

*The 41<sup>st</sup> Symposium of Veterinary Medicine: Animal Welfare*  
*Koret School of Veterinary Medicine,*  
*The Hebrew University of Jerusalem, Israel*

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INVITED LECTURES

## How Management of the Length of Stay of Shelter Animals can Influence the Capacity of an Animal Shelter and Save More Lives

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Shelters are trying their best to get as many animals released alive by returning them to their owners, getting them adopted, relocated or returned to the wild. From the first day of intake into an animal shelter however, the days are counting for a shelter animal. The Length of Stay (LOS) per animal is a crucial factor for its wellbeing, for an increased LOS will negatively affect the physical and behavioral health of the animal. And when animals get ill, their treatment even prolongs their shelter stay while it impairs their welfare.

For health and welfare reasons animals should spend the shortest time possible in animal shelters. The LOS per animal can be influenced by adjustment of the intake and rehoming procedures combined with improvements of preventive health management. A decrease in the LOS can result in a decrease in the daily shelter population, in healthier animals, lower shelter care costs per animal and an increase in the shelter's capacity to save lives.

## Outbreak Management of Infectious Diseases in Animal Shelters

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The principle of offering care and shelter to homeless animals is intrinsically connected with the increased risks of outbreaks of infectious diseases. These risks are especially high in overpopulated shelters. An outbreak affects the health of animals, impairs their welfare and might have lethal implications. It also results in an increased workload with high emotional demands for shelter staff and volunteers, with negative effects on their quality of care. Treatment of sick animals and a temporal closure of the facilities has financial consequences for a shelter.

An effective outbreak management will contain the spread of the infectious disease by using the following measures:

- Diagnosis and isolation of diseased animals.
- Identification and management of exposed/at-risk animals.
- Environmental decontamination.
- Protection of newly admitted animals.
- Documentation.
- Communication with staff, stakeholders, adopters and the public.
- Evaluation and prevention

The evaluation of an outbreak can result in adjustments of shelter procedures and management in order to prevent outbreaks in the future.

## ABSTRACTS

# Rabies Antibody Titer Survey among Post-Rabies Vaccinated Dogs Imported to Israel

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Assessing immunity level of humans and animals is important for preventive medicine research and regulation. A minimum serum level of 0.5 IU/mL rabies neutralizing antibodies (RFFIT) is considered the minimum protective level against rabies. Identifying risk factors for low antibody levels is necessary for debating and understanding the vaccination policy in Israel and worldwide. This policy has public health and animal welfare implications. In our study we have examined serum of 109 dogs imported to Israel, vaccinated at least once against rabies,  $\geq 30$  days prior to the test. The risk factor for test failure (antibody level lower than threshold) was a single vaccination during the dog's life. Background factors, including importation from non-OECD countries and being of pure breed were examined as well. Fisher exact test was used to examine these factors. Of the 109 dogs, 59 have been vaccinated only once against rabies, of which 21 (36%) have failed the test. This was found significant with failing the test ( $P < 0.001$ ). Background factors were not associated with failing the test. In one dog, antibody titer was  $\leq 0.5$  IU/mL even after three consecutive rabies vaccinations. This dog belongs to a group of dogs known as "Non-responders". In conclusion, we found a significant association between low anti-rabies antibody titer and an only single rabies vaccination in dogs imported to Israel. Similar findings were also in a previous survey conducted among dogs examined pre-exportation. The results reinforce a regulatory decision to add a rabies vaccination "booster" for dogs after the first rabies vaccination.

## The Israel Journal of Veterinary Medicine: A Reflection of Israel Veterinarians' Achievements

**Waner, T.**

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The Israel Veterinary Medical Association was founded in 1922 and the journal was first launched in 1939. The journal is peer reviewed and its scope covers the Middle East and the Mediterranean Basin. The website of the journal ([www.ijvm.org.il](http://www.ijvm.org.il)) is included in Google's search allowing easy access to the articles. The number of monthly visits to our website in June, July and August 2018 was 279, 233 and 302, respectively. When I took over as editor 9 years ago, the number of citations per year was only 2. Over the years with challenging situations, the number of annual citations steadily increased and over 2017 reached 178. The journal now ranks 117 out of 140 veterinary journal. Also the IJVM is included in the libraries of the VIN (Veterinary Information Network) and the IVIS (International Veterinary Information Service). The veterinary school and the surge of veterinary medicine in Israel is a relatively recent episode. Proudly we are all in some ways the pioneers in the growth of veterinary medicine in Israel. Everyone can make an historic contribution during this momentous epoch by documenting their achievements. Writing articles is also a learning experience enhancing knowledge and recording what one has personally achieved in our science. I encourage all to write and record experiences be they case reports, research articles or reviews. At the same time IJVM offers the opportunity and the added benefit of enriching the name of Israel as a leader in the field of veterinary medicine.

## Carriage of Extended Spectrum $\beta$ -Lactamase and AmpC-Producing *Enterobacteriaceae* (ESBL/AmpC-E) in Petting Zoos in Israel: A Zoonotic Hazard?

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Extended-spectrum beta-lactamases and AmpC-producing *Enterobacteriaceae* (ESBL/AmpC-E) have become a great concern in human and veterinary medicine. We aimed to investigate the prevalence, molecular epidemiology and risk factors for ESBL/AmpC-E carriage in petting zoos. A prospective cross-sectional study (December 2016-May 2018) was performed in eight petting zoos. Fecal, skin, fur and feather samples were enriched, plated onto CHROMagarESBL and sub-cultured. ESBL/AmpC-production was determined according to CLSI standards. Bacterial species identification and antibiotic susceptibility profiles were assessed using Vitek-2. Bacterial sequence types were determined using Multi-Locus Sequence Typing. ESBL and AmpC genes (CTX-M, SHV, TEM, CMY-2) were identified via PCR and sequencing. Owners' questionnaires were reviewed for risk factor analysis. ESBL/AmpC-E carriage rate was 12% (28/228), with 35 recovered bacteria: 77% from fecal samples (27/35) and 23% from skin/fur/feathers (8/35). The most common bacterial species included *Enterobacter* spp. (55%), *Escherichia coli* (31%) and *Citrobacter* spp. (14%). The main ESBL gene was CTX-M-1

group (17%), and 20% of the AmpC-E were CMY-2-positive. MLST revealed eight different *E. cloacae* sequence types and six *E. coli* sequence types, including ST656 and ST127, of ETEC and UPEC, respectively, posing a zoonotic potential. In a univariate analysis, carriage was associated with antibiotic therapy ( $P = 0.038$  and  $P = 0.011$  for fecal and skin/fur/feather samples, respectively) and with petting permission policy ( $P = 0.023$ ). In a logistic regression model, antimicrobial therapy was a risk factor (OR, 7.34, 95% CI 1.88–28.564). Our findings demonstrate the diverse potential ESBL/AmpC-E reservoir in petting zoos in Israel. The occurrence of resistant pathogens in petting zoos with high contact with children is alarming.

## Urinary Heat Shock Protein as an Early Marker for Kidney Injury in Dogs Undergoing Different Types of Surgery

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Early recognition of acute kidney injury (AKI) in surgical patients is important, since early treatment might decrease mortality rate. The aim of this study was to assess the increase of Heat shock protein-72 (HSP72), an early urine biomarker for AKI, pre versus postoperatively and within 4 American Society of Anesthesiology (ASA) – status dogs. Urinary HSP72-to-creatinine ratio (uHSP72/uCr) and serum creatinine (sCr) were measured pre and 24 hours post-operatively in 61 dogs from ASA1–4. There was a significant difference ( $P < 0.001$ ) in uHSP72/uCr between dogs of ASA2, ASA3, ASA4 and the controls prior to surgery. Nine of 19 dogs (47%) in ASA4 group, 4/18 (22%) in ASA3, 1/13 (8%) in ASA2 and 1/9 (11%) of the dogs of ASA1 had elevated uHSP72/uCr prior to surgery. Comparing uHSP72/uCr pre and post-anesthesia within every ASA-status group, there was a significant increase only in ASA3 group ( $P = 0.006$ ). The proportion of dogs with abnormal preoperative uHSP72/uCr concentration increased with ASA status ( $P = 0.043$ ). No significant difference in sCr was found between the groups before or after surgery ( $P = 0.215$ ,  $P = 0.151$ ). Increase in ASA status showed increased proportion of dogs with elevated uHSP72/uCr but sCr within the reference range, pointing at ongoing kidney injury in more critical patients which is undetected by sCr levels. Evaluating sensitive urine biomarkers can help the clinician to improve the assessment of kidney status prior to surgery. Elevation of uHSP72 reflects kidney damage and patients which present elevated uHSP are at higher risk to progress to overt kidney damage and should be monitored closely.

## Novel Bulking Agent for the Treatment of Urinary Incontinence in Dogs

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Bulking agents are an alternative treatment for urinary incontinence in dogs. The objective of this study is to evaluate CellFoam as a novel bulking agent for the treatment of urinary incontinence due to urethral sphincter mechanism incompetence in female dogs. Dogs diagnosed with urethral sphincter mechanism incompetence, refractory or unamenable to medical treatment were prospectively enrolled. CellFoam was injected under endoscopic guidance to the urethral submucosa. Continence score was evaluated before

and after the procedure and monthly thereafter. Fifteen spayed female dogs were prospectively enrolled, and a total of 23 injection procedures were performed (six dogs underwent more than one procedure). Median age of all dogs at the time of the procedure was 111 months (range, 18-180). Dogs had a significant increase in median continence score before compared to after CellFoam implantation (1.5, range, 1-3.5 vs. 5, range, 1.5-5). Continence was achieved in 13/15 (87%) dogs after the first procedure and in 8/8 (100%) after repeated procedures; overall, 21/23 (91%) procedures resulted in good continence scores at time of first follow up. Median duration of continence was 5.5 months (range, 0.5-24). No notable side effects or clinicopathologic changes were apparent in any of the dogs, except for one dog (7%) who experienced mild, spontaneously resolving, stranguria. CellFoam, is a safe and effective novel bulking agent, which can potentially be used as an alternative, cost effective treatment option for dogs with urethral sphincter mechanism incompetence.

## The Contribution of Baseline Cortisol to Interpretation of the ACTH Stimulation Test in the Diagnosis of Canine Hyperadrenocorticism

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The latest ACVIM consensus statement on diagnosis of hyperadrenocorticism advocates the collection of baseline cortisol, but no references for interpretation thereof exist. Therefore, we aimed to evaluate the contribution of baseline cortisol to ACTH stimulation test (ACTHST) interpretation and diagnosis of hyperadrenocorticism. Samples from 73 dogs, submitted to a referral laboratory for evaluation of hyperadrenocorticism by ACTHST (Group 1) and 57 dogs from a referral hospital evaluated with ACTHST for suspected hyperadrenocorticism (Group 2) were included. General practitioners were asked to complete a questionnaire regarding ACTHST interpretation (Group 1). Records of dogs (Group 2) with suspected hyperadrenocorticism were reviewed. Receiver operator characteristics (ROC) analyses were used to assess the performance of baseline and post-stimulation cortisol concentrations, and delta and ratio post-to-baseline cortisol concentrations for the diagnosis of hyperadrenocorticism. Areas under the ROC curve (AUC) for baseline, ratio, delta, and post-stimulation cortisol concentrations were 0.63, 0.58, 0.77 and 0.85, respectively, for dogs from Group 1, and 0.70, 0.53, 0.85 and 0.90, respectively, for Group 2. Post-stimulation cortisol AUCs were significantly higher than all other AUCs except for delta cortisol concentration in Group 2, which performed similarly to post-stimulation cortisol. For the diagnosis of hyperadrenocorticism in dogs from Group 2, an optimal cut-off value of 683.1 nmol/L for post-stimulation cortisol yielded a sensitivity/specificity of 81.6%/94.7%, respectively. In conclusion, post-stimulation cortisol demonstrated good discriminatory ability for hyperadrenocorticism diagnosis. It was comparable to delta cortisol, while baseline cortisol and ratio cortisol were ineffective. Current recommendations to collect baseline cortisol samples appear redundant.

# Trends in Serum Cobalamin, Folate and Total Iron Binding Capacity Concentrations in Pregnant Bitches and Their Association with Hematological Parameters and Neonatal Survival

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This study investigated serum cobalamin, folate, total iron binding capacity (TIBC) and complete blood count analytes at mid- and late-pregnancy in 48 healthy bitches, from two breeders, and their association with pregnancy-related anemia (PRA) and neonatal survival (NS). The effects of pregnancy stage, parity, litter-size and breeder were assessed. Compared to mid-pregnancy, serum cobalamin (adjusted mean [95%CI]) decreased at late-pregnancy (430 pg/mL [394-466] vs. 330 pg/mL [303-357], respectively;  $P < 0.001$ ). Parity ( $P = 0.015$ ) and litter-size ( $P < 0.001$ ) had significant effects on serum cobalamin concentration, with every 1-increment in parity number or litter-size corresponding to 28.6 pg/mL (95%CI, 5.6-51.6) and 20.3 pg/mL (95%CI 10.9-29.7) serum cobalamin concentration decrease, respectively. At late-pregnancy, compared to mid-pregnancy, TIBC increased in dogs of one breeder (4.40 mg/L [4.14-4.66] vs. 3.63 mg/L [3.381-3.87], respectively;  $P < 0.001$ ), while in another, every 1-increment in litter-size resulted in 0.09 mg/L (95%CI, 0.002-0.179) serum TIBC decrease ( $P = 0.042$ ). The significant decrease in red blood cell (RBC) count was significantly, albeit weakly, correlated with decrease in serum folate concentration ( $r = 0.33$ ;  $P = 0.023$ ) and increase of serum TIBC ( $r = 0.40$ ;  $P = 0.004$ ). None of the measures was associated with neonatal survival. In conclusion, in healthy bitches, hypocobalaminemia frequently developed during pregnancy and was aggravated with increased litter size and higher parity. Unlike women, TIBC increases as pregnancy progresses, but this trend is offset by increased litter-size. The decreases of serum folate concentration and RBC count were positively correlated, warranting future studies to investigate whether prophylactic folate administration to pregnant bitches mitigates PRA.

# The Effect of a Cricoid Stent on the Rima Glottidis and Intraluminal Laryngeal Pressure in Dogs – A Cadaveric Study

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Brachycephalic dogs with severe laryngeal collapse are unable to breath and permanent tracheostomy is the only surgical option available. Clients are generally unaccepting of permanent tracheostomy and will often choose to euthanize their dogs. We have developed a novel extraluminal subcartilaginous cricoid stent for the treatment of laryngeal collapse. The aim of this study was to evaluate the effect of the stent on the area of the Rima glottidis and intraluminal laryngeal pressure. Cricoid stents designed to conform to the shape of the cricoid cartilage of normocephalic dogs were tested in 8 cadaveric larynxes. The stent was placed, via a surgical approach, in the space created by separating the cricoid cartilage from its overlying mucosa. Inspiratory airflow through the larynx was generated by a custom made machine, and the resultant intraluminal pressure was measured using a manometer. The Rima glottidis was photographed during testing using the identical technique for all specimens, the photos were used to determine the area of the Rima glottidis. Intraluminal pressure and area of the Rima glottidis was determined both prior and subsequent to placement of the cricoid stent. Placing the

stent resulted in a significant increase in the area of the Rima glottides (median-5.38 mm<sup>2</sup>), and significant decrease in the negative intraluminal pressure (median-8.75 Pa), in addition, moderate correlation ( $R^2=0.664$ ) was found between the area of the Rima glottidis and intraluminal pressure. Cricoid stent for Grade II/III laryngeal collapse in dogs may provide an alternative treatment to current available surgical options.

## Recurrent seasonal morbidity of *Staphylococcus Aureus* in fruit bats (*Rousettus Aegyptiacus*)

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*Staphylococcus aureus* (S.A) is a Gram-positive bacteria which there is not a long term immune process against. It is frequently found in respiratory tract and on skin of humans and many animals. However S.A. is rarely found on bats' fur (0.006% in 250 bats fur samples in nature and captivity). Despite being mostly a commensal; it is a common cause of skin infections including abscesses. In the past five years, we see a significant seasonal spreading illness in fruit bats colonies, in captivity as in nature, following the reproduction seasons, twice a year. Classical clinical signs are abscess in cervical and inguinal lymph nodes, or upper back abscess, with or without joint swelling, but might as well appear as abscess in other body parts. Culture and sensitivity taken from abscess demonstrate mostly S.A. sensitive to several antibiotics but sometimes contain Strep beta hemolytic as well. Treatment is mostly successful with oral Augmentin 7 to 10 days according to severity plus specific abscess drainage in case of breathing difficulties. In the current season (August 2018) in our captive colony out of 48 bats (10 males 20 females 18 pups) 3 females died (15% of females) 3 pup died (16.6% of pups.) 8 sick females were treated and healed (72% of sick females) 13 sick pups were treated and healed (81.2% of sick pups) and 2 sick males were treated and healed (20% of males). Bats might get infected from other animals that carry the bacteria including humans as retro zoonosis.

## A New Sustained Release Formulation of Florfenicol for Use in Pigs

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Swine respiratory disease (SRD) is amongst the most serious disease problems in modern swine production and often requires antimicrobial therapy. In most cases, antimicrobial administration is given to groups of pigs via feed or water, and by using this method there are animals that are not ill but receive treatment. This unnecessary administration increases the chance of resistant bacteria formation. Administration by injection is less common and often requires multiple injections which is time consuming and involves intensive use of labor. Florfenicol (FFC) is not in human use and is highly effective for treatment of infections in SRD. The aim of this study was to develop and evaluate an injectable, bio-degradable, sustained release intramuscular (IM) FFC formulation to achieve single dose therapy of pigs. Single dose injectable administration of antimicrobials provides the advantage of rapid and targeted, individual animal treatment without the need to re-handle each pig for subsequent injections. The concept is based on an injectable FFC, which is incorporated in a reverse

thermal gelation system using poloxamer polymers. We have compared the plasma concentrations of FFC in pigs after IM injection of our prototype formulation with that after two separate doses of a commercial product. While commercial FFC plasma concentrations decreased below MIC<sub>90</sub> after less than 12 hours, our prototype maintained plasma levels above the desired concentration for more than 4 days. These preliminary results are very promising and we plan to complete additional studies in order to strengthen the concept.

## Peak Antebrachiocarpal Joint Amikacin Concentration Following Cephalic Vein Regional Limb Perfusion in Standing Horses

**Gustafsson, K.,<sup>1</sup> Tatz, A.J.,<sup>1</sup> Dahan, R.,<sup>1</sup> Britzi, M.,<sup>2</sup> Abu Ahmad, W.<sup>3</sup> and Kelmer, G.<sup>1</sup>**

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Regional limb perfusion is a well-known method for delivering high antimicrobials concentration to the distal limb synovial structures in horses. The accepted and typically reported tourniquet application time is 30 minutes, although several studies concluded that application time of 10-15 minutes may be sufficient. Decreasing the application time, may be beneficial, by avoiding repeated sedation and discomfort. The objective of this study was to determine the time to maximum concentration ( $T_{max}$ ) of amikacin in the antebrachialcarpal (ABC) joint following cephalic intravenous regional perfusion (IVRLP). Six adult horses underwent IVRLP with 2 g of amikacin diluted in 100 mL of NaCl 0.9%. A wide esmarch rubber tourniquet was applied, and amikacin solution was injected through a 23G butterfly catheter. Synovial fluid was sampled from the ABC joint at 0, 5, 10, 15, 20, 25 and 30 minutes post IVRLP. Synovial amikacin concentration was determined by liquid chromatography/tandem mass spectrometry. Statistical analysis were made using a statistical software package (Stata 14). Median  $T_{max}$  was 15 minutes (range 10-20 min) and median  $C_{max}$  for the ABC joint was 1153 $\mu$ g/mL (range 630-1950  $\mu$ g/mL). Although median  $T_{max}$  was calculated to be 15 minutes, 2/6 horses had  $T_{max}$  of 20 minutes. Therefore, a tourniquet application time of 20 minutes is preferred. The study limitations include a small study group, with large synovial concentration variability. In conclusion, a tourniquet application time of 20 minutes is sufficient for completion of amikacin IVRLP in order to reach  $C_{max}$  in the ABC joint in standing horses.

## Synovial Fluid Concentration of Metronidazole Following Standing Regional Limb Perfusion in Horses

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Metronidazole is a lipid soluble bactericidal antimicrobial for treatment of anaerobic bacteria and protozoa including *Bacteroides spp*, an identified agent in synovial sepsis in horses. *Bacteroides spp* has high resistance

against commonly used antibacterial drugs including cephalosporins, aminoglycosides and trimethoprim-sulfa but is highly susceptible to metronidazole. Intra-venous-regional-limb-perfusion (IVRLP) is an established method of delivering high concentrations of antibiotics to synovial structures of the distal limb in horses. The objective of this study was to evaluate the pharmacokinetic properties of metronidazole in synovial fluid following IVRLP in standing horses. Seven adult horses underwent IVRLP with 500mg of Metronidazole diluted in 0.9% NaCl with a total volume of 108ml. Synovial fluid was collected from the distal interphalangeal joint at T=0, 15min, 30min, 2h, 12h and 24h. Synovial metronidazole concentrations were determined by liquid chromatography/tandem mass spectrometry. Statistic calculations were made through statistical computer programs (IBM statistics SPSS 24, STATA 14). Mean and median C<sub>max</sub> was 327 and 259 µg/ml. Mean and median T<sub>max</sub> was at 26 and 30 min. Mean and median concentration at T=2h was 6.2 and 8.3 µg/ml. At the following time points all measurements were below the clinical resistance breakpoint of 4 µg/ml. Although IVRLP with metronidazole results in high synovial concentration for 2 hours, concentrations decrease quickly below therapeutic levels making it unsuitable as a sole therapy. IVRLP with metronidazole can be considered pre-operatively in penetrating foot injuries but for further treatment of diagnosed anaerobic infections IVRLP with metronidazole seems ineffective and other routes should be explored.

## Identification and Genetic Characterization of West Nile Virus Strains in Israel with Respect to Their Virulence and Pathogenesis

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West Nile Virus (WNV) is a mosquito borne zoonotic arbovirus transmitted by mosquito vectors. Several outbreaks occurred in Israel, with a high number of neuro-invasive cases in human and animals, rendering WNV one of the most widely distributed encephalitic flaviviruses in humans and horses. In 2018 WNV outbreak occurred in human patients as well as increased morbidity and or mortality in wild avian species. This study is focused on molecular and genetic characterization and comparison between Israeli isolates of West Nile Virus, originating from birds and horses if available with respect to their immunogenic potential and virulence. In 2017-2018 several independent PCR tests for detection of WNV were developed, calibrated and used together with established assays to screen samples from horses, avian species and mosquitoes that were trapped in suspected stables. Samples from locations and collecting dates were screened and examined using partial genomic sequencing. To establish genetic differences between WNV strains. Of 270 samples from birds and 110 from horses, WNV was detected in 9 wild birds and 3 horses, all in 2018, belongs to Lineage 1. Future plans include phylogenetic analysis of local isolates that will be examined for their capacity to replicate and generate a cytopathic effect, under different conditions, in mammalian, avian and insect cell cultures. The integration of data from the genetic and virulence analysis will help to improve the current understanding of the effect of genetic differences on viral distribution and propagation in different hosts.

# Use of Anti-Salmonella Antibodies as a Mean to Evaluate Efficacy of Maternal Antibody Transfer in Broilers

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In the hatching chick the adaptive immune system is immature, and its immune protection is passively acquired following transfer of maternal antibodies (mostly IgY) to the egg. Maternal antibody protection is of great importance in broilers: First, a very young marketing age doesn't allow for effective "active" vaccination programs. Second, it can prevent the chicks from becoming carriers of zoonotic pathogens such as *Salmonella* strains, thus contributing to public health. In Israel, meat type breeders are routinely vaccinated against *Salmonella* strains from day one to the 18<sup>th</sup> week of life, while the laying period begins approximately at 24 weeks and ends at 64 weeks of life. Consequently, the duration between the last vaccination and the laying period is long. This interval raises the question on how successful is the vaccination program in producing long-lasting protection in both hens and chicks against *Salmonella* infectivity?

To address this, IgY transfer from hen to chick was examined in two breeder groups: 24 and 64 weeks of age. While total IgY levels increased with age (4778mg/chicken+/-172.9 Vs 9264.3mg/chicken+/-290.5), those specific for zoonotic *Salmonella* strains were markedly low and decreased age (2.78% Vs 1.43% out of total IgY), thus indicating that natural exposure was inefficient in maintaining antibody levels. Antibody levels in chicks proportionally reflected those found in the hens (9.17% Vs 14% on day 1 and near to 0 on 21 days of age), thereby rendering the protection concept to be questionable. These observations, especially the low response to *Salmonella* strains, notwithstanding vaccination and the appropriate transfer of maternal antibodies, raise a major concern on the protectively of broiler chicks against zoonotic *Salmonella* infections.