 10,000 citizens per year). Length of daylight was moderately correlated with the rate of complaints of kittens in the same month ( $r = 0.64$ ) and parturition in the previous month ( $r = 0.54$ ) ( $P < 0.001$ ). Both kitten- and parturition-related complaints showed a prominent seasonal pattern, peaking in April and May, respectively, and declining gradually until November. 'Kittens' or 'parturition' were explicitly mentioned in 38%, 39% and 19% of the complaints, regarding cat aggressiveness toward people, cat invasion to human facilities and cat injuries and distress, respectively. In most of the cities, the rate of citizen complaints regarding carcasses, aggression, invasion and injuries were still significantly correlated with the rate of complaints regarding kittens after omission of these joint complaints, and remained significant after controlling for seasonality. These findings imply an association of cat welfare impairment and nuisances with FRC reproduction intensity. The current study revealed the high rate of nuisance and potential public health hazards related to FRC, as well as impairment of cat welfare, which might be merely be 'the tip of the iceberg' of the real welfare situation of these cats. Further studies should examine the effectiveness of FRC population control strategies for the reducing the rate of FRC-related nuisances and public health risks, as well as for improving FRC welfare.

## Evaluation of Five Medetomidine-Midazolam-Based Anesthetic Combinations in Egyptian Fruit Bats (*Rousettus Aegyptiacus*)

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The objectives of this study were to evaluate and compare the anesthesia and recovery induced with medetomidine-midazolam-based combinations in Egyptian fruit bats (*Rousettus aegyptiacus*). Eight bats were randomly assigned by a crossover design to an anesthetic combination: medetomidine-midazolam-saline (MM-Sal), medetomidine-midazolam-ketamine (MM-Ket), medetomidine-midazolam-fentanyl (MM-Fen), medetomidine-midazolam-morphine (MM-Mor) or medetomidine-midazolam-butorphanol (MM-But), all administered subcutaneously. Each bat was studied five times with a  $\geq 10$ -day washout period. Time to recumbency and recovery were recorded. The anesthetic depth, heart rate (HR), respiratory rate (RR), and rectal temperature (RT) were monitored at baseline and every 10 minutes until bats recovered spontaneously (i.e., flying). Atipamezole was administered if bats did not recover within 3 hours. Mean induction times were similar with all regimens (range 7-11.5 minutes). All combinations produced anesthesia (following a short period of twitching), with significantly decreased HR (from 400 bpm to 200 bpm), and RR (from 140/min to 36-65/min). Opioid-based combinations resulted in significantly lower RR compared to MM-Sal and MM-Ket. Time to first movement was significantly longer in MM-But and MM-Ket (99 min) compared to MM-Sal (69 min). Mean recovery time was significantly longer in the MM-But (159 min) compared to other treatments, and significantly shorter in the MM-Sal (88 min) compared to other treatments. Atipamezole was administered to 4 bats, all from the MM-But treatment. In conclusion, all five combinations can be used to anesthetize Egyptian fruit bats, however, cardiorespiratory depression occurs. MM-Ket and MM-But produce longer anesthesia. Reversal of the MM-But combination is recommended.

## Messi: A Modified Equine Surgical Shock Index for Prognosis of Colic in Horses

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Shock is a common symptom in horses with colic, mainly due to endotoxemia. It is negatively associated with survival, and is an important aspect in their medical management. Shock index (SI), the ratio between heart rate (HR) and systolic blood pressure, has been shown to be an indicator of shock severity in several species. The aim of this study was to evaluate whether a modified SI, the ratio between HR and mean arterial pressure (MAP), measured during equine colic surgery, may serve as a prognostic indicator for outcome. Surgical records of horses that underwent colic surgery between January 2011 and December 2103 with a complete anesthetic record were evaluated retrospectively. The Modified Equine Surgical Shock Index (MESSI) was calculated as HR/MAP. MESSI measured at the first MAP measurement (fMESSI) and the maximal ratio calculated during surgery (mMESSI), were compared between survivor and non-survivor horses. ROC analysis was used to establish the area under the curve (AUC), sensitivity and specificity of both MESSIs as outcome predictors. Of 125 horses studied, 76.8% survived to discharge. fMESSI and mMESSI were significantly higher in non-survivors compared to horses surviving to discharge ( $P = 0.01$ ;  $P = 0.008$ , respectively). The AUC for mMESSI as a predictor of a negative outcome was 0.731 (95% confidence interval 0.616-0.845). mMESSI above 1.0 had a specificity of 99% ,and a positive likelihood of 19.86-fold for non-survival. Horses with mMESSI>1.11 had 100% mortality rate. In conclusion, MESSI may be a valuable prognostic tool for horses undergoing colic surgery.

## Comparison of Three Acute Colic Pain Scales

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A valid, reliable and usable scale is needed for assessing the severity of acute pain in equine colic. The aim of this study was to compare three scales: the six-point Equine Acute Abdominal Pain Scale (EAAPS), a four-point scale described by Mair and Smith (M&S), and a six-point numerical rating scale (NRS). Forty short films of horses, 35 colic cases and 5 control horses, were randomly presented to 46 equine clinicians through a website. Randomly allocated into three groups, participants used one of the three scales. Five films, randomly selected, were shown twice for intra-observer reliability. Speed, ease of use and face validity of the scales were evaluated. Response rates were 89% (16/18) for EAAPS and 100% for both M&S (18/18) and NRS (10/10). The intraclass correlations (ICC) of the EAAPS [0.87; 95% confidence interval (95% CI): 0.80-0.92] was significantly higher compared to M&S and NRS (0.68, 0.71, respectively), demonstrating better inter-observer reliability. The kappa for the intra-observer reliability of the EAAPS was 0.95 (95%CI: 0.92-0.98) compared to 0.77 for the other two scales. Convergent, extreme group and predictive validities were similar for all three



scales. The usability showed no significant difference between the three scales with respect to the time taken to score the films (speed) or their ease of use. However, the face validity of the M&S scale was significantly better than the EAAPS scale. In conclusion, the EAAPS showed superior reliability, the M&S scale showed better face validity, while usability and other validity tests were comparable among scales.

## West Nile Virus in Equids in Israel and the Palestinian Authority – From Prevalence to Incidence

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West Nile Virus (WNV) is an endemic disease in Israel and the Palestinian Authority (PA). Clinical manifestations include flu-like syndrome or neuroinvasive disease. Horses are the animal species most clinically affected with WNV, and used as sentinels in surveillance programs. The aims of this study were to evaluate the prevalence of WNV in horses and donkeys in Israel and the PA, and to evaluate its yearly incidence in horses by comparing carriage at the beginning and at the end of the mosquito season. Equine serum samples were obtained from 185 horses in Israel, and 66 in the PA during July–September 2014. All horses in Israel and 26 in the PA were re-sampled during November–December 2014. Serums samples were obtained from 44 donkeys in Israel and 44 in the PA between August 2014 and May 2015. Exposure to WNV was estimated using commercial cELISA that detects specific IgG antibodies. The overall prevalence of WNV in horses was 80%. In Israel the prevalence was 85%, none seroconverted. In the PA the prevalence was 65%, and 3 horses seroconverted, which indicates an incidence of 33%. The overall prevalence in donkeys was 44%, 61% in Israel and 27% in the PA. Risk factors associated with increased prevalence were the farm ( $P < 0.001$ ), Israel versus PA ( $OR = 4.11$ ,  $P < 0.001$ ), horses over donkeys ( $OR = 4.92$ ,  $P < 0.001$ ), and age ( $P < 0.001$ ). WNV prevalence in the PA is lower and its incidence is higher than in Israel. Donkeys have lower carriage rates than horses and may serve as better sentinels than horses in Israel.

## Phylogenetic Characterization of *Theileria equi* Species Based on the 18S Ribosomal RNA Gene in Horses in Israel and Neighboring Countries

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Equine Piroplasmosis (EP) is a tick-borne disease caused by the apicomplexan hemoprotozoa *Theileria equi* and *Babesia caballi*. It is transmitted by ixodid ticks of the genera *Rhipicephalus*, *Hyalomma* and *Dermacentor*. Manifestations of the acute disease include severe anemia, hemoglobinuria, jaundice and edema. The disease may be fatal. Untreated infection with *T. equi* is considered to be life-longed, therefore, sub-clinically and chronically infected horses may serve as reservoirs. EP is endemic in tropical and subtropical areas worldwide, including

Israel. The purpose of this study was to perform a phylogenetic analysis of the 18S rRNA gene of *T. equi* due to its low substitution rate, high conservation and occurrence of multiple copies, in order to better understand the epidemiology of piroplasmiasis in our region. As part of an ongoing molecular survey, horses from farms in Israel, the Palestinian Authority (PA), Jordan and Turkey were sampled. A total of 403 blood samples were screened for *T. equi* using PCR. The entire 18S ribosomal RNA gene (~1600bp) was amplified and sequenced, from 21 preselected samples from northern, central and southern Israel and neighboring countries. Samples were aligned and compared with previously published sequences from public sequence databases. Phylogenetic analyses by the three algorithms yielded topology-similar trees, revealing three distinct previously established clades of the parasite in Israel (A, C, D). Surprisingly, no association between genotype and geographical distribution was found. Conversely, there was a high similarity within clade D between sequences from Israel and the PA and previously published sequences from Sudan (GenBank accession number: AB515311, AB515312). Clade C contained three sequences from Israel, together with sequences from the USA (JQ390047), South Africa (EU888903, EU642511) and Brazil (KJ573372), while the clade A revealed similarities between the Israeli and Jordanian sequences, and sequences from Spain (AY150062), USA (JX177671, JX177672, JX177673) and South Africa (EU888906, Z15105). In conclusion, this is the most comprehensive phylogenetic analysis of the 18S ribosomal RNA gene of *T. equi* done in Israel, and the first full genotype sequencing. The genetic heterogeneity within the *T. equi* sequences found in Israel and neighboring countries may play a distinctive role in developing diagnostic assays for EP, and therefore, may contribute to the effort of monitoring the spread of the parasite. Additionally, phylogenetic analysis, as demonstrated herein, may help to better understand the epidemiology of piroplasmiasis.

## Equine Botulism in Israel

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Equine botulism is clinically defined as a gradually progressive myasthenia and is associated with a high mortality rate. It occurs by ingestion of botulinum toxin or *Clostridium botulinum* spores or wounds contamination with the bacterium. Clinical signs are insufficient for a definitive diagnosis. Hence, laboratory confirmation is essential. Preventing exposure, immunization and early aggressive treatment including administration of antitoxin may decrease mortality. In 2015, an outbreak of type-D botulism involved 12 horses in an equine farm in Israel. All animals developed progressive weakness, ataxia and, decreased tongue and tail tonus, which are consistent with exposure to *C. botulinum* neurotoxin (BoNT). Supportive care was provided, while antitoxin, which is unavailable in Israel, was not administered. Within nine days post emergence of clinical signs, 10 horses died or were euthanized due to clinical deterioration. A sample of the gastrointestinal content from one of these horses tested positive for preformed type-D BoNT by a mouse bioassay test. Type-D botulism is rarely reported in horses. This is the first report of botulism in horses in Israel. Efforts should be made to improve awareness among horse owners, veterinarians and veterinary authorities. Correct management and availability of antitoxin and vaccines are essential for prevention of future cases.

## RUMINANTS AND PUBLIC HEALTH

## Epidemiology and Risk Factors for Pyelonephritis in Israeli Dairy Cattle

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Urinary tract infections (UTI) in cattle are caused by ascending infections. The disease is relatively rare, with lactation incidence rate (LIR) of 0.5-2%. The bacteria involved are mainly *Escherichia coli* and *Corynebacterium renale*. Urethral catheterization is described as a possible risk factor for UTI in cows. This study was a retrospective case-control study conducted on 3 commercial Israeli dairy herds. Seventy four cows (1.05%) were clinically diagnosed with pyelonephritis out of 7052 cows that calved during the study period. The odds ratio of second and third or more lactation cows to suffer from pyelonephritis compared to 1<sup>st</sup> calf heifers is 2.28 and 3.48 respectively ( $P < 0.001$ ). *E. coli* was isolated from the urine in all cases where bacterial culture was positive. *C. renale* was not isolated in any case of the study population. No recurrences of pyelonephritis were recorded. Pyelonephritis was not associated with increased culling relatively to calving. Most of the pyelonephritis cases were diagnosed near calving, with 40% and 60% within the first month and the first three months, respectively. Urethral catheterization was not associated with pyelonephritis. Urine sampling by catheterization for ketosis diagnosis performed after a soap water scrub of the vulva was not demonstrated a risk factor for pyelonephritis. *E. coli* is the dominant bacterium involved in pyelonephritis in dairy cattle in Israel. The primary empirical antibiotic treatment, pending bacterial culture results, should therefore be chosen accordingly.

## Evaluation of Topical Sustained Release Formulation Containing Chlorhexidine in Prevention of Intra-Mammary Bacterial Infections in Dairy Cows During the Dry Period

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Mastitis is the most economically effecting disease of dairy animals and is caused mostly by pathogenic bacterial infection of the mammary gland. In Israel, "Dry Cow Treatment" (DCT), an antibiotic injection into each quarter of the mammary gland upon entry to the dry period, is the common treatment. Wide use of antibiotic treatment can lead to failure to comply food safety regulations and to bacteria antibiotic resistant in animals and humans. The objective of the study was to evaluate a new antiseptic sustained release formulation, which contains chlorohexidine (CHX) on dairy cows that enter the DCT. In an "*in vitro*" study, immersed mammary tissues with the new formulation were placed on three different bacteria plates: *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus dysgalctiae*. Bacteria grown were found only among mammary tissue that was not treated with the formulation indicating that the new formulation inhibited the bacteria grown. In the next stage, "*in vivo*" experiments took place. Cows' mammary glands were immersed in the formulation 24 hours prior to being



sent to the slaughterhouse. One quarter of the mammary gland was not immersed and served as a control. The tissue was frozen, sliced and placed on agar plates with *Escherichia coli*. A growth inhibition was visible compared to the control plate after 24 hours only in places where the formulation was visible on the tissue. The slices were transferred to new agar plates on day 3 and 6. Inhibition was detected only where the formulation still existed.

## The Impact of the Selective use of an Internal Teat Sealant Material to Improve Udder Health in the Israeli Dairy Herd

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The study was conducted over a 2-year period in a commercial dairy farm (approximately 280 milking cows). The cows that reached the “dry-off day” had individually undergone the following preliminary examinations: milk somatic cell count (SCC), clinical infection events and milk retention events, any injuries and teat sphincter defects, California mastitis test (CMT) and bacterial milk culture for pathogens. Definition of the “status” of the cow in accordance with the findings: “healthy” or “irregular.” Four different treatments were prescribed: no treatment; provision of a teat sealant only; standard “dry-off” antibiotic treatment and a combination of antibiotics and a teat sealant. The study included 282 cows, of which the results of 38 cows were obtained from two successive lactations. On the dry-off day, 90 cows were defined as “healthy” (32%) in accordance with the stringent metrics examined. Most of these were from heifers (75%), and the remainder from adults ( $\geq$  calvings). Among the “healthy” cows, prevention of new infection during the “dry period” and the recovery rates after calving showed similar results in untreated cows compared to those treated only with antibiotics, or only with a teat sealant. The “irregular” group included 192 cows (68%), which received two different dry treatments. Some of these received the commonly used treatment in the region (“Nafbenzal”), while others received in addition a teat sealant (i.e., two injections into the teat). In the “irregular” cows, the integrated treatment of antibiotics and teat sealant increased the chance of preventing new infections 5-fold and increased the chance of recovery 2.4-fold compared with cows treated only with antibiotics. This study suggests making a judgment and choice of alternative treatment for the dry period based on findings and real-time results of each individual herd as of the level of the single udder quarter. The breeder can determine the status of the cow, the udder and the quarter as “healthy” or “irregular” and can then choose the preferred treatment option, including no treatment. This system does require the preliminary collection of historical data of the cow, performing clinical examination of the udder, milk sampling prior to, and after drying out, but, at the same time, it allows selective treatment, a dramatic decrease in antibiotics use (25%), and above all, continued maintenance of udder and public health.

# The Association of Intravenous Glucose Bolus as Treatment for Ketosis and Left Displaced Abomasum in Israeli Dairy Cows

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Postpartum dairy cows present a negative energy balance due to high milk production. Oxaloacetate deficiency prevents utilization of acetyl CoA, leading to increased ketone bodies concentrations and ketosis. Ketosis treatment in Israel includes combinations of propylene glycol, IV glucose and glucocorticoids. The literature mentions that cows diagnosed with ketosis are at higher risk of Left Displaced Abomasum (LDA). This study aims to evaluate the association between ketosis and the risk for LDA, and examine whether IV glucose treatment is linked to increased risk for LDA. A case control study included 70 case cows and 70 control cows. No correlation was found between administration of IV glucose and occurrence of LDA ( $P=0.347$ ); however, there was a distinct association between ketosis severity and the risk for LDA. A retrospective study was performed in 26 dairy farms. 1,350 cases of LDA were reported, of which 462 cases were not diagnosed for ketosis prior to LDA, 654 were diagnosed for ketosis but were not treated with IV glucose prior to occurrence of LDA, and 234 were diagnosed for ketosis and received IV glucose treatment prior to occurrence of LDA. The proportion of LDA was higher in those untreated with IV glucose for ketosis compared to treated cases. Glucose treatment was not a risk factor for LDA ( $P<0.001$ ). In conclusion, there is no evidence of IV glucose treatment inefficiency in ketosis treatment. The low cost of glucose and the lack of association between this treatment with LDA, suggest that this treatment is probably beneficial.

# The Cost of Lameness of Dairy Cows in the Era of Intense Husbandry

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In this era of intensified dairy herd husbandry, the incidence of lameness in dairy cows is increasing. This study investigated the cost of lameness between 2005 and 2007. The study included 14,872 dairy cows from 43 cooperative (Kibbutz) and private herds (consisting of approximately 18% of all Israeli dairy cattle). Data on the direct and indirect costs of cases were provided by the herd managers. The average herd size was 346 cows (range: 50-1000). The average number of lameness cases per herd during the study period was 36, with a lameness index of 10.31%. The direct cost/case comprised of 90% of the total income loss, with decreased milk production being the major cause of income loss (88.4%). During the study period, the average cost per lameness case was 2,409 NIS and the average annual loss per herd was 83,897 NIS (541 and 18,846 USD, respectively). The study demonstrates that the economic loss due to lameness in dairy cows in Israeli dairy herd under intense husbandry, particularly loss due to decreased milk production, is considerable, and that should be controlled under intensive management conditions.

# A Case of Foodborne Listeriosis Associated with Contamination in a Restaurant

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On August 2015, Jerusalem District Health Office team was notified of a pregnant woman with listeriosis (blood culture positive for *Listeria monocytogenes*). The infection was revealed and diagnosed upon reception for delivery. Symptoms, including intermittent high fever, occurred in the week prior to the delivery day. Environmental and epidemiological investigation were initiated, aiming to determine the possible infection (listeriosis) source, and are summarized herein. The patient reported that she ate chicken liver, and poultry meat in a restaurant 10 days prior to the appearance of the initial clinical symptoms. Environmental inspection actions, followed by laboratory analysis of food samples from the restaurant, revealed extremely poor hygiene conditions. Food samples prepared in the restaurant during the inspection included Kebab in Pita bread (ready to eat grilled minced meat in bread), cabbage salad, raw minced meat, humus salad with finely chopped parsley, and tomato salad. *L. monocytogenes* was detected in all food samples, except the tomato salad. *Salmonella enterica* *Montevideo* (group c1) was detected in the Kebab in Pita and raw minced meat. Other pathogens and fecal contamination was detected in all food samples, except the tomato salad. The clinical and food isolates were referred to the Israeli National Listeria Reference Center for laboratory investigation. Three of four *L. monocytogenes* food isolates were found to be closely related genetically to the clinical isolate by Pulse-Field-Gel-Electrophoresis (100 % similarity). Both, the food and clinical isolates were characterized as serotype 4b. However, the fourth isolate (from the kebab in pita) was identified as *L. monocytogenes* 1/2a and was not related to the clinical isolate by molecular PFGE typing. *L. monocytogenes* is a food borne pathogen, and a potential zoonotic agent, which causes life-threatening infections in elderly, immunocompromised patients, and in pregnant women. In pregnancy it may cause fetal loss or a preterm delivery, and the neonate is prone to neonatal sepsis meningitis and mortality. In this case the pregnant woman delivered in her due date by C-section, the newborn was not infected. Control measures were recommended at the restaurant in order to prevent such events.

## POULTRY AND FISH

# Development of a Differential Diagnostic Assay (Diva) Between Vaccine and Wild-Type Turkey Meningoencephalitis Viruses (Tmev)

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The flaviviruses (genus *Flavivirus*) are important pathogens of wild birds, domestic poultry and humans, and several members are zoonotic. TMEV belongs to the mosquito-borne cluster, clade XI and the Ntaya flavivirus, antigenic complex VI. Turkey meningoencephalitis virus (TMEV) causes a neuroparalytic disease of commercial turkeys, expressed by paresis, incoordination, dropping wings and mortality that may reach up to 80% of the

flock. The virus infection is controlled by vaccination with a live attenuated virus. Recently we determined the full viral genomes and polyprotein amino acid sequences of 5 TMEV isolates (one dated from the year 1995 that served for the development of the commercial vaccine, the actual vaccine virus and three isolates from the outbreak of the year 2010). Based on the genomic differences between the isolates we developed the DIVA (Differentiating Infected from Vaccinated Animals) assay, consisting of three real-time amplification systems: 1) a general assay that detects all TMEV strains; 2) an assay that detects only the 1995 strain and the vaccine virus; 3) an assay that detects the 2010 TMEV strain. The assays were evaluated on three types of samples: brains of experimentally-infected turkeys with TMEV strains dated from the years 1995 and 2010, clinical samples collected between the years 1995-2015, and commercial vaccines of two manufacturers. Based on the findings a sustainable DIVA assay was developed, to identify the general and the vaccine TMEV strains, and to neutralize genomic changes that might happen in field viruses.

## Genotypic Characterization of Avian Reovirus Isolates in Israel

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Avian reoviruses are involved in a wide variety of diseases in chickens; Viral Arthritis is the most important in broiler chickens. The disease occurs mainly in broiler chickens and causes swelling of one or both hock joints, and induces acute lameness and reduced growth rate, leading to decreased performance of the birds. "Tendon cross syndrome" with symptoms of rupture of Gastrocnemius muscle tendons was reported to be caused by Avian Reovirus. That syndrome is discovered usually close to slaughter therefore it negatively impacts kosher slaughter of poultry in Israel due to condemnation of the carcasses, leading to heavy economic losses to broiler growers. According to a study carried out in 2010 in Israel, avian reoviruses are divided into four genotypic groups. Group 1 includes the commercial vaccine strain s1133. In recent years we monitored outbreaks of avian reovirus by RT-PCR method and sequencing of outer shell gene sigma C. Since January 2015, 205 samples were tested for avian reovirus in our laboratory in Kimron Veterinary Institute, while 85% of them were positive to the pathogen. Alterations that have been occurred in avian reovirus sigma C gene classified 90% of the isolates to genotype 2 with up to 10-12% difference in comparison to the 2008 isolates. Following these results, we recommend to replace the reovirus isolates in the commercial avian reovirus vaccine.

## Deep Pectoral Myopathy in Broilers in Israel – A Case Report

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Deep Pectoral Myopathy (DPM) is an ischemic necrosis of the *Supracoracoideus* (pectoralis minor) muscles of poultry, first described in adult turkeys (1968), later in adult broiler breeders (1975), and finally in broiler chickens (1980). DPM develops in the deep pectoral muscle (*supracoracoideus* or *pectoralis minor* muscle), mainly because this muscle is surrounded by inelastic fascia and the sternum (Osteofascial compartment = inelastic compartment), which do not allow the muscle mass to swell in response to the physiological changes occurring when muscles are exercised, as in wing flapping. Although this disease was first recognized in adult meat-type turkey and chicken breeders, it is becoming progressively more common in meat-type growing birds, with a

genetic predisposition of large-breasted birds to the disease. DPM occurs exclusively in birds selected for breast muscle development. Its incidence is higher in Ross 508 relative to Cobb 500 lines, suggesting a genetic role as important factor. The lesions do not impair the general health of the birds, and are generally found during cut-up and deboning; they may be both unilateral and bilateral. The fillet should be removed due to organoleptic changes. Breast fillet and tenders are economically the most important muscles of poultry. No public health significance is associated with this pathology. A probable analogy can be drawn between this disease of poultry and the so-called "march gangrene" (anterior tibial syndrome) in humans. An ischemic necrosis of the muscle seen in athletes or military personnel, in the myofascial compartment of the human anterior tibia following increased intra-compartmental pressure, clinically characterized by uncontrollable swelling, isolated muscle cramping and pain that lasts for several hours or days. Surgical treatment is available for acute and chronic compartment syndrome. *The case reported here was revealed* at the processing plant in July 2015 in Broilers in Israel. Broilers of 45 days old - Ross breed. The report describes the pathological features of a bilateral myopathy of the deep pectoral muscles in broilers. This study is the first report of DPM in broiler chickens in Israel.

## A New Subgroup of Velogenic New Castle Disease Virus with a Potential to create the 5<sup>th</sup> Panzootic.

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Newcastle disease can affect more than 250 species of birds. The most sensitive are commercial poultry, while water-fowls and wild birds which can serve as reservoir. The disease is caused by Newcastle Disease Virus (NDV), which belongs to the Paramyxoviruses. Viral incubation period ranges between 5-6 days. Transmission can be made by secretions and fomites. Clinical signs vary according to the virulence of the strain. Mild or unapparent respiratory and digestive signs for low virulence – lentogenic strains, and severe respiratory and digestive signs, nervous signs, drop in egg production and mortality high as 100% for high virulence-velogenic strains (vNDV). The definition of panzootic requires the existence of 3 criteria: 1) emergence of disease into the population 2) presence of a severe disease 3) easy and continues spread. Newcastle disease virus genotypes are present around the world. Viruses from genotypes II, III, IV, were responsible for the first panzootic of the disease during the years 1920-1960. The 2<sup>nd</sup> panzootic, in Europe in the late 1960's originated from viruses of genotype V. Viruses from subgroup VIb originating from the Middle East, were connected to the 3<sup>rd</sup> panzootic in pigeons in the 80'. Genotypes VII and VIII were responsible to outbreaks in Asia including Pakistan and in Europe since 1984. Viruses from these genotypes caused the 4<sup>th</sup> panzootic that spread to Asia, Africa and Europe and were isolated also in South America. The 4<sup>th</sup> panzootic begun in 1985 in south-east Asia and has spread to the rest of the world. Viruses from genotype VII are common in outbreaks around the Middle east, Asia and Africa and are of special concern since they cause a significant illness and death in vaccinated poultry, and some have broaden their host range and can cause disease even in geese. NDV isolates in Israel are classified in genotype VII. The emergence and spread of new genotypes around the world implies that the virus is evolving and changing. In a joint phylogenetic work which purpose was to compare viruses originating from Indonesia and spreading to Pakistan and Israel, it was estimated that we maybe facing the 5<sup>th</sup> panzootic. Virus isolates belonging to a new subgroup within genotype VII are rapidly spreading in Asia and the Middle East. These viruses cause significant outbreaks in poultry and have the characteristics of a panzootic state.



They belong to subgroup VIIi and it seems that their origin is not from genotype VII but rather they relate to viruses diagnosed in the 80' in Indonesia and originated from wild birds. Since 2011 and during 2012 many viruses belonging to this subgroup were isolated from various birds throughout Indonesia, Pakistan and Israel and the number of isolates from this group is increasing. The similarity percentage between isolates from this subgroup is 99% and they are closer to viruses isolated in Indonesia from 1983 and throughout the 1990', than to isolates from genotype VII circulating in these regions in the last years. In addition there are two other new subgroups VIIh and XIIIb which also related to viruses previously isolated in Indonesia, Malaysia, China, and Cambodia, as well as viruses isolated in Iran, Russia, Europe, India, and South America, and related to viruses previously isolated in Pakistan. These data suggests that at least 3 new subgroups that have evolved at the same period from different sources are very close to viruses present at the same time at different geographic regions.

## Outbreak of Avian Influenza (H5N1) in Israel, 2015

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Avian influenza viruses, have been known since the early 20<sup>th</sup> century, however, the avian influenza virus subtype H5N1 was first isolated in the 1950's to 1960's from wild birds in South Africa, and then from birds in China, while during that period, it did not infected humans. In 1996, the virus appeared in the Guangdong province, China, where it killed poultry, and one year later, it infected nearly 20 people in Hong Kong, some of whom died. In years 2003 to 2006 the virus began to spread in Asia, moving also to Europe, Africa and the Middle East, and then captured the public attention as an avian virus dangerous to humans. Its spread was largely attributed to migratory wild birds. The virus is a single-stranded RNA virus of the family *Orthomyxoviridae* with an envelope of glycoprotein branches of Hemagglutinin HA protein responsible for its binding to host cells, and neuraminidase (NA) responsible for viral particle release from cells, and their further dissemination. H5N1 virus is considered very virulent and a highly pathogenic virus. Up to now, it has affected millions of domesticated birds in Asia and the Middle East, spreading the virus through aerosol, nasal and mouth discharge and droppings. In controlled inoculation experiments, within about 48 hours from the infection, the flock mortality rate approaches 100%. Human infection usually occurs by direct contact with infected birds, their secretions, blood, or secretion-contaminated objects. Since 2003, the World Health Organization (WHO) reported over 840 virus-affected people, in 16 countries, with 450 deaths. Possibly, the main exposure route of affected people was during slaughter or with feather plucking of infected birds, bird cleaning and preparation for cooking, consuming raw meat and contact with infected blood of birds. The main concern is that avian influenza virus will mutate, such as by transferring genes from human influenza virus or other influenza viruses, enabling receptor binding alterations, thereby creating a virus that could easily pass between people (pandemic). In suspected birds, tracheal and cloacal swabs from suspected flocks or individual birds are tested. The test method used in the Laboratory of the Avian and Fish Diseases Division (LAFDD), Kimron Veterinary Institute is real-time RT-PCR, for the general MATRIX and HA genes, and in HA (H5 strain)-positive cases, the NA gene is also tested. Thereafter, virus isolation in SPF chicken embryos is performed, with phylogenetic sequence analysis of the isolate and intravenous pathogenicity index test (IVPI) in pullets. In January 16<sup>th</sup> 2015, avian influenza virus subtype H5N1 returned to Israel, after a break since April 2012, when it was detected in turkeys in Shalva and in cats that consumed infected carcasses. Since the first diagnosis of the 2015 outbreak, 10 outbreaks were diagnosed

in Israel, in two waves; the first between January and February 2016, between Binyamina to Netanya, and the second in May, in Betzet, Western Galilee. Premises with focal outbreaks, as well as other close premises, based on risk analysis, were destroyed. In addition, three outbreak foci were diagnosed in the LAFDD from Judea and Samaria region of the Palestinian Authority (January to March 2015). In April 2015, LAFDD began to examine also outbreaks in the Gaza Strip, and until October 2015 almost 40 events were diagnosed, most of which included turkey meat premises, with two events in heavy breeders. In Judea and Samaria, there were some events in layers, and one in turkeys. In the first affected a turkey poultry pen, following evacuation of the birds an avian influenza-infected free-living Spur-winged Lapwing (*Vanellus spinosus*) bird was detected. The events in the Gaza strip included mostly backyard layers, broilers, turkeys, waterfowl and mainly ducks. All isolated viruses belonged to subtype H5N1. Values of IVPI were of a highly pathogenic virus (Mean SD  $2.88 \pm 0.04$ ). Analysis of amino acid sequence of the HA gene cleavage site showed were similar among isolates and compared to previous virus isolates from Israel of yrs. 2011 to 2012, Egyptian poultry isolates of years 2013 to 2015, and West Bank and the Gaza Strip isolates from the current outbreak. A similar picture was found in the NA gene. The genetic distance (% genenucleotide difference) among all these isolates was <0.5%. Conversely, the genetic distance was >5% compared to viruses of previous (2006 to 2012) outbreaks in Israel, and a greater distance from virus isolates from other parts of the world (e.g., Turkey, China, Indonesia and Thailand). Epidemiological outbreaks analysis indicated that the first affected farm was the likely virus infection and spread source in the January to February 2015 outbreak, and that the May 2015 (Betzet) outbreak was probably a separate one. In conclusion, in all the 2015 events of in Israel and the Palestinian Authority were of the same genotype strain (clade 2.2.1). The 2015 H5N1 strain is almost identical to the Egyptian human 2015 isolates and previous bird same region (especially Egypt) isolates.

## Suspected Melamine-Cyanuric Acid Intoxication in Gilt Head Sea Bream

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A brood stock of gilt head sea bream was subdued to a chronic onset of reduced body condition and continuous long-standing low rate mortality. On clinical examination, there was high prevalence of reduced body condition, hyperpigmentation and lethargy. One of the fish was humanely sacrificed and necropsied on the farm, and samples were collected and submitted for histopathologic examination and ancillary tests. No gross lesions were detected during the field necropsy. Microscopically, the main lesions were in the kidney with numerous intratubular crystals with tubular epithelial degeneration and necrosis, along with moderate myeloid hyperplasia and increased melano-macrophagic centers. The crystals were up to 80 micron in diameter, birefringent, brown-green, and varied from fan-shaped to starburst radial spokes arranged in concentric circles. This morphology is consistent with melamine cyanuric acid crystals. A feed sample was submitted for chemical residues analysis. The sample tested borderline positive (approximately 1 PPM) for melamine in its free pathologic form. A test for the cyanuric acid-melamine complex was not performed. As far as is known, this is the first report of suspected melamine cyanuric acid intoxication in gilt head sea bream fish.