

SELECTED ABSTRACTS OF THE 5TH ANNUAL MEETING OF THE ISRAEL ASSOCIATION OF VETERINARY MICROBIOLOGY AND IMMUNOLOGY, JANUARY 2009

Chairman: Elad, D.

DO ANTI- *PORPHYROMONAS LEVII* ANTIBODIES IN PREPARATORY HEIFERS AFFECT THE OCCURRENCE OF BOVINE NECROTIC VULVUVAGINITIS POST-PARTURITION?

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Bovine necrotic vulvovaginitis (BNVV) is an important syndrome in some dairy herds in Israel, which can potentially lead to severe economic losses (3). Primiparous cows are at the highest risk. Clinical signs appear three days after calving, starting with erythematic lesions that progress to hemorrhages leading to inflammation and necrosis of the vaginal mucosa. Severe cases of BNVV can result in peritonitis and death. Thus far there is no successful treatment of BNVV(1).

BNVV was first diagnosed in 2001 in several dairy herds in the north of Israel and subsequent additional herds in the country became affected. All the infected herds had a history of introducing a large number of cows during consolidation with other farms during unification with small herds.

The etiological agent of the syndrome is *Porphyromonas levii*, a Gram-negative anaerobe, commensal of the digestive tract that colonizes mucosal lesions of the post-parturient vagina. The microorganism is unable to cause BNVV without the help of additional predisposing factors. The principal factor is assumed to be stress associated with animal transfer and calving (4). However, BNVV has become endemic on two farms, with new cases appearing for several years after the consolidation of the farm. This indicates that factors other than the aforementioned stresses are necessary for predisposition to the syndrome.

The aim of this study was to characterize the humoral response to *P. levii* during the periparturient period in order to assess whether a correlation exists between the presence of anti-*P. levii* antibodies before parturition and the appearance of BNVV afterwards. The study was conducted on three herds: A-endemic (54 cows), B-outbreak (51 cows) and C-unaffected (17 cows).

Serum samples were taken on the weeks around days 210, 240 and 270 of pregnancy, and the first and fourth weeks post-parturition. ELISA tests were conducted to determine the levels and type of anti-*P. levi* antibodies in each sample.

Antibodies were found in 59/105 (56.2%) heifers pre-parturition, indicating previous exposure. No correlation was found between the presence of antibodies pre-parturition and development of BNVV. However, only four (28.6%) of 14 cases with the high preparturition titers, developed BNVV after parturition. In contrast, among cows with lower or no titer, 41.9% (36/86) developed BNVV. The humoral pattern of clean herds was similar to infected ones. Also, heifers that developed BNVV had a higher mean antibody titer after calving than healthy animals.

In conclusion, the presence of antibodies before parturition does not explain the difference between infected and uninfected herds and between afflicted and healthy heifers in the infected herds. However, the relatively low number of BNVV-positive heifers among the animals with high preparturient antibody titer, indicates that an prevention of the syndrome by vaccination would be justified.

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THE FIRST EVIDENCE OF BVDV-2 IN AN ISRAELI HERD.

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Bovine viral diarrhea viruses belong to the pestivirus genus of the Flaviviridae. Analysis of genome sequences of BVD viruses distinguishes between two genotypes, BVDV-1 and BVDV-2. Antigenic differences occur between the genotypes, but there is considerable cross-reactivity, so that pestiviruses are not classified into serotypes. The incursion of both genotypes of BVDV into a herd is usually inapparent and the main damage is the result of fetal infection. However there have been reports of severe clinical disease caused by virulent strains.

The 5' UTR region of Israeli BVDV isolates, collected from 1996 to 2008 from various geographic locations, was sequenced and analyzed in order to characterize and

map the isolates. BVDV-1 and -2 were both identified; BVDV-1 was the predominant genotype and could further be subtyped. Type 1b was the most common, but 1a and 1b were also found.

An outbreak of severe acute BVDV infection occurred in an Israeli dairy herd in the summer of 2008. The outbreak presented 2 distinct clinical syndromes, characterized by hemorrhagic enteritis in adult cattle and severe pneumonia in young calves. Nine BVDV isolates from the outbreak were analyzed. All isolates from both clinical syndromes were identified as BVDV- 2 and were nearly identical phylogenetically.

USE OF IN VIVO INDUCED ANTIGEN TECHNOLOGY (IVIAT) FOR IDENTIFICATION AND CHARACTERIZATION OF *MYCOPLASMA GALLISEPTICUM* ANTIGENS PREFERENTIALLY EXPRESSED DURING INFECTION

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Mycoplasma gallisepticum is known to cause chronic respiratory disease in chickens, infectious sinusitis in turkeys and is a major component of the respiratory complex in domestic poultry. Several virulence factors of *M. gallisepticum* have been characterized and shown to be involved in the pathogenicity of this organism. The focus of this study was to discover novel virulence-associated determinants of *M. gallisepticum* induced in the host on infection, using *in vivo* induced antigen technology (IVIAT). In order to prepare antibodies detecting only *in vivo* induced (*ivi*) genes, serum samples from ten chickens experimentally infected with *M. gallisepticum* R (8p) were pooled and absorbed with the pathogen grown *in vitro*. An inducible expression library of *M. gallisepticum* R (25p) screened with anti-*ivi* serum yielded 10 positive clones. Identification of the *ivi* clones was performed by comparison of the nucleotide sequences of the mycoplasmal inserts with the available *M. gallisepticum* R genome sequence data. Analysis of the putative *ivi* clones revealed striking results.

Five *ivi*-genes encode previously identified putative virulence factors of *M. gallisepticum*: phase variable VlhA lipoproteins (VlhA 1.07 and 4.01), the cytoadhesin molecule GapA and two homologues of *M. pneumoniae* cytoskeletal proteins P65 and HMW3 (PlpA and Hlp3, respectively), which exhibit fibronectin binding capabilities. Identification of these known pathogenic determinants of *M. gallisepticum* by IVIAT validates this experimental approach. Interestingly, five additional IVIs proteins not previously implicated in infectivity were identified, including ABC predicted permease (MGA 0654), a hypothetical protein (MGA 0241), two 50S ribosomal proteins L23 and L2 (MGA 0711 and 0712), and a chaperonin GroEL protein. We believe that identification and characterization of *ivi* genes, which most likely encode virulence factors essential for survival, bacterial cell division and multiplication *in situ*, may provide new insights into *M. gallisepticum* pathogenesis as well as the development of efficacious vaccines, novel targets for antimicrobial therapy and innovative diagnostic tools.

WHAT DO LUMPY SKIN DISEASE, PSEUDO LUMPY SKIN DISEASE AND ULCERATIVE HERPES MAMMILLITIS HAVE IN COMMON?

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BHV-2 belongs to the *Alphaherpesviridae* sub-family and produces mild to moderate disease in ruminants. It is probably transmitted mechanically while the other viruses belonging to this subfamily are transmitted by the respiratory route. Herpes viruses generally cause local diseases, with barely detectable levels of viremia. Bovine ulcerative herpes mammillitis (BHUM) and pseudo lumpy skin disease (PLSD), two clinically distinct diseases that have not been reported to appear on the same infected cow at the same time. BHUM is associated with a painful localized ulcerative udder, and although it seems to be present in Israel, it has not been reported officially.

PLSD also known as Allerton virus infection is characterized as a generalized BHV-2 infection of cattle.. To the authors' knowledge, the present outbreak is the fourth recorded case of PLSD outside Africa.

In May 2008, a close examination of a primiparous dairy cow, presented lumps around and on the perianal region. The internal part of the tail revealed additional skin lesions. These were presented as scattered circular

multifocal lesions, approximately 2 cm in diameter. Other lesions had lost hair and resembled ringworm lesions, were scattered among the lumps of the caudal region and on the dorsal region, between the neck and the hips, and were interspersed with a few well-circumscribed hairless lesions. PLSD, as the generalized cutaneous form of BHV-2 infection does not seem to be a very serious disease in itself, but it raises concern at differential diagnosis between PLSD and LSDV infection, ringworm (*Thricophyton verrucosum*), and *Dermatophilus congolensis* infection.

Lumpy skin disease (LSD) presents as an acute, sub-acute or latent infectious disease of cattle caused by a single strain of capripox virus known as Neethling virus. LSD is characterized by the rapid eruption of multiple circumscribed skin nodules, generalized lymphadenitis and fever. Although the field experience and circumstantial evidence suggest that transmission of LSDV occurs primarily by biting insects the mode of field transmission is not fully understood.

Table 1- Characteristics of PLSD, LSD and BUHM infections

Clinical characterization	PLSD	LSD	BUHM
Causal agent	BHV-2 Allerton virus	Capripox Neethling virus	BHV-2 Allerton virus
Anatomical appearance of the lesions	Generalized skin lesions, caudal upper region mainly	Generalized skin lesions, all over the body	Local skin teat & udder only
Aspect of the lesion	Lumps (pseudo) necrosis sloughs off?	Lumps necrosis slough off	Granular ulcerative
In between etiology	BHV-2 + Capripox -	BHV-2 ± Capripox +	BHV-2 + Capripox -
Sharing intermediate clinical feature	Never appears with BUHM	Lumps mixed with pseudo- lumps	Never appears with PLSD
Differential diagnosis:	LSD	PLSD	Unique

MICROBIOLOGY IN THE POST-GENOMIC ERA

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Genomics has revolutionized every aspect of microbiology. Now, 13 years after the first bacterial genome was sequenced, it is important to pause and consider what has changed in microbiology research as a consequence of genomics. Over the past decade, genomic technologies have revolutionized microbiology and will probably continue to do so during the next decade. The information that is being added to sequence databases is increasing exponentially and every day we are in a better position to describe microorganisms by their genome, bacterial species by their pan-genome, and even complex microbial environments by their metagenomes. Advances

in genomics are also beginning to drive the discovery of novel diagnostics, drug targets and vaccines. In this talk, I will review various aspects of the impact of genomics on microbiology in general and veterinary science in particular, including in the evolving field of bacterial typing, and the genomic technologies that enable the comparative analysis of multiple genomes.

I will also address the implications of the genomic era for the future of veterinary microbiology and how this could assist and advance the veterinary practitioner in the day- to- day clinic.

CHARACTERIZATION OF QUINOLONE RESISTANCE-DETERMINING REGIONS IN *MYCOPLASMA BOVIS* CLINICAL ISOLATES WITH DIFFERENT SUSCEPTIBILITY TO ENROFLOXACIN

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Emergence of resistance to fluoroquinolones is mainly due to chromosomal mutations in genes encoding the subunits of the drug's target enzymes, DNA gyrase (GyrA and GyrB) and topoisomerase IV (ParC and ParE), which are essential for DNA replication. The quinolone resistance-determining regions (QRDRs) of these genes were characterized in 42 *Mycoplasma bovis* strains isolated between 2005 and 2008 from different clinical conditions and exhibiting various levels of susceptibility to fluoroquinolones. All enrofloxacin-resistant isolates harbored amino acid substitutions in the QRDRs of each of three proteins (GyrA, ParC and ParE). However, the main difference between susceptible and resistant *M. bovis* strains was the change of nt G to A

at position 265 of the *parC* amplicon, resulting in the substitution of 84-Asp/Asn. The *parC*-PCR-Restriction Fragment Length Polymorphism (RFLP) assay using *PsiI* restriction enzyme clearly differentiates between enrofloxacin resistant and susceptible *M. bovis* field strains. This feature may serve as a molecular marker for fluoroquinolone resistance.

In addition, molecular typing of enrofloxacin resistant and susceptible *M. bovis* strains by insertion sequence-like elements-RFLP analysis, suggest that the currently prevalent enrofloxacin-resistant strain evolved by selection under field conditions from one of the susceptible strains.

EMERGENCE OF DOG RABIES IN NORTHERN ISRAEL

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Between the years 1979 to 2000, foxes constituted the main reservoirs of rabies in the Northern regions of Israel. Following the implementation of fox targeted oral vaccination programme (ORV) in 1998, rabies was eradicated from this area. Subsequently in the years 2004 to 2007, the biological and molecular characterization of the rabies isolates showed that stray dogs emerged as the main animal reservoir in Northern Israel while lower

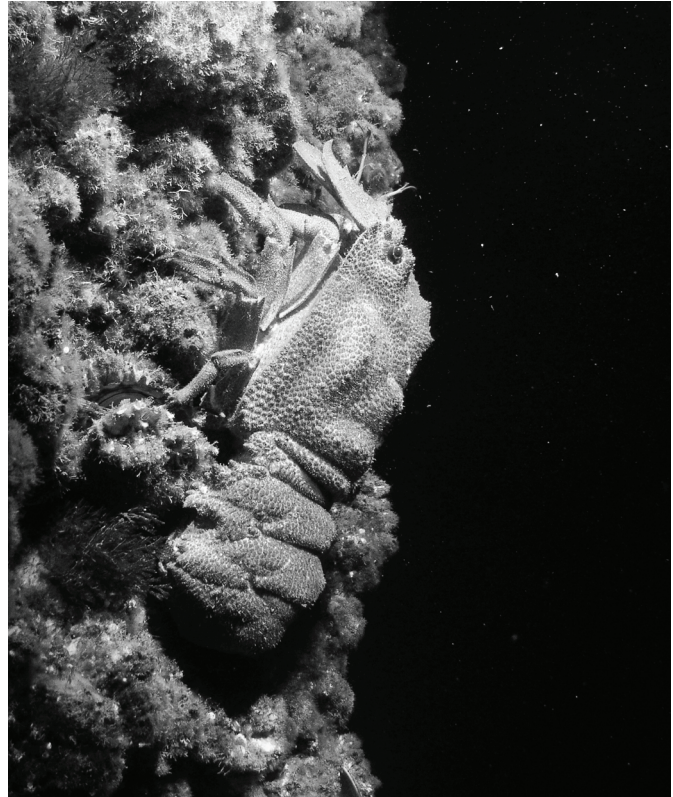
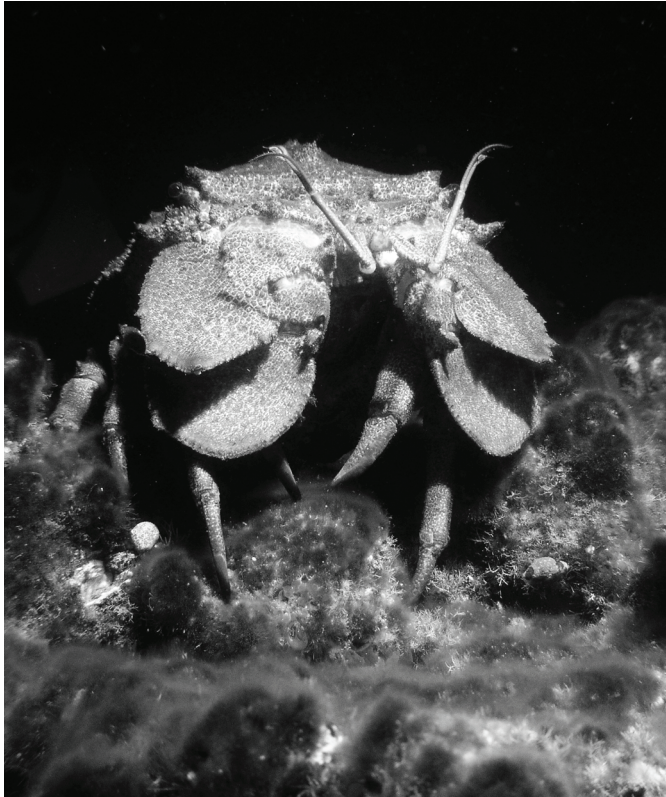
numbers of cases were reported in domestic animals. The virus isolates from foxes and dogs differed, in their molecular characterization, suggesting two distinct separate lineages. The transition from fox mediated rabies to dog-mediated rabies is a great concern to public health because of the close contact between dogs and the human population.

DETECTION OF *BARTONELLA* SPP. IN WILD RODENTS IN ISRAEL USING HRM REAL-TIME PCR.

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The prevalence of *Bartonella* spp. in wild rodents was studied in four zoogeographical areas in Israel. One hundred and twelve rodents belonging to five species: *Mus musculus*, *Rattus rattus*, *Microtus socialis*, *Acomys cahirinus* and *Apodemus sylvaticus* were included in the survey. In addition, 152 external parasites belonging to three species: *Pulex irritans*, *Xenopsyla cheopis* and *Haemaphysalis* sp., and arthropod species belonging to one of the three following suborders: Anoplura, Gamasina and Ixodidae were collected from the rodents. DNA was extracted from spleen aspirates from each of the rodents and from pools of external parasites and examined for the presence of *Bartonella* spp. DNA. The diagnosis and molecular identification of *Bartonella* spp. was done by

high resolution melt (HRM) real time PCR. The method, originally used for single nucleotide polymorphism (SNP) genotyping, was designed for the differentiation of 12 *Bartonella* spp. according to the nucleotide variation in two gene fragments, *rpoB* and *gltA*, and the 16S-23S intergenic spacer (ITS) locus simultaneously. *Bartonella* DNA was detected in spleen samples of 19 out of 79 (24%) black rats (*R. rattus*) and in 1 of 4 (25%) Cairo spiny mice (*A. cahirinus*). *Bartonella* DNA was detected in 15 of 34 (44%) flea-pools. All positive flea pools contained only rat-fleas (*X. cheopis*) and were collected from black rats (*R. rattus*). The *Bartonella* sp. detected in most samples was closely related to both *B. tribocorum* and the zoonotic *B. elizabethae*.



Cover image – Slipper lobster | *Scyllarides latus*

Slipper lobsters are a family of decapod crustaceans found in all warm oceans and seas.

Despite their name, they are not true lobsters but are more closely related to spiny lobsters and furry lobsters, and they lack prominent claws. Slipper lobsters are easily identified by their enlarged antennae, which project forward from the head as wide plates.

In the eastern Mediterranean their size rarely exceeds 40 cm. and they live on rocky substrates in depths up to 100 m.

They are active at night and during the day they hide frequently inside caves and crevices. They feed on molluscs, mainly limpets and other sessile marine snails.

The species has become rare in most parts of the Mediterranean Sea due to severe over-fishing for human food.

In some places along Israel's shores slipper lobsters can be spotted during the night, especially in the vicinity of artificial substrates like ship wrecks on the sea bed.