SELECTED ABSTRACTS OF THE 5TH ANNUAL SYMPOSIOM OF VETERINARY MICROBIOLOGY AND IMMUNOLOGY BET-DAGAN, ISRAEL. DECEMBER 2007

CHAIRMAN: ELAD .D

HISTOPHILUS SOMNI: A NEW, UNINVITED RESIDENT IN ISRAEL

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Histophilus somni is a normal inhabitant of the urogenital and the upper respiratory mucosa of ruminants, and is the causal agent of a varied range of diseases (1). H.somni was first described in Israel in 1991, from a case of bovine mastitis (2). Further cases were not reported until 2002, when it was isolated from the lungs of a pneumonic calf. From 2002 to 2007, cases of H. somni in Israel have notably increased each year: three isolates in 2003 (two from mastitis and one from pneumonic lungs), eight in 2004 (four from mastitis and four from pneumonic lungs), eight in 2005 (one from bovine metritis, three from mastitis and four from pneumonic lungs), 16 in 2006 (two from mastitis and 14 from pneumonic lungs), and 19 in 2007 (10 from mastitis and nine from pneumonic lungs). Most isolates were obtained from cattle; only four isolates were obtained from sheep, all of them from cases of mastitis. Cases of H. somni do not seem to be seasonal or concentrated geographically. Moreover, the number of flocks where H. somni has been isolated increased proportionally to the number of isolates, indicating that cases are often sporadic, and not enzootic. Yet, the importance of H. somni as a respiratory pathogen should not be overlooked. In 2007, 80% of the cases where it was cultured only from lungs it was the only pathogen isolated, a steep increase from the respective rates in 2005 and 2006 (20%). The observed trend suggests that H. somni may be a new pathogen in Israel. Intensive management, merging of flocks, and the introduction of new strains, for example, could all possibly be related to the growing incidence of this pathogen.

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COMPARATIVE STUDY OF AVIAN EGG WHITE GLYCANS AND THEIR ABILITY TO ABROGATE *PSEUDOMONAS AERUGINOSA* AND *CHROMOBACTERIUM VIOLACEUM* ADHESION TO TARGET HOST CELLS USING THE LECTINS OF THESE PATHOGENS

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The soil-borne life-threatening opportunistic pathogens Pseudomonas aeruginosa and Chromobacterium violaceum possess lectins that mediate their homing to target animal cells. R aeruginosa produces a galactophilic PA-IL lectin and a fucose>mannose-specific PA-IIL lectin. C. violaceum produces a PA-IIL-like (structurally homologous) lectin, CV-IIL. Blocking the binding of these lectins to their cell receptors, prevents bacterial adhesion to host cells and the resulting facilitated cell-damaging effects of their virulence factors. The well-characterized isolated bacterial lectins have been used to study the ability of animal secretions to prevent lectin-dependent bacterial adhesion. The present communication describes their usage for comparison of anti-adhesion activities of avian egg-white glycans that naturally function as decoys, competing with cell receptors for protecting their immunodeficient (due to immature immune system) fetus from infections. The chicken egg-white glycans, which are known to react most strongly with the mannose/glucose-binding plant lectin Con A (due to their glycoprotein complex-type carbohydrate chains), were shown to strongly inhibit PA-IIL, followed by CV-IIL, but not PA-IL. Quail egg-whites, which are rich in polymannosylated glycoprotein chains, also most strongly inhibited the fucose>mannose-binding lectins CV-IIL and PA-IIL, as well as Con A, but not PA-IL. In contrast, pigeon egg-white blocked most strongly PA-IL (owing to its known terminally galactosylated P_1/P^k-type glycoproteins), and also PA-IIL and CV-IIL. The present results show that diverse avian egg-whites differ in PA-IL-, PA-IIL-, and CV-IIL- inhibiting efficacy and that these lectins are useful tools for their study.

The work is part of K.D.Z-Y PhD thesis.

EMERGENCE OF NOVEL *STREPTOCOCCUS INIAE* EXOPOLYSACHARIDE-PRODUCING STRAINS FOLLOWING VACCINATION WITH NON-PRODUCING STRAINS

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Streptococcus iniae is a major pathogen of fish, producing fatal disease among fish species living in diverse environments. Variations in bacterial populations, with the emergence of novel vaccine-escape serotypes have been shown to occur as a consequence of vaccination. Recently, reoccurrence of disease was recorded in rainbow trout *(Oncorhynchus mykiss,* Walbaum) farms where the entire fish population was routinely vaccinated. New strains are distinguished from existing strains by their ability to produce large amounts of extracellular polysaccharide that is released into the medium. Present findings indicate that extracellular polysaccharide is a major antigenic and pathogenic factor (as indicated by proinflammatory cytokine release), suggesting the evolutionary selection of strains capable of extracellular polysaccharide production.

APPEARANCE OF SKIN LESIONS IN CATTLE POPULATIONS VACCINATED AGAINST LUMPY SKIN DISEASE; A STATUTORY CHALLENGE

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Lumpy skin disease (LSD) appears as an acute, sub-acute or inapparent infectious disease of cattle which is caused by a single strain of capripox virus known as Neethling, and characterized by the rapid eruption of multiple circumscribed skin nodules, generalized lymphadenitis and fever. Other lesions, that are found on post-mortem examination are necrotic plaques in the membranes, chiefly of the upper respiratory tract and oral cavity, mastitis and orchitis. Since the other lesions appear in internal organs, they are not "valid" for diagnosis in field cases, which is made chiefly by the field practitioners who base their diagnosis only on skin lesions. Veterinary officers statutorily-based decisions are taken only on external i.e.,cutaneous lesions appearance. Such decisions include whether to impose a strict quarantine, and/or to destroy an animal and/or to continue statutory order. Such decisions seem rather simple when the affected population is a naïve unvaccinated herd. In these cases, specificity, (namely false-positive) will be very high and almost no affected animals will be culled from the farm. Between June and November 2007, numerous cases of LSD characteristic lesions were reported in herds that had had been vaccinated with one dose of RM65 sheep poxvirus vaccine. This unexpected situation poses a real challenge to the VO, and entailed harsh statutory decisions. This is also a situation where a large margin of security is required in order to diminish further spread of the virus. Consequently a larger number of cows are culled from the herd(s). In a field situation a variety of lesions must be taken into account in the differential diagnosis. These include: specific lumpy skin circumscribed lesions, that are characteristic and specific lesions of LSD, and with regional enlarged lymph nodes; generally the same clinical picture but without enlarged lymph nodes; intra-cutaneous nodules not hard enough to be considered as characteristic LSD lesions; and a variety of subcutaneous nodules, without defined borders, hard or soft on touch, firmly connected to the subcutis or movable when palpated, and nodular lesion of Hypoderma bovis. In addition, urticaria, cutaneous hypersensitivity or Streptothrycosis must also be considered.

At the 3 affected sites the primary LSD lesions un the unvaccinated population totaled 227 out of 1249 (22.1%) with a range of 0.004-37.5% on the 17 affected farms, while at the 8 vaccinated farms, a total of 479 animals with lesions were reported out of 3917 animals (12.2%) with a range of 0.3-55%. Based on the observation that the occurrence of animals considered sick, namely, presenting lumps, were of a similar proportion within the fully susceptible (unvaccinated) cohort and in that supposedly protected (vaccinated) population, we could state that vaccination of cattle with RM 65-sheep pox vaccine neither ensures full protection against clinical LSDV infection nor does it afford clinical prevention. These field observations confirm the conclusions reached 15 years ago, after the results of a controlled study to assess RM65 vaccine efficacy, which were published by the Kimron Veterinary Institute (Brenner et al., Isr. J. Vet. Med. 47: 17-21. 1992).

INITIAL STEPS TOWARDS IMPLEMENTING A BVD CONTROL PROGRAM IN ISRAELI DAIRY HERDS.

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Developments in understanding the biology and epidemiology of BVDV (1), the development of novel laboratory techniques (3), and the success of Scandinavian countries in eradicating the disease (4) prompted a cooperation between the Hachaklait Veterinary Services, Kimron Veterinary Institute and the Israeli Dairy Board to develop a systematic BVDV control program for Israeli dairy herds.

The first step consisted of a serological spot survey (1, 2) in which 5 sentinel heifers aged 6-12 mo. were tested (ELISA) for the presence of BVDV antibodies. We adopted the Danish experience (1, 2) for herds where >= 3 of the 5 heifers were seropositive and were classified as herds with a high probability of persistently infected (PI) animals. By the end of 2007 about 600 herds were tested, of which 40% were classified as infected. The second step of detecting and removing PI animals was attempted in 10 herds, which, in addition of being infected according to this criterion, experienced a high level of abortions and / or high morbidity in the young stock.

Defining PI is based on the antigen capture ELISA (ACE) test of a skin biopsy – ear notch. This technique has the advantages of detection of antigen and not antibodies, high sensitivity and specificity, low sensitivity to transient infections (TI) and not being affected by colostral antibodies, which enables us to test one day old calves.

One herd of 350 milking cows has fully completed the process of detecting and removing Pi's, 24 animals were detected as Pi's and removed from the herd.

There were no signs of reinfection one year after the last PI was removed from this herd .Future projects include completion of the serological survey and analyzing the results together with abortion, morbidity, and biosecurity data, developing PCR test for milk and blood samples, serological survey of milk samples, comparing ACE test of pooled ear notch with single ear notch samples and typing the isolated viruses

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FLUORESCENT BRIGHTENERS AS AN ALTERNATIVE TREATMENT FOR FISH SAPROLEGNIASIS

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Fish pathogen water-molds (oomycetes), such as *Saprolegnia parasitica* cause vast economic losses to aquaculture business worldwide. As Malachite Green, the preferred agent to control *Saprolegnia* infection has been banned from routine use due to its high toxicity, a dramatic increase of this infection has occurred. Therefore, there is an urgent need for alternative chemical agents. Advantageously, this compound should be highly water-soluble, safe to living organisms and the environment, degradable, and economical. In the course of our search for an alternative treatment, several Fluorescent Brighteners (diaminostilbene derivative compounds) were tested for their anti-*Saprolegnia* activity. We hypothesize that these compounds, used as whitening agents in the textile and paper industry, bind and interfere with further synthesis of *Saprolegnia* cell wall cellulose or have an effect on the integrity of the cell wall so that it does not have enough strength to guarantee cell survival. In vitro susceptibility tests indicated that *S. parasitica* T-1 was susceptible to the stilbene derivative, Blankophor® BA (MIC=100 mg/L). This compound possessed a low toxicity for Tilapia (high LC_{50} value >1,000 mg/L, at 25, 18 and 15°C) and was highly effective in preventing *Saprolegnia* infection in the Tilapia-Saprolegniasis model, even at a low concentration of 25 mg/L. The effective concentration was lower than the MIC value. The high therapeutic index of Blankophor® BA in combination with its documented safety to mammals and the environment and its low cost, indicated that treatment with Blankophor® BA for fish Saprolegniasis could be an excellent alternative for Malachite Green.

IMMUNIZATION AGAINST NEWCASTLE DISEASE AND SENDAI VIRUSES USING CINNAMON FRACTION

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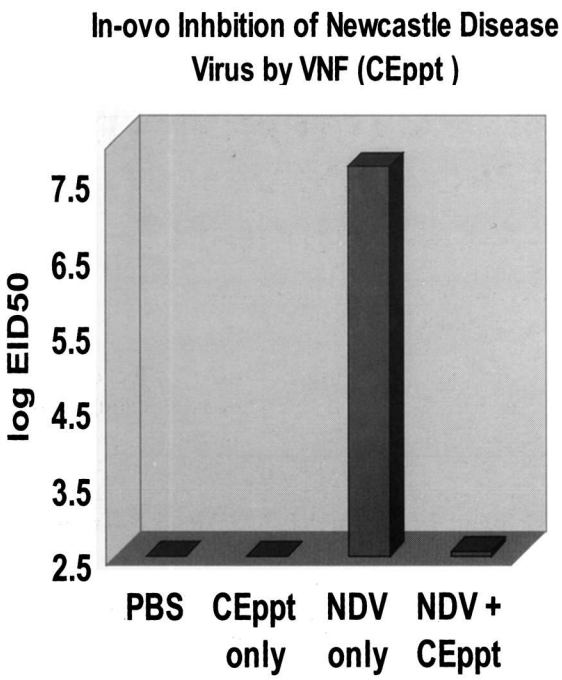
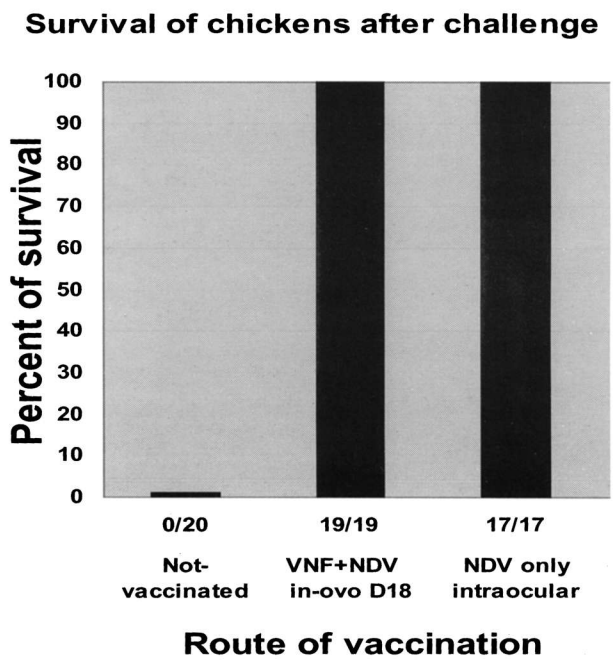
Newcastle disease virus (NDV) and Sendai virus (SV) are avian and mammalian members of the family Paramyxoviridae (Parainfluenza), respectively. NDV causes a contagious and fatal disease of most species of birds. It is so virulent that many birds die before showing clinical signs. Previous studies have demonstrated the antiviral activity of cinnamon extract against human influenza H1N1, avian influenza H9N2 and Sendai virus (Barak and Ovadia, 2005; Ovadia, 2007; Gueta and Ovadia, 2005). The aims of the present research were: 1) to examine the ability of the Viral Neutralizing Fraction (VNF) = CEppt fraction, which was precipitated from the crude Cinnamon Extract, to inhibit NDV. 2) to develop immunization against NDV by in-ovo vaccination, as an alternative approach to post-hatching vaccination of chicks; and 3) to use VNF for immunization against Sendai virus.

Injection of NDV incubated with CEppt into 11-day old SPF chicken embryos resulted in a decrease in the viral hemagglutinating activity and infectivity by 5 logs, and a significant increase in survival; the embryos resisted 10^8 EID_{50} of the virus, whereas 10^2-10^3 EID_{50} of the virus alone killed the embryos.

In-ovo vaccination of 18-day old SPF chicken embryos with NDV preincubated with CEppt induced high titers of antibodies during the following month, as high as with the standard intraocular vaccination of 1-2-day old chicks. Challenge with NDV (velogenic strain) was given 35 days post-immunization. All 19 chickens immunized in-ovo, resisted the velogenic virus challenge while all 20 non-vaccinated chickens died.

Similar results were obtained with Sendai virus. Mice received a mixture of CEppt and Sendai virus inoculated either intranasally,or intramuscularly, or orally, or subcutaneously. Three weeks post-immunization, the mice were challenged with the naïve virus alone. The mice were weighed every 2-3 days through the experiment. The immunized mice were hardly affected by the subsequent virus infection and continued to gain weight. The best immunization was obtained by the intranasal route.





FIRST REPORT IN ISRAEL OF *BARTONELLA BOVIS* IN BLOOD CULTURES FROM A BEEF CATTLE HERD: EPIDEMIOLOGICAL, MICROBIOLOGICAL, AND CLINICAL CHARACTERISTICS

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Background: Members of the genus Bartonella are fastidious, aerobic, gram negative bacteria that are considered as emerging zoonotic pathogens. The reservoir of Bartonella includes a wide range of mammals including cats, dogs, coyotes, wild rabbits, rats and other species of rodents. Various vectors including fleas and ticks have been implicated in disease transmission. Four species of Bartonella have been isolated from wild and domestic asymptomatic bacteremic ruminants: B. bovis and B. chomelii from cattle, and B. shoenbuchensis and B. capreoli from European roe deer. Although a single case of human endocarditis due to B. bovis has been described, the actual significance of B. bovis bacteremia for animals or humans is unclear.

Aim: To determine whether Bartonella bacteremia occurs in cattle in Israel and to describe its epidemiological, microbiological, and clinical characteristics.

Methods: Caudal blood samles were taken from two separate herds of 8 dairy cows and 17 beef cattle into sterile tubes with EDTA and frozen at -80°C. A few days later the blood was thawed, cultured on chocolate agar plates and incubated at 35°C for 8 weeks. Colonies with morphologic and gram stain consistent with Bartonella spp. were subjected to PCR of the citrate synthase (gltA) gene. Species identification was performed by sequencing of the PCR product. Unusual symptoms and signs were recorded. Each animal was searched for arthropods.

Results: None of the 8 dairy cows and 14 of the 17 beef cattle were found to have bacteria with Bartonella spp. Sequencing of 334 bp of the citrate synthase gene PCR product had 99.9% homology with B. bovis confirming B. bovis bacteriemia in the beef cattle herd. The concentration of B. bovis in the blood ranged from 1 to >100,000 cfu/mL. None of the milk cows and 7 of the 16 beef cows was infested with ticks. Neither herd showed symptoms of infection and both herds seemed to behave normally.

Conclusion: This is the first report of B. bovis bacteremia among beef cattle in Israel. B. bovis bacteremia was not found in milk cows and was closely associated with tick infestation. The zoonotic and enzootic potential of B. bovis infection is yet to be determined; however, the high rate of infection among beef cattle and the rare but serious morbidity caused by B. bovis in humans call for attention and should encourage research on the Bartonella cattle-human relationship

APPLICATION OF *PSEUDOMONAS AERUGINOSA* AND *CROMOBACTERIUM VIOLACEUM* LECTINS IN A COMPARATIVE STUDY OF SEVERAL MAMMALIAN MILK GLYCAN REPERTOIRES AND THEIR ANTIPATHOGEN ADHESION POTENTIAL

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seudomonas aeruginosa and *Chromobacterium violaceum* are opportunistic pathogens involved in human and animal morbidity and mortality. Both produce lectins that mediate their homing to animal cell surface glycans. *P. aeruginosa* possesses a galactophilic lectin, PA-IL, and a fucose- (preferentially of Lewis epitopes) and mannose-binding lectin, PA-iii, and *C. violaceum* produces a PA-IIL like (both in structure and specificity) lectin, CV-IIL. The binding of these lectins to host cells facilitates the pathogen toxin and enzyme function on them. Interest in lectins as targets for antibacterial adhesion has arisen following the decrease in antibiotic efficacy due to acquired bacterial resistance. This resistance resulted in a search of strategies for abrogating bacterial binding to host cells. The natural anti-adhesion function of human milk saccharides, which function as receptor - mimicking decoys that attract the bacterial lectins, was chosen as a model. Their binding to bacterial adhesins competitively blocks lectin attachment to the newborn cells until their immune system matures. Use of PA-IL, PA-IIL and CV-IIL has shown that human milk is their best inhibitor, displaying highest affinity to PA-IIL. In addition, PA-IL was also strongly inhibited by milks of alpaca, mare, buffalo and monkey, and moderately by those of cow, ewe (sheep), goat, camel, and fallow deer (mainly by their low molecular glycoconjugates). Only rabbit milk did not inhibit it. PA-IIL was also considerably sensitive to milks of alpaca, monkey, rabbit and camel, more weakly to those of goat and mare, while being almost insensitive to buffalo, cow, and fallow deer. CV-IIL was also nicely blocked by camel, rabbit, alpaca, buffalo, ewe, and monkey milk, followed by fallow deer, mare and cow. The present results show that diverse milks differ in PA-IL-, PA-IIL- and CV-IIL- inhibiting efficacy and that these lectins are useful tools for their study.

The work is part of K.D.Z-Y's PhD thesis

AN OUTBREAK OF RESPIRATORY INFECTIONS CAUSED BY *STREPTOCOCCUS EQUI SUBSP ZOOEPIDEMICUS* IN A CATTERY

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Streptococcus equi subsp. *zooepidemicus* (*S. zooepidemicus*) is a commensal of the upper respiratory tract mucosa of mammals, notably equine, and is associated with infections in a variety of animals and humans. We describe an outbreak of *S. zooepidemicus* in a cattery, where approximately 700 cats are raised freely in two wide, covered enclosures (previously used to raise poultry). Cats are castrated/neutered, but not vaccinated prior to entry, and sick cats are separated into a delineated area inside one of the enclosures. Few cats are delivered for adoption from this cattery, and public access is limited. Since its establishment in 2006, an outbreak of respiratory disease, which was firstly characterized by purulent nasal discharge, was reported in the cattery. Nasal and throat swabs were sent to the bacteriology laboratory in KVI and *S. zooepidemicus* was isolated from three out of six samples at that time, whereas no pathogenic bacteria were obtained from the three remaining samples. Following those first cases, dead cats started to be sent for *post mortem* examination in KVI, and internal organs were sampled for bacteriology. The most common pathological findings were sinusitis, pyothorax, and necrosuppurative or pyogranulomatous bronchopneumonia. *S. zooepidemicus* was isolated from the lungs and purulent discharges from the sinus, as well as from the brain, in a few cases of pyogranulomatous meningoencephalitis, in approximately half the cases with a positive bacterial growth. Other pathogens, namely *Escherichia coli*, *Pasteurella multocida*, *Mycoplasma felis* and *M. gatae*, were isolated sporadically from the lungs. A few *Chlamydia* spp. basal bodies were seen in lung smears stained by immunofluorescence. Twenty-six isolates of *S. zooepidemicus* obtained in the last two years were further investigated by PCR amplification and sequencing of 518 bp of 16S rRNA gene for confirmation of identification and molecular typing. Phylogenetic analysis revealed two genogroups of *S. zooepidemicus* present in the cattery. Both genogroups were isolated during the same period and they did not differ in virulence (determined by source – carcass or nasal swab) or biochemical characteristics. To the best of our knowledge, this is the first report of an outbreak of *S. zooepidemicus* infection in cats.