ID: 9

PREVALENCE AND CHEMOTHERAPY OF EQUINE THEILERIOSIS IN AND AROUND LAHORE-PAKISTAN

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Topic: 6. Epidemiology and Infectious Diseases / Vector-Borne Diseases

The prevalence of theileriosis in clinical affected equines of Lahore district was examined and the efficacy of three treatments measured. A total of 300 (horses=100; donkeys=100; mules=100) blood samples were collected from equines and examined microscopically; Overall, 174 (58%) were positive for Theileria equi whereas in horses (54%), mules (64%) and donkeys (56%) The final efficacy of treatment with Imidicarb dipropionate, Buparvaquone or Aak (Calotropis procera) was 91.7%, 66.7%, and 58.3%, respectively, making Imidicarb dipropionate the most effective treatment. This is the first report of Theileria equi infection in equines of Pakistan.
ID: 12

GASTROINTESTINAL HELMINTHES AND PROTOZOA IN MIGRATORY CAMELS IN MULTAN, PAKISTAN

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Topic: 6. Epidemiology and Infectious Diseases / Spread of Pathogens through International Trade

A Survey of gastrointestinal helminthes/ parasites in camel migrated from Tehsil Jalapur Pir Wala to Multan Tehsil, was carried out during May, 2012. A total number of 50 samples (20 males and 30 females) were collected from various places at Multan. The revealed parasites were mixed helminthic infection and identified as strongylidae spp., trichostrongyle spp., coccidian/ eimeria spp. and isospora spp.

Keywords: Gastrointestinal helminthes, Camel, Fecal sample, Migratory camels.
An epidemiological study on brucellosis in humans and domestic ruminants was carried out between 2007 to 2011 in Bangladesh. One thousand three hundred and sixty (1360) cattle, 636 goat, 1044 sheep and 800 human sera were tested in parallel by using indirect ELISA (iELISA), Rose Bengal Test (RBT) and Slow Agglutination Test (SAT) (in Human Standard Tube Agglutination Test). True prevalence of brucellosis in cattle, goat, sheep, humans and sensitivities and specificities of iELISA, RBT and SAT (STAT in humans) were determined by Bayesian analysis. Risk factors were identified multiple random effect logistic regression. Seventeen MRT positive pooled milk samples, 23 placentas, 17 vaginal swabs, 5 semen samples and 20 seropositive human sera were tested for the detection of Brucella at species level by using IS711 realtime PCR.

The overall herd level prevalence of bovine brucellosis was 35.10% (95% Probability Interval (PI): 30.3, 40.0) (n=388). The true prevalence of brucellosis among humans, cattle, goats and sheep estimated were 2.6% (95% CrI: 1.2-4.5), 1.8% (95% Credible Interval (CI): 0.8-3.1), 0.7% (95% CrI: 0.2-1.6) and 1.2% (95% CrI: 0.6-2.2) respectively. The sensitivity of iELISA, RBT and SAT (Standard Tube Agglutination Test in human) were estimated.

Herd size and breeding were found to be significantly associated with herd level risk factors of bovine brucellosis.

Duration of contact with livestock was significantly associated with brucellosis in human. Only Brucella abortus was detected from both humans and animals by using real time PCR for the first time in Bangladesh.
POSITIVE AND NEGATIVE CONSEQUENCES OF A THERMAL SHOCK ON CERCARIAL SHEDDING OF INFECTED GALBA TRUNCATULA

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Topic: 6. Epidemiology and Infectious Diseases / Emerging Diseases

Thermal shock was often used in the past to stimulate cercarial shedding from infected lymnaeids such as \textit{Galba truncatula}. If this species was constantly bred at 20°C the use of a <10°C temperature applied two or three times a week rapidly caused the death of infected \textit{G. truncatula} during the first two or three weeks of the patent period. To induce cercarial shedding for this snail a less stressful temperature (12°C) applied once a week (3 h) was selected to study positive and negative consequences of this process in snails infected with \textit{Fasciola hepatica} or \textit{Paramphistomum daubneyi}. The results were compared with those of control snails infected according to the same protocol and always maintained at 20°C. A significant increase in the number of cercariae-shedding snails, a significantly longer patent period, and significantly greater cercarial production were noted in temperature-challenged (TC) snails, regardless of the type of digenean infection. In contrast, the number of incompletely formed metacercariae (mcc) was significantly higher in TC snails than in controls. Incompletely formed mcc of \textit{F. hepatica} consisted of cysts whose colour remained whitish after shedding (25.4% for TC snails) or whose dome was flattened after encystment (74.6%). Those of \textit{P. daubneyi} were totally dark brown after formation. These incomplete mcc might originate from young differentiating cercariae within the snail body (\textit{F. hepatica}) or from cercariae which died just after encystment (\textit{P. daubneyi}). The use of regular temperature changes for snails infected with \textit{F. hepatica} or \textit{P. daubneyi} must be monitored carefully during mcc collection to select completely formed cysts for infecting definitive hosts.
LEVEL OF COMPLIANCE WITH VACCINATION AGAINST RABIES AMONG DOGS IN ILORIN, NIGERIA

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Topic: 6. Epidemiology and Infectious Diseases / Zoonoses

Rabies is an acute encephalomyelitis of high fatality affecting all-warm blooded animals including man. It has been classified as eleventh (11th) killer disease of the world, killing 60-100,000 human beings annually. It is a zoonosis that poses global public health challenge in terms of its control and prevention.

This study provides vaccination profile of dogs in Ilorin as 51.05% vaccinated at private clinics, 18.42% vaccinated in government campaigns, 8.42% vaccinated at both private clinic and government campaign, 10.00% unknown vaccination status and 12.11% not vaccinated respectively.

Quantitative indirect enzyme-linked immunosorbent assay (I-ELISA) was used to detect antibody against rabies in sera from 190 randomly sampled dogs between June and December 2010 in Ilorin.

The study shows low vaccination compliance among dogs in Ilorin Nigeria.

Keywords: Dog, Rabies, Vaccination, Compliance, Ilorin.
SURVEY OF RISK FACTORS OF RABIES IN HUMAN IN ILORIN, NIGERIA

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Rabies is an acute viral encephalomyelitis of high fatality affecting all warm blooded animal including man. Rabies is caused by a bullet shape, enveloped, single-stranded RNA virus 130-240nm known as lyssa virus type1 and marked with variable incubation periods.

Survey of risk factors of rabies in human was conducted in Ilorin in October 2010 using direct street count and questionnaire survey. Five hundred questionnaires were administered to obtain primary data on risk factors. Hospital data from January 2002-December 2010 were used to identify spatial locations of dog bites in human whose victims were presented at the University of Ilorin Teaching Hospital for clinical care. Geographic co-ordinates of the identified location were captured and used to create spatial models of dog bite and rabies transmission in Ilorin.

A total of 162 cases of dog bite were retrieved. Some 21 (13%) patients were confirmed as clinical cases with fatal outcome. There were 14(66.5%), 5(24%) and 2(9.5%) fatal cases in children (age 0-15year old), youth (16-30 years old) and adult (>31 year old). Spatial clusters (p<0.001) of rabies virus infected dog bites was identified at Ilorin central abattoir and its adjoining open beef market.

Key words: Rabies, survey, Risk factor, Geographic co ordinates, Ilorin.
Brucellosis is considered as one of the major zoonotic infections worldwide. The aim of the study was to assess the knowledge, attitudes and practices (KAPs) of livestock owners with regards to brucellosis and to estimate seroprevalence of brucellosis in cattle and their owners in households. A cross-sectional study was carried out from June to August 2011 in four associated small villages of East Lahijan in Piranshahr, West Azarbaijan, Iran. One hundred households were selected using systematic random sampling and owner family and lactating cattle present in the household were randomly sampled and tested with ELISA for antibodies against *Brucella* spp. In addition, questionnaire regarding KAPs associated with brucellosis was administered. Household and cattle seroprevalence were estimated to be 33% and 27.2% respectively. Livestock owners had weak to moderate general knowledge of brucellosis.
The thermostability of rabies virus was studied by subjecting the ERA strain produced in Vero cells to accelerated aging (7 days at 37 °C) in the presence of stabilizers. The analysis of the obtained results by in vivo titration method (inoculation of rabies virus to young mouses) revealed that the rabies virus ERA/Vero keeps well without stabilizers at -20 °C or -70 °C, fairly well to +4 °C, but it is sensitive to temperatures above 25 °C and 37 °C. The rabies virus ERA/Vero stored at 37 °C for 7 days, is found with an acceptable titre. Apart from the PBS and the saccharose at 2% carried by the ENDEERS buffer, all the other media used in this study: ENDEERS buffer, sorbitol at 1% carried by ENDEERS buffer, sorbitol at 4% carried by the PBS, sorbitol at 4% carried by distilled water, have a stabilizing effect on the rabies virus ERA/Vero after 7 days of incubation at 37 °C. The best stabilizing effect on the rabies virus is obtained with the sorbitol at 4% carried by the PBS. The saccharose was ineffective; its use to a concentration at 2% carried by the ENDEERS buffer gave a negative effect on rabies virus. The sorbitol solution at 4% carried by the PBS can be an ideal formulation, adequate and economical to be incorporated in vaccine preparations, in order to reach the production of a rabies thermostable vaccine.
CONCORDANCE OF COMPETITIVE ELISA AND NESTED-POLYMERASE CHAIN REACTION IN THE DETECTION OF CAPRINE ARTHRITIS-ENCEPHALITIS VIRUS

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Topic: 6. Epidemiology and Infectious Diseases / Emerging Diseases

The study detected the presence of caprine arthritis-encephalitis virus (CAEV) in blood samples from 262 blood samples of goats and compared the results using competitive ELISA (cELISA) and nested-Polymerase Chain Reaction (nested-PCR) assay. Moreover, it determined the agreement using kappa (κ) statistic, analyze the genetic sequence of CAEV and describe the histopathologic features using carpal joint, brain, lung and mammary gland samples of CAEV positive animals. CAEV antibodies were detected in 15/262 (5.73%) of goat serum samples using cELISA, based on the use of monoclonal antibody binding to CAEV gp135 or SU glycoprotein. In nested-PCR assay targeting the CAE proviral gag region, 9/262 (3.44%) goats were positive which increased the number of positive animals detected to 19 (7.25%). Kappa statistic showed fair agreement between cELISA and nested PCR (κ= 0.39). DNA sequence of PCR product showed 91-98% homology among the reported CAEV genome in the GenBank. Histopathological findings were characterized by varying degrees of mononuclear cell infiltrations that conformed to the typical features of lentivirus infection.
PATHOGENIC PROPERTIES OF SWINE INFLUENZA A/VLADIMIR/2009(H3N2) VIRUS ISOLATE AT EXPERIMENTAL INFECTION

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Topic: 6. Epidemiology and Infectious Diseases / Emerging Diseases

In 2009, during swine influenza monitoring in one of the pig holdings of the Vladimir Oblast an influenza A/swine/Vladimir/2009(H3N2) virus isolate was detected. Phylogenetic analysis demonstrated that primary structure of HA (JX879784) and NA (JX879791) genomic segments were significantly different from all influenza A virus strains deposited in GenBank/EMBL. Pathogenic properties of A/swine/Vladimir/2009(H3N2) isolate were examined following experimental infection of 2-month-old influenza virus seronegative pigs of Large White breed (body weight: 12-16 kg). The isolate induced acute respiratory infection in pigs irrespective of the route of infection: intranasal or contact. Pig infection with the isolate resulted in acute respiratory disease manifested with such clinical signs as depression, aggravation of overall health condition, anorexia, serous rhinitis, conjunctivitis, coughing and hyperthermia. Pigs developed the disease symptoms on 2 d.p.i. and they lasted for 5 days. The disease concluded with absolute recovery. Post mortem examination of these animals euthanized after the experiment termination demonstrated no significant lesions in lungs or upper respiratory tract. Influenza virus was isolated by Real-time PCR from nasal swabs of infected pigs from day 2 to day 5 post infection/contact. The duration of influenza virus replication in the infected animals depends on general condition of the immune system: pigs with adequate immunity become virus-free within 5 days and in animals with compromised immunity the virus can replicate for much longer period of time. The virus replication in pigs was limited to respiratory organs; there was no viremia.

The works was performed within the ISTC Project # 2800.
SCHMALLENBERG VIRUS - MORE WIDESPREAD THAN HAS BEEN REPORTED?

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**Topic:** 6. Epidemiology and Infectious Diseases / Emerging Diseases

Diseases do not respect borders. It is therefore quite understandable that Schmallenberg virus may occur in countries bordering those in which herds and flocks have tested positive. Schmallenberg virus is transmitted by vectors and strong winds are said to be responsible for blowing these vectors from Europe to the United Kingdom. Is it possible that the virus is present in countries that do not share a border with those that have positive animals?

There are many viruses that produce similar defects in lambs and kids including blue tongue, Rift Valley fever, Wesselsbron, Middleburg and Akabane. Schmallenberg virus is very closely related to the Akabane virus which is widespread over Africa, Asia, Australia, the Middle East, and has been suspected in cases elsewhere. Is it possible that Schmallenberg mutated from Akabane? If so, it is likely that the prevalence of Schmallenberg virus could be more widespread than has been reported.

Without adequate tests that can identify viruses it is easy to ascribe certain malformations to other viruses, toxins or genetic abnormalities. It is therefore important that veterinarians attempt to identify the causes of malformations in offspring. This is often difficult as the initial infection, especially in the case of viruses, has taken place some months prior to the birth of the malformed young. However, if such cases are not investigated it may be some time before the causative agent is identified and thus substantial economic losses may occur before the disease is identified and brought under control.
EMERGING GENETIC DISORDERS IN MERINO SHEEP

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Topic: 6. Epidemiology and Infectious Diseases / Emerging Diseases

Congenital defects or neurological conditions may be due to viruses, toxins, or genetic abnormalities. Malformations of the branchial arches, clefts and grooves, congenital myoclonus, familial episodic ataxia and segmental axonopathy have recently been reported in Merino sheep.

Malformations of the branchial arches, clefts or grooves can lead to either incomplete or complete fistules. Where multiples are born, it is possible for the siblings to have different malformations, be mummified, or born with a normal appearance. The occurrence in a small flock of another case almost a decade earlier lends support to the likelihood that the defect has an hereditary origin.

Segmental axonopathy develops over time with most cases only presenting clinical signs at 14 to 15 months of age. It is hypothesised that nutritional imbalances may amplify the clinical manifestations of this disorder.

Congenital myoclonus and familial episodic ataxia present similarly although congenital myoclonus appears to be more severe. Repetition of stimulus results in a decrease in reaction until no reaction is observed in both conditions. By three to four months of age most lambs born with familial episodic ataxia appear normal thus increasing the risk of breeding with animals which carry this genetic disorder.

Continuing to breed with possible gene carriers of any of these conditions can lead to a gradual buildup of deleterious genes. Therefore, ignoring such cases have a negative impact on finances and profitability as a result of increasing prevalence of cases and loss of production due to a decrease in heterosis and gene diversity.
A NEW (DHPPI/L4R) CANINE COMBINATION VACCINE PREVENTS INFECTION, SHEDDING AND CLINICAL SIGNS FOLLOWING EXPERIMENTAL HETEROLOGOUS CHALLENGE WITH FOUR LEPTOSPIRA SEROVARS

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Topic: 6. Epidemiology and Infectious Diseases / Control, Prevention and Immunophylaxis

Results from four EU monograph challenge studies following vaccination of dogs with a multivalent vaccine containing Leptospira canicola, icterohaemorrhagiae, Bratislava and grippotyphosa are reported. Six week old dogs received two vaccinations, three weeks apart; control dogs received sterile water. Dogs were challenged 25 days after the second vaccination with heterologous strains of each Leptospira serovar, and observed for 28 days. Clinical observations were recorded, and blood samples collected throughout the study for Leptospira re-isolation and for biochemical and haematological analysis. Urine samples were collected throughout the study, and liver and kidney samples collected at necropsy for re-isolation of Leptospira.

Following challenge, controls dogs demonstrated various clinical signs while no vaccinated dogs were affected; each study had significant differences in the mean clinical scores (P=0.0005). Only following challenge with Leptospira icterohaemorrhagiae were there significant differences between vaccinates and controls in mean haematological scores (P=0.0279), no other significant differences were observed. Antibody titres to each Leptospira antigen were seen in vaccinated dogs 21 days following the first vaccination, further increases in titres were observed after challenge; controls remained seronegative until challenge. Leptospira were re-isolated from the blood, urine, kidney and liver of all control dogs following each challenge, but from none of the vaccinated dogs; significant differences were seen in days with positive isolation (for blood and urine) and the number of dogs with positive samples (kidney and liver).

In conclusion, vaccination of dogs with DHPPi/L4R induces immunity three weeks after second vaccination preventing infection, urinary shedding and clinical signs following challenge.
ID: 104

THE COST-BENEFIT ANALYSIS OF ALTERNATIVE BRUCELLOSIS CONTROL STRATEGIES IN TURKEY

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Topic: 6. Epidemiology and Infectious Diseases / Zoonoses

The aim of this study is to determine the most financially rational brucellosis control strategy for Turkey by means of cost-benefit analyses. In this study, four different infection control strategies were designed under three different scenarios named optimistic, expected and pessimistic scenarios. The most financially rational infection control option for Turkey was found to be the second strategy, which is “only young animals, three to six month old female bovine and three to six month old male and female ovine, have been vaccinated and after reaching the target prevalence for each species, vaccinations will be terminated and in the same year test and compulsory slaughter methods will be implemented throughout the country”. For the optimistic, expected and pessimistic scenarios according to second strategy the Net Present Value was estimated as -$3.1 million, $29.2 million and $41.9 million respectively, the Benefit/Cost ratio was estimated 0.86, 2.26 and 2.84 respectively. The results of these studies indicated that fighting with brucellosis is financially rational for expected and pessimistic scenarios. However, it is very important to consider a strategy that is optimal financially, but may not be suitable technically or in respect to public health.
In this study, it is aimed to estimate financial losses resulting from \textit{B. abortus} and \textit{B. melitensis} infections in bovines, ovines and humans for Turkey, according to optimistic, expected and pessimistic scenarios. The material of this research obtained from Delphi Expert Opinion Surveys (DEOS) and Targeted Groups Surveys (TGS). DEOS were carried out with specialist veterinarians and medical doctors, Targeted Groups Surveys were carried out with owners of officially reported diseased animals and the patients who had brucellosis. Results from this research demonstrated that according to weighted averages, the estimated financial losses of the optimistic, expected and pessimistic scenarios for an infected bovine were, respectively, around $38, $165 and $538; for an infected ovine around $10, $23 and $44; and for an infected human around $494, $807 and $1206. The total financial losses in Turkey resulting from \textit{B. abortus} and \textit{B. melitensis} infections were estimated for optimistic, expected and pessimistic scenarios respectively as $20, $41.3 and $61.7 million. The results of this research indicated that the authorities responsible should review the existing control strategies for dealing with brucellosis, determine technically and/or economically rational control strategies and also allocate more budget funds for the fight against brucellosis.
COMPARISON OF HUMERAL IMMUNITY IN TWO USUAL VACCINE IN FOOT AND MOUTH DISEASE IN LORI-BAKHTIARI SHEEP

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Topic: 6. Epidemiology and Infectious Diseases / Control, Prevention and Immunoprophylaxis

For humeral immunity comparison of usual FMD vaccines in Lori-Bakhtiari lamb selected 60 animals in three groups with mean weight 50 kg and without any previously vaccination against foot and mouth diseases and aged 6-12 months. Blood sample take before any vaccination for detect of antibody level. Then group 1 vaccinated with polyvalent Razi produced vaccine (A₀₅IR, Asia, O, A₈₇IR), group 2 vaccinated with Merial co. vaccine (O, AIR₈₇, A₂₂IRQ, Asia) and group 3 (control group) not vaccinated. Blood samples were taken in 21, 51, 81, 110, 140, 170, 230 and 350 days after vaccination. Serums were examined by serum neutralization test and detected different strains virus anti body titer. The data were analysis by SAS program. The result showed that different between group 1 (1.58 ± 0.03) and group 2 (1.47 ± 0.03) and with control group (0.73 ± 0.03) were significant (p<0.05). Protection immunity was remained at least until 230 days after vaccination or 8 month after vaccination (protective immunity =1.2).
The present study was planned to investigate the prevalence of Sarcocystis spp. among water buffaloes (Bubalus bubalis) at Alexandria province, Egypt. Enzyme linked immuno sorbent assay (ELISA) and indirect haemoagglutination assay (IHA) tests were used to evaluate the serological incidence of Sarcocystis spp. The result unveiled that 203 (67.6%) of the tested serum samples were seropositive to Sarcocystis spp. by ELISA while there were 191 (63.6%) positive samples by IHA among naturally infected water buffaloes. The sensitivity and specificity for both IHA and ELISA for detection of antibodies against Sarcocystis were determined by 94% and 100%, respectively. The mentioned result was confirmed by post-mortum (slaughtering) of the examined animals. Taken both results together, it was clear that the ELISA and IHA tests could be used as reliable screening tests for diagnosis of Sarcocystis spp. infection in living water buffaloes. The inter and intra species genetic polymorphism within Egyptian isolate of Sarcocystis spp. were also molecularly characterised using PCR and PCR-RFLPs to differentiate between isolates from two different geographical regions. Alexandria isolates (large and small size of the same host) and Assuite isolates (Upper Egypt) (large and small size of the same host). It was turned out that far distance between the two local isolates (small and large sized) in contrast there were no differences between the large size isolates. Moreover, a morphological study was made describing Sarcocystis spp. cyst. Throughout this investigation, the visual examination uncovered two completely different types of macroscopic cysts one was large sized and the other was small.
USE OF NANOPARTICULATE AND CONVENTIONAL ANTIMICROBIALS FOR TREATMENT OF SUBCLINICAL MASTITIS IN SHEEP AT DRYING OFF

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Topic: 6. Epidemiology and Infectious Diseases / Control, Prevention and Immunoprophylaxis

There are few studies in Brazilian herds about the control methods for mastitis in sheep as there is lack of specific products for treatment of this disease in ewes. The aim of this study is to determine the efficacy of subclinical mastitis treatment immediately before the dry period in ewes, as well the rates of spontaneous recovery in an untreated control group. Two antimicrobial formulations, a conventional and a nanoparticulate cloxacillin are being studied. Santa Ines ewes have been distributed in three groups, considering a 50% prevalence of subclinical mastitis: G1 (control); G2 (intramammary infusion of 100 mg of cloxacillin); G3 (intramammary infusion of 50 mg of cloxacillin nanoparticulate). The subclinical mastitis was previously diagnosed by California Mastitis Test (CMT) and somatic cell count (SCC). The infectious mastitis was confirmed by microbiological analysis before and after treatments likewise all treatments were performed after susceptibility tests in vitro. Preliminary results show that infectious subclinical mastitis was identified in 44 ewes. Up to now, coagulase-negative Staphylococci were the most prevalent microorganisms before treatments. SCC before treatment to G1, G2 and G3 were 1,622,000; 1,035,000 and 171,000 cells/mL and after treatment were 2,356,000; 1,025,000 and 384,000 cells/mL, respectively. The cure rates were 26.3% to G1; 45.0% to G2; and 60.0% to G3. So far, the cure rate for ewes treated by nanoparticulate antimicrobial have been higher than other groups. However, further investigation will be performed to evaluate possible differences among the groups and the association of the cure rates according to microorganisms.
EFFICACY OF VERSIFEL® FELV COMPARED TO PUREVAX® FELV IN MINIMUM AGE CATS FOLLOWING VIRULENT FELV CHALLENGE

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Topic: 6. Epidemiology and Infectious Diseases / Control, Prevention and Immunoprophylaxis

Objective To determine the efficacy of Versifel® FeLV Vaccine (Zoetis) when compared to PureVax® FeLV Vaccine (Merial Animal Health).

Design A challenge study under laboratory conditions was designed according to the requirements of the European Pharmacopoeia 6.0 Monograph 01/2008:1321 – Feline Leukaemia Vaccine (Inactivated). The study was carried out in compliance with national legislation and subject to local ethical review.

Fifty cats (8-9 weeks old) were vaccinated subcutaneously on two occasions, three weeks apart on Day 0 and Day 21, with either placebo (treatment group T01), Versifel® FeLV Vaccine (treatment group T02), or PureVax® FeLV Vaccine (treatment group T03). Cats were challenged three weeks after the second vaccination with a virulent FeLV (Feline Leukemia Virus) isolate (61E strain). Persistent FeLV antigenemia was determined from 3 to 15 weeks post-challenge. Bone marrow samples were tested for the presence of FeLV proviral DNA to determine FeLV latent infection.

Results: At week 15 after challenge with virulent FeLV 61E strain, the Versifel® FeLV Vaccine conferred 89.5% protection to FeLV persistent antigenemia and 94.7% protection to FeLV proviral DNA integration in bone marrow cells. In comparison, the PureVax® FeLV Vaccine conferred 20% protection to FeLV persistent antigenemia and 35% protection to FeLV proviral DNA integration in bone marrow cells following challenge.

Conclusions The data of this study indicate that, under laboratory conditions with this particular challenge model, the Versifel® FeLV Vaccine was more efficacious than the PureVax® FeLV Vaccine in preventing both FeLV persistent antigenemia and FeLV proviral DNA integration in bone marrow cells.
THE EVALUATION OF DOGS AND CATS IN THE JABOTICABAL CITY, BRAZIL

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**Topic:** 6. Epidemiology and Infectious Diseases / Zoonoses

The interaction of humans with pets is becoming increasingly narrow. However, adequate knowledge about responsible ownership, zoonoses and animal behavior does not seem to follow this partnership. Situations like increase in abandoned animals, domestic accidents with dogs and cats, uninformed owners, among others, are present in all socioeconomic levels. To minimize the problem, it is essential to improve actions and provide resources for population control programs, animal disease control and health education. Therefore, it is necessary to know the characteristics of canine and feline population, and the level of knowledge of people about responsible ownership. Georeferencing techniques, such as building a geographic database based diagnostic situation arise as an aid for planning that involves efficient measures to circumvent this whole pictures.
The objectives of this study were to set specific dog breed and sex type standards for total cholesterol (T-Cho) and total triglyceride (T-TG) concentrations, and to quantify the associations between age in different breeds and concentrations of both types of lipids. Blood samples of 496 dogs from 51 breeds were collected from a veterinary clinic between January and August in 2012. Plasma T-Cho and T-TG concentrations in the samples were determined by the clinic using the automated spectrophotometric analyzer. A generalized linear model was used to examine associations between concentrations of the two types of lipids and dog age, breed and sex type. Mean age (range) was 6.3 years old (0 to 16 years old), and mean (±SEM) T-Cho and T-TG concentrations were 205.4 ± 3.22 and 86.9 ± 3.42 mg/dL, respectively. Higher age was associated with higher T-Cho concentrations in all breeds (P < 0.05). As age increased from 0 to 16 years old, the T-Cho concentrations increased by 65.7 mg/dL. In addition, the T-TG concentrations increased by 5.0 mg/dL per year of age (P < 0.05). Miniature Schnauzers had the highest T-Cho concentrations of any breed, and Miniature Dachshunds had the lowest concentrations (P < 0.05). There was no association between T-TG concentrations and breed (P = 0.05). Additionally, there were no associations between sex types and T-Cho or T-TG concentrations (P > 0.11). Therefore, it is recommended that veterinarians consider dog age and breed when they use the T-Cho and T-TG concentrations for diagnostic purposes.
METABOLIC DISEASES IN DOGS RELATED WITH HYPERLIPIDEMI A AND RISK FACTORS FOR HYPERLIPIDEMIA

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The objectives of this study were 1) to examine the relationships between hyperlipidemia (HL) and metabolic diseases, 2) to determine factors associated with HL, and 3) to compare lipoprotein cholesterol and triglyceride concentrations between HL dogs and non-HL dogs. The study used medical records, including lipoprotein profiles in serum samples, of 3,942 dogs in 102 breeds obtained from 735 clinics between December 2005 and September 2011. Hyperlipidemia was defined as 300 mg/dL or higher of total cholesterol, or 150 mg/dL or higher of total triglyceride. Cholesterol and triglyceride concentrations were measured in four lipoprotein classes. Two-level mixed-effects models were applied using a clinic at level 2 and an individual dog at level 1. Of the 2,865 dogs with HL (72.7% of all dogs), 3.6, 6.8 and 3.7% had diabetes mellitus, hypothyroidism and hyperadrenocorticism, respectively. Factors associated with HL were dogs aged 6 years old or higher, spayed females and specific breeds such as Miniature Schnauzers and Shetland Sheepdogs (P < 0.05), but there was no association with sampling season (P = 0.49). The HL dogs had higher concentrations of cholesterol and triglyceride in each of the four lipoprotein classes (P < 0.05). Therefore, veterinarians are recommended to pay particular attention to HL dogs with high cholesterol or triglyceride concentrations in any of the lipoprotein classes, and also the high risk factor groups including spayed females, dogs aged 6 years or older and specific breeds such as Miniature Schnauzers and Shetland Sheepdogs.
RISK FACTORS ASSOCIATED WITH DOGS IN JAPAN HAVING HYPOTHYROIDISM EFFECTS ON LIPOPROTEIN CHOLESTEROL AND TRIGLYCERIDE CONCENTRATIONS

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The objectives of this study were to examine factors associated with dog hypothyroidism (HT), and to compare cholesterol and triglyceride concentrations in four lipoprotein classes between HT dogs and non-HT dogs. The study used medical records of 3,783 dogs in 101 breeds obtained from 735 clinics between December 2005 and September 2011. Diagnosis of HT by veterinarians was based on serum total thyroxine concentrations (< 0.5 µg/dL) and free thyroxine concentrations (< 5 pmol/L). The dataset included age, sex, breed and body condition score (BCS). Two-level mixed-effects models were applied using a clinic at level 2 and an individual dog at level 1. Hypothyroidism was diagnosed in 9.8% of the dogs studied. Factors associated with HT were higher age, non-hyperlipidemia and specific breeds including Shetland Sheepdogs (P < 0.05). The probability of dogs having HT increased from 5.0 to 10.2% as dog age rose from 3 to 10 years old. However, HT was not associated with BCS, sex type or sampling season (P > 0.07). The HT dogs had lower high density lipoprotein (HDL) cholesterol concentrations than non-HT dogs (P < 0.05), but there were no differences between HT and non-HT dogs for chylomicron, very low density lipoprotein and low density lipoprotein cholesterol concentrations, or any class of lipoprotein triglyceride concentrations (P > 0.07). Therefore, in order to improve HT diagnosis it is recommended that veterinarians pay particular attention to higher age dogs in specific breeds that have lower HDL cholesterol concentrations.
Epilepsy and or seizure are among the most common neurological disorders all over the world, including Nigeria and the aetiology may vary. However, the International League Against Epilepsy recognizes neurocysticercosis as a growing problem in many tropical countries and as a leading cause of epilepsy and seizures. Neurocysticercosis (NCC) is an infection of the central nervous system (CNS) caused by the metacestode stage (Cysticercus cellulosae) of the pig tapeworm of man *Taenia solium*. It is probably the most common helminth parasite incriminated in central nervous system parasitic infections in human beings. *T. solium* taeniasis and therefore, NCC are common in many of the world’s poorer countries especially where the environmental hygiene is poor and families raise free-roaming pigs that have easy access to and consume human faeces. In Nigeria, *T. solium* cysticercosis is common in pigs but there is little information on human *T. solium* taeniasis and virtually none on human *T. solium* cysticercosis (ocular- and neuro-cysticercosis). Conversely, epilepsy is the commonest neurological condition diagnosed in adults in most Teaching Hospitals in Nigeria but whether it could be attributed to neurocysticercosis is not clear. This paper examines neurocysticercosis in relation to epilepsy and seizures and draws attention to this important zoonosis which it seems in Nigeria is an unknown, a neglected or a completely forgotten major causative agent of neurological conditions.
Interleukin-12 (IL-12) can stimulate the natural killer cells and T lymphocytes which induce interferon-γ (IFN-γ), promote Th1 immunity and modulate immune reactions. The previous reports show that chicken IL-12 (chIL-12) plays a pivotal regulatory role for different antigens in the immune response. The goal addressed in this project is to provide a means to investigate regulatory mechanisms of chIL-12. The single chain chIL-12 encoding gene was cloned into pcDNA3 expression vector and transfected into the chicken embryo fibroblast DF-1 to develop a stable cell line (DF-1/chIL-12) expressing chIL-12 protein. The chIL-12 gene constructs was successfully expressed, proven and isolated by western blotting and Nickel ion affinity chromatography respectively. Interestingly, dimethyl sulphoxide could be an inducer that stimulates the stable cell to over-express the chIL-12 detected by fluorescence microscope. In addition, the bioactivity of chIL-12 protein significantly enhanced IFN-γ expressions in cultured primary chicken splenocytes. Taken together increased IFN-γ production might suggest the enhancement of the cell-mediated immunity. These innovation strategies of expression and purification of chIL-12 protein showed high bioactivity and consistently reproducible quantities. Importantly to the future that chIL-12 can be use as a potential biological adjuvant applied into the development of poultry vaccines.
EFFECT OF DOG’S VACCINATION (ANTI RABIES) RECORD KEEPING ON COST OF POST EXPOSURE HUMAN RABIES PROPHYLAXIS IN SRI LANKA

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Topic: 6. Epidemiology and Infectious Diseases / Zoonoses

Sri Lanka spends US$ 4.31 million annually for rabies post exposure prophylaxis (PEP) treatments for dog bite victims: stray dog bites, unvaccinated domestic dog bites and vaccinated domestic dog bites without proven records. Currently, dog’s vaccination record against rabies is used to decide whether PEP is indicated to the dog bite victim. Therefore some victims have to undergo PEP, if dog’s vaccination record is not produced even though dog is actually vaccinated.

The main objective of this study was to find out, how many dog owners after obtaining vaccination services from field clinics, kept their dog’s anti rabies vaccination records till the following year due date.

The details of dog owners came for anti rabies booster vaccinations without previous year vaccination records to field clinics in Dehiwala area from January 2012 to December 2012, were recorded.

Data revealed that out of 1858 dogs vaccinated against rabies, 1684 dogs were booster vaccinated. 25% (422) of the booster vaccinated dogs did not have previous year records ($P = 0.25, CI \pm 0.02$). Shifting to new houses (9%), displacement due to prevailing floods (18%), misplacement (72%), damages caused by rats (0.6%) and other (0.4%) were the given reasons for loss of records.

In conclusion, establishment and linking of electronic record keeping system between Government health care institutions in the area, persuading dog owners to keep vaccination records and enforcement of existing legislations are vital to reduce cost and risks of non-indicated PEP.

Key words: Rabies, vaccination, prophylaxis,
THE WITNESS® PARVO TEST, AN EFFECTIVE TOOL FOR DIAGNOSIS OF THE EMERGING CANINE PARVOVIRUS TYPE 2C

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We report the results of a study carried out to evaluate the analytical performances of the WITNESS® Parvo test (Synbiotics Corporation, Zoetis Inc.), an in-clinic test for detection of canine parvovirus (CPV). A total of 150 faecal samples collected from dogs displaying acute diarrhoea were submitted to the WITNESS® Parvo test, haemagglutination (HA) and real-time PCR assays. CPV was detected in 66, 73, and 101 faecal samples by means of in-clinic, HA and TaqMan testing, respectively. The relative sensibility and specificity of the WITNESS® Parvo test were 86.3% and 96.1%, respectively, when the test was compared to HA, and 65.3% and 100%, respectively, in comparison to real-time PCR (Fig. 1). The percentage of positive in-house tests was 70.5% for CPV-2a, 72.7% for CPV-2b and 75.0% for CPV-2c (p>0.05). Assuming real-time PCR as the gold standard for CPV detection, the WITNESS® Parvo test had higher specificity and comparable sensitivity with respect to HA, showing detection rates similar to those previously observed for other in-house rapid tests. The test was also able to detect with the same efficiency all CPV types, including the new variant CPV-2c (Fig. 2).

Although the WITNESS® Parvo tests was proven to detect the new variant CPV-2c with the same efficiency as the other variants, it is strongly recommended that faecal samples that are CPV negative by means of in-clinic assays are tested by PCR-based methods, especially when there is a strong clinical suspect of parvovirus.

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Fig. 1: Comparison of the WITNESS® Parvo test with haemagglutination (a) and real-time PCR (b). Numbers indicate the samples positive (+) or negative (−) for CPV. Results according to the compared techniques are shown in bold. The overall agreement, sensitivity and specificity of the in-clinic testing compared with the other assays were calculated and are indicated.
Fig. 2: Distribution of the WITNESS® Parvo test results by viral load (DNA copies mg⁻² of faeces) and CPV strain.
EPIDEMIOLOGICAL ASPECTS OF CANINE GRANULOCYTIC ANAPLASMOSIS IN CENTRAL ITALY

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Topic: 6. Epidemiology and Infectious Diseases / Vector-Borne Diseases

*Anaplasma phagocytophilum*, an intracellular obligate Gram – bacterium, infects granulocytic neutrophils and eosinophils of several animal species, including humans. The transmission is maintained by the hard tick *Ixodes ricinus* and the canine species plays an important role of sentinel in the epidemiological circuit of the pathogen. Aim of the present work was to conduct a cross-sectional survey on the prevalence and potential risk factors linked to this infection in a randomly sampled canine population in Central Italy. Individually blood and serum samples were obtained from 240 dogs, asymptomatic at clinical examination, and used for stained blood smears, serological test (IFAT) for IgG and IgM detection and PCR. Univariate’s analysis was performed to evaluate risk factors statistically associated to serological positivity. The results showed a seropositivity by IgG IFAT (titers from 1/80 to 1/320) in no. 24 dogs (10%), confirmed by PCR only in 6 dogs. Stained blood smears resulted negative for the pathogen. The variables statistically associated (p< 0.05) consisted on: age (OR: 5.1 for > 10 years old), breed/attitude (OR: 2.14 guard dog and OR:2.43 hunting dog), use of spot-on and collars as prophylactic measures to prevent tick’s infestation (OR:0.2 and OR:0.32), usual habitat (OR: 4.14 for dogs maintained outdoor), any contact with wild-life animals (OR:0.29) and any travel through endemic areas (OR: 0.2). The data obtained (in Italy only 2 studies have been conducted on this topic) confirmed the possibility of using the canine population as epidemiological sentinel for this zoonotic pathogen’s monitoring.
This study aimed to describe the occurrence of antibodies against leptospires in Pantanal and Caatinga, Brazilian biomes quite distinct in geo-climatic conditions. In each farm, blood and urine samples of free-living wild animals, domestic animals and humans were collected to perform serological test and Leptospira spp. culture. Antibodies to Leptospira spp. were detected by microscopic agglutination test (MAT). The urine cultures were evaluated weekly. When growth of Leptospira spp. was observed, the samples were characterized by VNTR (variable number tandem repeat analysis) and then submitted to serological typing. Of 84 serum samples analyzed in Pantanal, 47 (55.95%) were positive and of 133 samples analyzed in Caatinga, 59 (44.36%) were positive. By Fisher's exact test, there was no significant difference at 95% between the occurrence of antibodies against Leptospira spp. in these two biomes (p = 0.063). In a farm of Sobral, state of Ceará, Leptospira interrogans was cultivated from a female Cercocyon thous and a female Dasypus septemcinctus, the latter being similar to Pyrogenes serovar. In a farm in Sobradinho, state of Bahia, Leptospira interrogans was cultivated from a male Cavia aperea. The non-significant difference in occurrence of antibodies against Leptospira spp. between Pantanal and Caatinga and the leptospiras isolation findings in Caatinga open the discussion to the fact of leptospirosis in rural areas being a zoonosis whose epidemiological chain is directly related to poor sanitation practices, regardless of the environment condition and seasonality.
FACTORS INFLUENCING ANTIBIOTIC PRESCRIBING HABITS AND USE OF SENSITIVITY TESTING AMONGST VETERINARIANS IN EUROPE

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Topic: 6. Epidemiology and Infectious Diseases / Zoonoses

The Heads of Medicines Agencies and the Federation of Veterinarians of Europe undertook a survey to gain a better insight into the decision making process of veterinarians in Europe when prescribing antibiotics.

The survey was completed by 3017 practitioners including responses from 25 European countries. Analysis was to the level of different types of practitioner (food producing animals, companion animals, equines) and country for Belgium, Czech Republic, France, Germany, Spain, Sweden and UK.

Responses indicated

- No single information source is universally considered critical, though training, published literature and experience were indicated as the most important.
- Factors which most strongly influence prescribing behaviour were recorded as sensitivity tests, own experience, the risk for antibiotic resistance developing and ease of administration.
- Most practitioners usually take into account responsible use warnings.
- Antibiotic sensitivity testing is usually performed where a treatment failure has occurred.

Significant differences were observed in the frequency of sensitivity testing between the different types of practitioners and the different countries studied.

The responses indicate that there is a need to improve sensitivity tests and services in this area, with the availability of rapid and cheaper testing being key factors.
The aim of this study was evaluated the active and passive immunity against rotavirus induced by vaccination with an inactive commercial vaccine in cows and their respective calves from a farm of Cravinhos, state of São Paulo. The analysis of IgG, IgG1, IgG2, IgM and IgA were done in serum 60 days before calving (before vaccination); 30 days before calving (before revaccination) and in the day of calving, with indirect ELISA. The levels of IgG, IgG1, IgG2, IgM and IgA in colostrum were evaluated in the day of calving, with the same technique. The levels of calves’ immunoglobulin were evaluated in the day of born and 1, 7, 21 and 28 days of age. The secretion of rotavirus was evaluated in fecal samples of calves by SDS-PAGE. In this study, levels of IgM and IgA were increased in cows that were not vaccinated, and their calves, fed their colostrums, have levels of IgM increased one day after born. Vaccinated cows have serum IgG1 and colostral IgG2 higher than non vaccinated cows but statistics differences in levels of immunoglobulin were not found in calves. In both groups, was detected rotavirus in feces of calves, and besides the low occurrence in this study, one calf of vaccinated cow excreted rotavirus in Day 21, while two calves born of non vaccinated cows excreted rotavirus with 7 and 14 days. In conclusion, the vaccination of cows with a commercial inactivated vaccine did not prevent the rotaviruses in calves.
IMPROVED GENETIC METHODS FOR RABIES DIAGNOSIS AND THE MOLECULAR EPIDEMIOLOGICAL ANALYSIS OF RABIES VIRUSES ISOLATED IN TOKYO

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**Topic:** 6. Epidemiology and Infectious Diseases / Zoonoses

Rabies has not been reported since 1956 except for 3 imported cases in Japan. Therefore, it is the important issue to diagnose it rapidly and certainly. We developed real-time PCR and RT-nested PCR methods for the detection of rabies viruses and the molecular epidemiological analysis of rabies viruses isolated from animals in Tokyo in the 1950’s was studied.

Real-time PCR primer/probe sets and RT-nested PCR primers were designed by using nucleoprotein(N) gene sequences held in NCBI database. Detection limit and relative sensitivity of both assay were compared with RT-PCR. As the results, real-time PCR and RT-nested PCR could detect a lower number of virus copies (1000 times more sensitive) compared with RT-PCR.

The N gene of 10 rabies viruses isolated from animals in Tokyo in the 1950 amplified by RT-nested PCR were subjected for sequencing. The phylogenetic analysis was performed on 96 rabies viruses including 10 isolates and 86 strains (including Japanese fixed viruses, Komatsugawa, Takamen and Nishigahara) on database.

The rabies virus strains isolated in Tokyo were divided into 2 groups (Tokyo1 and Tokyo2), and grouped into the world wide distribution cluster. Tokyo1 was grouped into the same subcluster as the viruses isolated from dogs in USA in the 1930’–40’s. Tokyo2 was grouped into the same subcluster as Komatsugawa strain which was known as subcluster of the viruses from wild animals in Russia. These data suggested that rabies viruses in Tokyo in the 1950’s might relate to the routes from Russia and USA respectively.
THE EFFECT OF AMINEX (A NUCLEOTIDES AND PEPTIDES BASED IMMUNOSTIMULATORY SUBSTANCE) ON VACCINATION RESPONSE IN CATS

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Topic: 6. Epidemiology and Infectious Diseases / Control, Prevention and Imunoprophylaxis

The objective of this study was to evaluate the effect of Aminex (a nucleotides and peptides based immunostimulatory substance), in potentiation of response to vaccination of cats against feline panleucopenia, feline calicivirus infection and feline herpesvirus infection. Cats were divided into group A which recieved Aminex over a period of three months, and control group C which recieved placebo. Antibody response after vaccination was evaluated using ELISA antibody tests and lymphocyte proliferation was tested using BrdU lymphocyte proliferation assay. Significant changes in antibody titers (from p<0.0022 to p<0.015) were observed in response to vaccination against feline herpesvirus, feline calicivirus and feline panleukopenia virus. Moreover, protective levels of antibodies were reached earlier in group A in comparison with group C. Lymphocyte proliferation expressed in stimulation index was found significantly higher in group A comparing group C. Our results have shown that substances based on nucleotides and peptides are useful in potentiation of response to vaccination of cats and may play an important role in increasing immunocompetence and prevention against feline infectious diseases.
ID: 230

AN EXPLORATORY STUDY OF HUMAN ACCIDENTS BY DOGS AND CATS BITES IN THE STATE OF MATO GROSSO, BRAZIL


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Topic: 6. Epidemiology and Infectious Diseases / Control, Prevention and Immunoprophylaxis

The intensification of the relationships between humans and animals is due to factors like the search for security, having fewer children and studies about the benefits that they can bring to family health. However, some problems can occur due to lack of knowledge about responsibility that the pet owner has and about zoonosis, for example, in situations with pet attacks. This study, with an exploratory and explanatory character, is essential for future researches that aimed an effective inversion of the reality, and evaluated the records of human rabies prophylaxis in the state of Mato Grosso, Brazil. In the year 2012, 8,300 people were registered after being involved in accidents with animals aggressors. Of these, 7,725 lived in urban areas and 575 in rural areas. The number of patients who received the vaccine rabies post-exposure was 7,186 and 13,168 doses were applied. The dogs were the most associated with the injuries, corresponding to 5,845 accidents in urban areas and 300 in rural areas. The cats were responsible for 658 accidents in urban and 55 in rural areas and the bats for 45 in urban and 32 in rural areas. The bovine was cited in 22 occurrences in urban and 35 in rural areas. These results are seen in other states of Brazil, which generates high economic injury levels, because the high costs with the vaccines and the treatments of the patients. It is suggested investments in educational campaigns to elucidate the population and health professionals about the epidemiology of this disease.
ID: 231

ZOO NOTIC AGENTS IN A DOMESTIC PIGEON (COLUMBA LIVIA) COLONY IN CENTRAL ITALY

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Topic: 6. Epidemiology and Infectious Diseases / Zoonoses

Urban pigeons has raised public health concern, being considered potential reservoirs of pathogenic organisms. A survey was conducted in a population of 100 domestic pigeons settled near a hospital in Central Italy. The pigeons were investigated for both zoonotic infectious agents (Salmonella spp., Campylobacter jejuni and C. coli, Coxiella burnetii, Chlamyphila spp.) and parasitic organisms (Cryptosporidium spp., Giardia spp., Toxoplasma gondii). Individual faecal samples were cultivated in different media according to the nutritive requirements of bacterial agents searched. C. burnetii, C. psittaci and Chlamyphila spp. DNAs were investigated in 25 faecal pools by PCR. ELISA screening test on individual faecal samples were performed for Giardia spp. and Cryptosporidium spp., while individual serological samples were tested with commercial MAT (Modified Agglutination Test) to highlight antibodies against T. gondii. Results showed that all fecal pools were negative for C. burnetii DNA, while one pool resulted positive for C. psittaci DNA and other two pools were positive for Chlamyphila genus. C. jejuni and C. coli were found respectively in 12.86% and 4.2% of cases. The 8% of the serum samples were positive for T. gondii. No Salmonella spp. was detected. The presence of C. jejuni and C. coli, T. gondii antibodies and C. psittaci DNA should be evaluated in relation to the maintenance of these organisms in urban environment. The excellent state of nutrition of the subjects confirmed the urban habitat as optimal environment for the available shelters and sources of nutrition.
Schmallenberg virus (SBV) is an Orthobunyavirus of the Simbu serogroup that has been recognized as the causative agent of a large outbreak in European ruminants. After its first detection in 2011, monitoring by serological analysis and real-time RT-PCR of SBV started in January 2013 in Croatia. Consequently, the presence of antibodies against SBV, as well real-time RT-PCR positive samples were recognized for the first time. Investigation of the SBV seroprevalence included 50 sera of cattle originating from different counties. Sera were checked by ID Screen®, Schmallenberg virus Indirect, (ID.vet, France), a commercially available indirect enzyme-linked immunosorbent assay (ELISA) according to manufactures instructions. Antibodies against SBV were detected in 52% of tested sera. The highest prevalence was found in the northern part of Croatia. The presence of SBV-RNA in organ samples of aborted foetuses, stillbirths or malformed newborns was conducted by the use of real time RT-PCR assays developed by the Friedrich-Loeffler Institute, Germany. Altogether, 33 brain samples of aborted calves, at the 5-7 month of gestation, 1 brain from malformed newborn calf, 7 cow placentas, 8 brains of aborted lambs and 3 brains of aborted kids were examined. Six samples, five from calf brains and one from cow placenta, were positive for SBV-RNA, with cycle threshold (Ct) values of 28.6–38.8. A relative high percentage of seropositive animals, as well as SBV positive abortions, stillbirths and malformed newborn in the first months of 2013 implicates that the infection has occurred in 2012.
EQUINE INFECTIOUS ANEMIA: RISK FACTORS IN CENTRAL ITALY 2007-2011

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Topic: 6. Epidemiology and Infectious Diseases / Control, Prevention and Immunoprophylaxis

Equine Infectious Anemia (EIA) is caused by a retrovirus related to the human immunodeficiency virus.

Data and serum samples from national surveillance plans in 2007-2011, were analyzed and tested to evaluate the EIA seroprevalence and the risk factors in equid populations in central Italy.

Equine sera were tested by the local laboratory using the Coggins test; positive samples were confirmed by the EIA national reference laboratory. Odds Ratio was used as a measure of association in the identification of age, specie (included mules although they are not as yet classified as a separate species), sex and seasonality as risk factors. The variables with \( p \)-values \(< 0.05 \) were entered into the multivariable model and the backward elimination technique was used.

All equines more than three months old in 2007 and six months old in 2008-2011, were tested.


Univariable analysis demonstrated that mules had 50 in 2007, 40 in 2008, 25 in 2010 and 40 in 2011 fold higher chance to be seropositive than other species.

In the final main-effect model of the multivariate analysis, species remained significant risk factor for all year 2007-2011 (they are used to transport wood in roadless mountainous areas); age (old animal) remained significant risk factor for 2007-2010; unexpectedly for a disease transmitted by biting insect, seasonality remained significant risk factor in 2007 and in 2010.
H1 subtype virus was one of the most abundant viruses routinely isolated from wild birds, swine and human, which can potentially cross species barriers. This study was to investigate the potential for cross-species transmission of influenza viruses through the comparison of antigenic, genetic and pathogenic characteristics using different host origin of H1 influenza viruses (AIVs) in Korea. In phylogenetic analysis, H1 AIVs circulating in Korea provides evidence of genetic similarity between the domestic ducks and wild bird viruses, whereas there are no relationships between the avian and swine viruses although there was viral gene transfer between swine and human viruses in part. In chicken infection experiment, viral replication was much higher in domestic duck origin viruses than wild bird origin viruses and in domestic ducks, both domestic duck origin viruses and wild bird viruses replicated poorly. While, none of swine origin viruses were replicated in chickens and domestic ducks. However, the replication of these H1 viruses was accompanied by relatively high titers in mice regardless of host origins and induced clinical signs such as ruffled fur and squatting with significantly weight loss. Thus, even though there is no evidence of any interspecies transmission between avian and swine in the isolated H1 viruses, these H1 viruses have a potential to expand the host range to mammals given conceivably increase of the contact chance. Therefore, our results suggest that avoiding the contact chance among the different animal species be a good way to prevent interspecies transmission.
NOTIONS ABOUT VISCERAL LEISHMANIOSIS: EVALUATION OF TEACHERS OF ELEMENTARY EDUCATION OF TWO MUNICIPAL SCHOOLS OF JABOTICABAL, SAO PAULO, BRAZIL

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Topic: 6. Epidemiology and Infectious Diseases / Zoonoses

Leishmaniasis is anthropozoonosis caused by *Leishmania spp* and can be transmitted to humans by the bite of a mosquito of the gender Lutzomyia. The main reservoir is the dog, and the most serious form of the disease is the Visceral Leishmaniasis (VL), characterized by a multifaceted syndrome that is fatal in 100% of cases clinically evident and untreated. In Brazil, it has been reported in 20 of 27 states, with autochthonous transmission in approximately 1600 of 5564 municipalities in the country. The disease spreads about 30 km per year, which can be explained by the intense migration of animals and build homes in forested areas, taking the vector to seek refuge in urban areas. This study aimed to evaluate if elementary school teachers from two schools in the municipality of Jaboticabal have knowledge about VL. The evaluation took place through the use of questionnaires to 42 teachers, and the results showed total lack of information about the disease: 64.3% of respondents believed that humans can not be affected by leishmaniasis, 42.9% did not know that transmission occurs through mosquitoes, 69% said they did not know if the dog has a stake in the disease cycle and 90.5% could not name any preventive measure. These data are alarming, since nearby municipalities already report cases of VL, being a matter of time before the disease reaches Jaboticabal and, without guidance, the population is totally unprepared to fight this disease.

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DERMATOPHTHOTOSIS RESPONDED TO IMMUNOTHERAPY IN CAPTIVE EXOTIC PRIMATES MACACA FUSCATA

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Topic: 6. Epidemiology and Infectious Diseases / Control, Prevention and Immunophylaxis

Dermatophytoses in exotic primates are poorly described in the literature. Here we describe a case study of dermatophytosis caused by Microsporum canis in two Japanese macaques (Macaca fuscata) and successfully treated with antidermatophytic vaccine Microderm.

Two Japanese macaques (Macaca fuscata) (males, age of 1,5 and 2 years) keeping in private house have been examined. Clinical manifestation was expressed by focal alopeces on the forearms, elbows and hands. Skin lesions were covered by gray crusts. Purulent inflammatory processes, permanents scratching were also observed.

Direct microscopy of both samples from affected sections revealed abundant arthrospores and fungal hyphae. On selective media the culture of Microsporum canis was isolated from one animal. In second case the dermatophyte was not isolated. It can be interpreted by the previous local treatment with 10% tincture of iodine. The satellite presence of Staphylococcus spp. in lesions was detected in both cases. Study of antibiotic susceptibility showed that erythromycin was the most effective agent against Staphylococcus culture.

Basing on the laboratory findings the therapeutic vaccination accompanied by erythromycin local treatment was prescribed. Commercial live vaccine “Microderm” for prophylaxis and therapy of dermatophytosis in small animals was administered. The vaccination was well-tolerated by macaques and provoked no adverse effects. On day 10 after vaccination the significant reduction of clinical signs was detected. Total clinical recovery was revealed on day 23-25 and was confirmed by mycological examination.

The study demonstrates susceptibility of macaques to Microsporum canis and applicability of live vaccine Microderm for treatment of dermatophytosis.
COMMERCIAL VACCINES AS A MAIN TOOL FOR TREATMENT AND PREVENTION OF ANIMAL DERMATOPHYTOSIS IN RUSSIA

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**Topic:** 6. Epidemiology and Infectious Diseases / Control, Prevention and Immunoprophylaxis

In recent years significant strides have been made in understanding the immunopathogenesis of fungal diseases and elaboration of medical antifungal vaccines although they are still out of wide-range practical implementation. The vaccines represent a preferable alternative to antifungal drug therapy which is often limited by toxicity, resistance and high cost. Moreover teratogenic and carcinogenic properties of antifugals have recently been revealed.

At the same time the veterinarians already possess effective antifungal vaccines. In Russia antidermatophytic vaccines are successfully used since 1970 y. In contrast to many other countries in Russia the vaccines but not antifungal drugs are the primary remedies in treatment of animal dermatophytoses.

Nowadays a variety of the vaccines are elaborated, approved and introduced to the clinical practice including LTF-130 against trichophytosis in cattle, Microderm against dermatophytosis in small animals (cats, dogs, rodents, fur animals), Equivac against dermatophytosis in horses. The above vaccines contain live highly-immunogenic attenuated fungal strains and promote both preventive and therapeutic effect. The immune tolerance lasts for at least one year.

The benefits of vaccines are safety, efficiency and economic feasibility. They are well tolerated by animals with no side effects. All the officially approved vaccines are monitored by governmental regulators for compliance with the quality and safety standards. Recently the vaccines Microderm and LTF-130 have been registered and officially approved for use in European Community.

Nowadays the antidermatophytic vaccines represent the strategic significance. Their wide-range usage able to decrease dramatically the incidence of dermatophytoses in animals and correspondently in humans.
LARGE-SCALE VACCINATION AND ELIMINATION OF LATENT FUNGAL CARRIAGE AS A MAIN MEASURES FOR CONTROL OF ZOONOTIC DERMATOPHYTOSES

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**Topic:** 6. Epidemiology and Infectious Diseases / Control, Prevention and Immunoprophylaxis

While the clinically affected animals are well-known source of dermatophytic infection the latent (asymptomatic) fungal carriers are still out of veterinary focus. Meanwhile the asymptomatic fungal carriage (AFC) play a role as a latent source of infection for humans and animals and as a source of environmental fungal contamination. The prevalence of AFC in pets is reported to be global (up to 90%).

AFC detection and treatment is still problematic in veterinary practice. In contrast to clinically affected animals the AFC have no any evident symptoms. An effective procedure for wide-ranging screening studies is necessary. This problem could be resolved by establishing an international “Veterinary Mycological Certificate” system as a part of existing system of veterinary inspection. Such a system will allow to confirm mycological safety of the animals basing in laboratory examination.

Fungal carrying can not be detected by routine express-methods such as luminescence and direct microscopy. Mycological analysis of hair samples collected with MacKenzie brush technique should be applied to detect hidden spores of dermatophytes. Further treatment with topic antifungals (shampoos or sprays allowing to treat the whole body) should be prescribed.

Another component of the strategy is the preventive immunization with antidermatophytic vaccines. The Russian experience demonstrated the dramatic reduction of cattle trichophytosis when total livestock vaccination was applied. Pet’s preventive vaccination is not total but still significant and leads to relatively low prevalence of dermatophytosis in Russia. Effective control of both human and animal dermatophytoses is impossible without preventive measures representing a socially-significant mission.
CHARACTERIZATION OF RUSSIAN FELINE IMMUNODEFICIENCY VIRUS ISOLATES AND USING REAL-TIME PCR FOR DIAGNOSTICS

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Topic: 6. Epidemiology and Infectious Diseases / Control, Prevention and Immunoprophylaxis

Feline immunodeficiency virus (FIV) is a lentivirus associated with an AIDS-like disease in cats. A major obstacle in developing PCR techniques for diagnostics of lentivirus infection is high variability of viral genome. The nucleotide sequence divergence is up to 20% among FIV field isolates. Assessment of some commercial PCR assays showed overall lower sensitivity for diagnosing FIV infection than serologic assays [Ammersbach M., 2013].

Real-time PCR kit «VIK-AmpliSens» was developed. The degenerate primers and probe were designed from the most conserved portions of the LTR region as defined by the sequence alignment of known subtypes and investigated Russian isolates. The sensitivity and specificity were estimated by comparing with commercial chromatographic immunoassays. It was shown that «VIK-AmpliSens» is more sensitive than «Anigen-Rapid-FIV-Ab» and «FIVTEST» kits. As it has been reported by other researchers [Krasnikova E.S., 2012] among patients with severe symptoms only 23.3% were positives with immunostrip test whereas 60% were positives with «VIK-AmpliSens». All PCR positive results correlated with abnormal blood test parameters and clinical signs.

Over 5000 cats were tested using «VIK-AmpliSens». The prevalence of infection varies from about 3% (in healthy cats) to as high as 12.5% (in symptomatic cats).

To gain insight into the genetic diversity of Russian isolates the nucleotide sequences of their gag and env genes were compared with those of previously described isolates. Surprising, the majority of Russian isolates clustered to subtype E which described only for Argentina [Pecoraro M.R., 1996] whereas the most widely distributed in Europe and Asia subtypes are A and B.
THE ENDEMIC STATUS OF ANAPLASMA MARGINALE IN CATTLE

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Topic: 6. Epidemiology and Infectious Diseases / Vector-Borne Diseases

This study was designed to determine the prevalence and endemic status of *Anaplasma marginale* in cattle in the province of Konya. A total of 700 cattle were randomly selected, assigned to different age groups (i.e. 0-6, 7-12, 13-24, 25-36 and >36 months) and examined in Kadinhani, Cumra and Beysehir provinces and in Konya city centre. *A. marginale* was diagnosed by competitive ELISA (cELISA) testing. A total of 223 (31.86%) serum samples were positive against *A. marginale* antibodies. The rates of seropositivity according to age group were 19.1, 24.39, 33.14, 44.21 and 37.36%, respectively. The endemic status of the disease was determined by calculating the inoculation rate (*h*) of each age group. The *h* values were detected to be lower than 0.005 in each groups and the endemic status of *A. marginale* was found to be instable.
DIAGNOSIS OF CYSTIC HYDATIDOSIS IN EXPERIMENTALLY INFECTED SHEEP BY ELISA AND IFAT

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**Topic:** 6. Epidemiology and Infectious Diseases / Zoonoses

This study was carried out to determine the sensitivities of ELISA and an IFA test in order to serologic diagnosis of cystic hydatidosis in experimentally infected sheep. A total of 20 sheep (4-5 months) were subjected in this study as 10 for control and another 10 for experimental group. A total of 15,000 live protoscolex collected from liver with hydatid cyst were administered into a dog via oral route, and at the end of two months, matured *E. granulosus* was collected from intestinal lumen. Approximately 2000 eggs were administered into each sheep in the experiment group. Sheep had been observed for 12 months. During this period, all the sheep were checked by using both tests for parasite specific antibodies. ELISA test used with partially purified cyst fluid antigen had shown 57% sensitivity and 67% specificity when compared to necropsy. Antibody responses in sheep during 12 months were shown different peaks in different times. The foremost antibody response was determined in two weeks after inoculation. No antibody response was determined in calcified cysts. In this study, no specific responses in IFA test in which protoscolex antigen was used.

In conclusion, more reliable results were observed in ELISA test in sheep with experimental infection for the diagnosis of cystic hydatidosis compared to IFA test. Necropsy findings can be used as reference for determination of sensitivity and specificity of serology.
PCR DIAGNOSTICS AND GENETIC DIVERSITY OF RUSSIAN BEAK AND FEATHER DISEASE VIRUS ISOLATES

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Topic: 6. Epidemiology and Infectious Diseases / Control of Diseases in Exotic Zoo Animals

Beak and feather disease (BFD) is one of the most common serious psittacine diseases caused by beak and feather disease virus (BFDV), a Circovirus of the family Circoviridae. To study an occurrence of BFDV in psittacine populations in Moscow region blood samples, feathers and fecal material from birds representing different psittacine species were examined using ORF C1 based PCR. BFDV was detected among 35% parrots show clinical signs of beak and feather disease as well among 15% clinically normal birds.

The nucleotide sequences of the ORF C1 of 11 unique BFDV isolates were determined and compared with the previously described sequences of BFDV. Phylogenetic analysis of isolates showed that some Russian BFDV isolates derived from different hosts in 2008 and 2012 grouped together. The most of our isolates from budgerigars showed close genetic relationship and formed 2 distinct clusters with budgerigar strains from Africa, Europe, Japan and China. Our results confirm that the BFDV host specificity is not absolute, the relationship between BFD viral strains, psittacine species and pathogenicity is very complex and BFDV detected in budgerigars differ from other BFDV strains.
PROPOSALS FOR IMPROVEMENT THE CONCEPTUAL ASPECTS OF RISK ANALYSIS USED FOR ANIMAL IMPORT RISK ANALYSIS

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Topic: 6. Epidemiology and Infectious Diseases / Spread of Pathogens through International Trade

Both, qualitative and quantitative risk analysis, were developed for animal import activities. While quantitative risk analysis is based on probabilistic approach using numeric estimation, the qualitative risk analysis is based on descriptive approach without a real conceptual base.

This paper is directed to explain the conceptual base for qualitative approach. In this respect, each event has positive or negative consequences, each of them having as well known and unknown elements. We propose to defined positive known elements as chance, the positive unknown elements as hazard, the negative known elements as risk and the negative unknown elements as misfortune or ill luck.

A danger consists in a sum of risk and hazard having consequences as chance or misfortune.

In this context, the risk is a notion that can be identified and known, so it can be quantified by description or probabilistic, it can be managed and treated/controlled.

By contrary, the hazard can not be identified and known and can not be quantified by description or probabilistic, it can be managed and treated/controlled.

The more detailed the risk is identified and defined; the less is the incidence of hazard within a danger.

This is consistent with ISO Guide 73: Risk management – Vocabulary referring to risk or hazard definition as well as likelihood and probability definitions: probability expressed as a number between 0 and 1 is submitted to general laws and more correlated with risk, while likelihood is not submitted to any law and mostly correlated with hazard.
DETERMINATION OF THE OPTIMAL CULTIVATION CONDITIONS FOR THE FIELD ISOLATES OF CHLAMIDIA PSITTACI ON THE CELL CULTURE

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Topic: 6. Epidemiology and Infectious Diseases / Control, Prevention and Immunophylaxis

Perspective method against animal’s clamidiosis is the vaccination. Regular vaccination in disadvantaged farms provides termination of abortion and premature termination of pregnancy.

The aim of this study was to adapt local strains of chlamydia on the primary and continuous cell lines to develop technology of production of cultural vaccine against animal’s clamidiosis.

Passaging of field isolates of Chl. psittaci on primary cell cultures of lamb’s kidney and testicular was carried out three times. During culturing of the infected culture a cytopathic effect (CPE) was not observed.

During passaging of field isolates on the continuous cell lines McCoy B, PS and M-Hella the CPE was observed. CPE in McCoy B cell line was not significant and appeared after 7-10 days, like rounded cells formation and destruction of monolayer. In M-Hella cell line CPE was observed after 4-5 days, by forming rounded cells with dark granular cytoplasm and destruction of the monolayer. In PS cell line CPE appeared after 12-14 days by symplast formation.

To determine the biological activity of cultural chlamydia, five passages on developing chicken embryos (DCE) were carried out. In the second passage specific death was observed on 8-9 days after infection, in the third passage the time reduced up to 5-6 days. On smears of yolk sac walls, elementary bodies of chlamydia were detected.

Also, complement fixation reaction was carried out. Results shows that activity of chlamydia grown on continuous cell lines higher than on primary cell lines, especially chlamydia grown on M-Hella cell culture showed the best results.
SEROEPIDEMIOLOGICAL AND MOLECULAR STUDY OF EQUINE INFLUENZA IN ALGERIA

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Topic: 6. Epidemiology and Infectious Diseases / Spread of Pathogens through International Trade

In order to determine the equine influenza seroprevalence in Algeria, 297 sera from vaccinated horses stemming from the Algerian West (Tiaret region) and from the Algerian East (region of EL-Eulma and Barika) were investigated using both an ELISA test and inhibition of haemagglutination (IHA) test.

The results obtained with ELISA are correlated with those obtained with the IHA test performed with H3N8/Russia/1983 strain. Differences in seropositivity rates for H3N8/Russia/1983 and H3N8/Miami/1963 strains by IHA suggest that horses have antibodies with improved affinity for the H3N8/Russia/1983 strain. The seroprevalence rates were significantly higher in old horses (above 10 years old) than in younger ones and in males Arabian thoroughbred horses than in females. Furthermore, a significantly increased susceptibility of Arabian thoroughbred horses compared to English thoroughbred and poorly represented breeds was noticed.

The equine influenza virus (H3N8) seems to circulate among the equine population in Algeria, unlike virus subyupe H7N7, like in the rest of the world, which does not seem to be present since the 5 sera found positive with IHA were from imported horses whose vaccination status was unknown.

This study describes the isolation and molecular characterization of the influenza virus that was responsible for the outbreak that surged in 2011 in iaret region.
DETECTION OF RADIOCESIUM IN THE FUKUSHIMA WILD JAPANESE MONKEY BY THE FUKUSHIMA DAIICHI NUCLEAR DISASTER

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Topic: 6. Epidemiology and Infectious Diseases / Emerging Diseases

Following the massive earthquake that struck eastern Japan on March 11, 2011, a nuclear reactor core meltdown occurred at the Fukushima Daiichi Nuclear Power Plant, operated by Tokyo Electric Power Company, and was followed by the release of large amounts of radioactive materials. Then, we predicted that Japanese monkey (Macaca fuscata) in this area was also polluted by some radioactive materials. Because scientists were not currently permitted to enter the restricted areas for animal research, we carried out with a population of Japanese monkeys inhabiting Fukushima City, located 70 km from the Fukushima Daiichi Nuclear Power Plant under the permission of the governor of Fukushima Prefecture, according to the Fukushima Japanese Monkey Management Plan which was established based on the Wildlife Protection and Hunting Management Law. The concentration of radiocesium 134Cs and 137Cs were measured in the muscle of individuals. Cesium concentration was high (10,000–25,000 Bq/kg) in April 2011 and decreased over 3 months to around 1,000 Bq/kg. However, the concentration increased again to 2,000–3,000 Bq/kg in some animals during and after December 2011 before returning to 1,000 Bq/kg in April 2012, after which it sustained. This pattern of change in muscle radiocesium concentration was similar to that of the change in radiocesium concentration in atmospheric fallout. The monkeys fed on winter buds and the cambium layer of tree bark containing a higher concentration of radiocesium during the winter season. The muscle radiocesium concentration in the monkeys related significantly with the level of soil contamination at the capture locations.
AUTUMN 2012, BLUETONGUE SEROTYPES 1 AND 4 IN SARDINIA: NEW INCURSIONS OR RE-INFECTION WITH OLD STRAINS?

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Topic: 6. Epidemiology and Infectious Diseases / Vector-Borne Diseases

Since 2000 several Bluetongue virus (BTV) incursions occurred in Sardinia (Italy) involving serotypes 1, 2, 4 and 16. In October 2012, new BT outbreaks caused by BTV-1 and BTV-4 were reported. Nearly 500 flocks were infected and 9238 sheep died because of the infection. When sequenced, Seg-10 of both strains share 99% similarity at nucleotide level with the BTV strains which have circulated in the Mediterranean basin in the last few years. Similarly, the Seg-5 sequences of the BTV-4 and BTV-1 newly isolated Sardinian strains are identical and cluster together with the recent BTV-1 circulating in the Mediterranean basin and the BTV-4 strains which were isolated in Tunisia in 2007 and 2009. These BTV-4 strains differ from those which circulated in Europe from 2003-2005 and appear to be reassortant strains.
EXPERIMENTAL INFECTION OF PIGEONS (COLUMBA LIVIA) WITH ITALIAN WEST NILE VIRUS LINEAGE 1 STRAINS

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Topic: 6. Epidemiology and Infectious Diseases / Zoonoses

To understand whether pigeons could represent a severe threat for public health after infection with West Nile virus (WNV) three groups of 8 pigeons were each infected subcutaneously with $10^6$ TCID$_{50}$/mL of the Italian WNV Lineage 1 strains responsible for the 2009, 2011 or 2012 WND outbreaks, respectively. Three pigeons were also sham-inoculated and left as a negative control. Serum samples, oropharyngeal and cloacal swabs were collected daily. Serum samples were tested for WNV antibodies by serum neutralisation, whereas sera and swabs were tested for the presence of infectious virus and WNV RNA, by virus isolation and real time RT-PCR, respectively. Viraemia and competence reservoir index (CRI) were also calculated.

All birds seroconverted starting from day 5 post infection (dpi) and antibodies were detected throughout the sampling period (35 dpi). All animals of the infected groups become viraemic from day 1 to day 4 pi. Mean viral titres ranged from $10^{1.99}$ (WNV 2009) to $10^{5.39}$ (WNV 2012) TCID$_{50}$/mL.

Competence reservoir indexes were 0.14 in animals infected with WNV 2009; 0.11 in those infected with 2011 and 0.12 in those infected with WNV 2012. No significant differences were found between the WNV strain CRI.

This study proved that pigeons are competent reservoir hosts for Italian WNV strains. For their particular ecology, they could represent a serious public health threat. They could carry WNV infection from rural to urban area and, once there, they could amplify the viral concentration increasing the risk of human infections.
COMPARATIVE EFFICACY OF MAROPITANT, METOCLOPRAMID AND ONDANSETRON IN PREVENT EMESIS INDUCED PARVOVIRAL ENTERITIS

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Topic: 6. Epidemiology and Infectious Diseases / Emerging Diseases

Parvoviral enteritis (PVE) is a viral disease which commonly seen in dogs between 6 weeks–12 months of age. The aim of this project was to evaluate the efficiencies of selected antiemetic drugs (metoclopramide, ondansetron and maropitant) to prevent vomiting in the treatment of canine parvoviral enteritis.

PVE quick ELISA test positive dogs between 4-12 months of age were included in the study. Each of metoclopramide, ondansetron, maropitant and control group had 8 dogs. Metoclopramide and ondansetron were administered with 0.5 mg/kg doses in three times a day via intravenous route, maropitant was administered with 1 mg/kg doses single in a day via subcutaneous route, but any antiemetic drug was not administered in control group. Hematology, serum biochemical examinations were done in 0. (before drug administration), 1., 3. and 5. days of treatment. The number and the severity of daily vomitings were recorded.

It was noted that, metoclopramide, ondansetron and maropitant decreased the severity of vomiting from the first day and the vomiting numbers from the third day in PVE treatment. Obtained results showed that maropitant which is a new drug in veterinary medicine, can be used successfully such as metoclopramide and ondansetron which are frequently used for PVE treatment. At the same time, it was discovered that metoclopramide, ondansetron and maropitant which are used in treatments as antiemetics, had no advantages against each other to reduce the number and the severity of vomiting.

Key words: Dog, parvoviral enteritis, antiemetic, metoclopramide, ondansetron, maropitant
EFFECT OF CO-INFECTION WITH A VIRUS ON EHRlichia Canis KINETICS IN TmICK AND MAMmALIAN CELLS IN VITRO

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Topic: 6. Epidemiology and Infectious Diseases / Vector-Borne Diseases

Ehrlichia canis, the causative agent of canine monocytic ehrlichiosis (CME), is a small gram-negative coccoid bacterium that infects circulating monocytes. The disease is transmitted by the brown dog tick Rhipicephalus sanguineus and is acknowledged as an important infectious disease of dogs and other members of the family Canidae. The distribution of CME is related to the distribution of the vector tick and the disease has been reported worldwide. E. canis is routinely cultured in vitro in canine macrophages (DH82) and non-vector Ixodes spp tick cell lines, but not in cells derived from its natural vector. In the R. sanguineus cell line RSE8 we established, for the first time, E. canis infection using bacteria isolated from Ixodes cells. The overall aim of our research is to investigate the effect of virus infection on growth of E. canis in tick cells and infectivity for mammalian cells. As a model we are using a construct of the arbovirus Semliki Forest virus (SFV) that expresses luciferase and replicates well in tick cells. E. canis load in RSE8 cells was determined by microscopy and qPCR, and SFV load by luciferase assay and qPCR. These techniques are being used to determine the growth kinetics of E. canis during SFV co-infection in RSE8 cells. This study is expected to contribute to the understanding of the dynamics of E. canis transmission in a model system and provide more information about the use of the RSE8 cell line for in vitro studies.
ANTIFUNGAL ACTIVITY OF THE EXTRACT PUNICA GRANATUM LIN. ON MALASSEZIA PACHYDERMATIS

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Topic: 6. Epidemiology and Infectious Diseases / Control, Prevention and Imunoprophylaxis

Otitis is a common disease in dogs, representing 42% of veterinary care. It is characterized as an inflammation caused by various microorganisms, amongst them the Malassezia pachydermatis. It is considered as an opportunistic fungal (yeast) that could act as a primary factor for perpetuating the infection. The indiscriminate use of antibiotics has contributed to the selection of resistant microorganisms and M. pachydermatis is already showing resistance to commercials antifungals. The objective of the present paper was to evaluate anti-fungal action of aqueous extract of Punica granatum Lin. on M. pachydermatis obtained from samples of auditory meathus of dogs. The extracts were prepared using the peel of the fruitage: dry (EAP dry) and in natura (EAP in natura), at concentrations of 10, 20, 30, 40 and 50%. The negative control treatment was made with saline solution. A suspension of 10³ UFC/mL of yeast, was subjected to pomegranate extract for 10, 20 and 30 minutes, and each sample was evaluated in quintuplicate. It was observed significant decrease in number of colonies by the use of EAP in nature, as from concentrations above 40% for 30 minutes of exposition. However when the time was below 30 minutes the extract was not be able to cause inhibition. The EAP dry did not presented antifungal action. In the conditions evaluated, the aqueous extract of EAP in nature presented potential antifungal activity against strains of M. pachydermatis.

Keywords: herbal medicine, dog’s ear infections, pomegranate extract.
Molecular Detection of Hepatozoon Species in Ixodid Ticks from Turkey

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**Topic:** 6. Epidemiology and Infectious Diseases / Vector-Borne Diseases

This study reports the distribution of tick infestation and the prevalence of *Hepatozoon* infection in ixodid ticks removed from humans living in two different climatic areas of Turkey. A total of 2333 ixodid ticks were examined for the presence of *Hepatozoon* DNA. The ticks were pooled according to species and developmental stages. Of the 169 tick pools tested, 36 (21.30%) pools were infected with *Hepatozoon* species, an overall maximum likelihood estimation of prevalence of 1.74% (95% CI= 1.25-2.37%) per 100 ticks. Maximum likelihood estimation of the infection rate varied by tick species, ranging from 1.13% (95% CI= 0.38-2.71%) in *Ixodes* spp. nymph (5/9 samples, 55.55%) to 9.55% (95% CI= 4.53-24.32%) in *Rhipicephalus sanguineus* (4/5 samples, 80%). All PCR positive samples were directly sequenced. Four amplicon were 100% identical to the sequence for *H. felis*. The remaining sequences shared 99 to 100% similarity with the corresponding *H. canis* isolates. This is the first detection of *Hepatozoon canis* and *Hepatozoon felis* in ixodid ticks in Turkey.
ID: 331

APPLICATION OF GENETIC ANALYSIS IN VACCINE SAFETY AND QUALITY CONTROL

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Topic: 6. Epidemiology and Infectious Diseases / Control, Prevention and Immunoprophylaxis

Purity testing is an essential quality requirement of immunological veterinary medicinal products and testing for extraneous agents usually includes monitoring for different viruses and mycoplasmas.

Mycoplasmas, particularly species of the genera Mycoplasma and Acholeplasma, are known to be occasional microbial contaminants of live vaccines and cell cultures. Also bovine viral diarrhoea virus is a potential contaminant of products of animal origin, including bovine serum. For detection of mycoplasmas and bovine viral diarrhoea virus commercial PCR tests has been used.

The molecular biology methods (PCR and DNA sequencing) have been used to identify and verify strains of Classical swine fever virus, Newcastle disease virus, Avian infectious laryngotracheitis virus, Avian infectious bronchitis virus, Turkey rhinotracheitis virus, Marek's disease virus and Avian infectious bursal disease virus in veterinary vaccines.

178 vaccines were tested in FGBU “VGNKI” during 2011-2012. Undeclared strains were detected in two vaccines against Newcastle disease. Three samples of Classical swine fever virus vaccine were contaminated with bovine viral diarrhoea virus and Mycoplasma species. Furthermore, five cell cultures were contaminated with Mycoplasma species.

Genetic methods may be successfully applied for extraneous agent testing in vaccines and other biological products because of a high sensitivity and a high level of microorganism discrimination.
MOLECULAR CHARACTERIZATION OF ROTAVIRUS IN DAIRY AND BEEF CATTLE IN PERIOD 2008-2012: GENOTYPES, ELECTRO PHENOTYPES AND TEMPORAL VARIATION

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Group A rotaviruses are recognized as important cause of enteritis in animals and humans. Member of family Reoviridae, genome consists of 11 segments of double-stranded RNA and on polyacrylamide gel electrophoresis (PAGE) have migration characteristic of profile divided into four classes according to molecular weight. Neutralizing antigens on outer capsid proteins, VP4 and VP7, has been used to classify rotavirus G and P types, respectively. Specific primers are used in genotyping by RT-PCR for characterizing genotypes P and G rotavirus. Types G6, G8 and G10 and P [1], P [5] and P [11] are most commonly found causing disease in cattle. This work included data on diversity of circulating genotypes in dairy and beef cattle in studies that were conducted in period from 2008 to 2012, in 65 municipalities of Brazil. Through PAGE, prevalence of 32.67% (50/153) among herds and 6.94% (151/2175) in sampled population. Genome profile analysis indicated seven distinct electro phenotypes, indicating genomic diversity. Seasonal distribution of positive samples showed higher incidence in rainy season - October to March 56.95% than in dry season - April to September 43.04%. Infected calves were diagnosed rotavirus in animals with signs of diarrhea 19.1% and clinically normal 1.59%. Feed management and installation type were factors influenced frequency of infection (P <0.05). Molecular characterization by RT-PCR indicated presence of genotypes: G10P [11] G6noP, G6P [11] G8P [5] and G11P [11], G6P [5] + [11], G6P [5], G6P [5] G8P [1], noGP [1], noGP [6] noGP [5] G8noP, G8 [P11] G6 and [P1].

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REDUCTION OF MICROORGANISMS IN THERMOPHILIC PROCESS OF ANAEROBIC DIGESTION OF CATTLE MANURE

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Topic: 6. Epidemiology and Infectious Diseases / Biosecurity

The changes in the quantities of microorganisms during continuous thermophilic anaerobic digestion of cattle manure at 45±1.5°C were studied. Test strains (TS) of Pseudomonas aeruginosa and Staphylococcus aureus, which are resistant to antibiotics from the groups of amphenicols and tetracyclines, were used. They were introduced in the digester in quantities by 10⁶CFU/ml of its content. After 5 days TS of P. aeruginosa disappeared in bioslame. By the 55th day of the experiment disappeared also Escherichia coli, and the amount of the TS of S. aureus and of the other microorganisms monitored was greatly reduced. Similar amendments were established and in parallel conducted analogous experiment mesophilic process of anaerobic digestion of the same manure, but to a lesser degree.

Key words: anaerobic digestion, thermophilic and mesophilic regime, test bacteria, decontamination.
RISK FACTORS FOR CARRIAGE OF ZOONOTIC PATHOGENS IN RATTUS NORVEGICUS RATS IN EIGHT MUNICIPALITIES IN BELGRADE, SERBIA

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Topic: 6. Epidemiology and Infectious Diseases / Vector-Borne Diseases

Background: Although carriage of zoonotic pathogens in urban brown rats has been well confirmed, nevertheless representative data of prevalence of microorganisms and its association with risk factors are missing for commensal \textit{Rattus norvegicus} rodents in Belgrade’s municipalities. \textbf{Aim:} The purpose of the current study was to assess the prevalence of zoonotic pathogens and its association with risk factors among rats in Belgrade. \textbf{Methods:} Data were collected as part of the first survey “Risk of urban zoonoses in relation to the presence of infective agents in commensal brown rats on the territory of Belgrade” conducted in 8 out of 17 municipalities in Belgrade. Data of pest control treatment in progress, demographic and environmental risk factors were collected. A total of 77 rats were live-trapped during summer-autumn season 2012 and screened for presence of \textit{Borrelia} spp. and \textit{Leptospira} spp. by dark-field microscopy and ectoparasites by binocular loupe. \textbf{Results:} The prevalence of zoonotic pathogens and infestation by ectoparasites were, 70.0\% (54/77) and 44.2\% (34/77), respectively. Carriage of infective agents was associated with, weight (p=0.039), trapping location (p=0.003), proximity to disadvantaged households (p=0.022), season (p=0.006) and pest control treatment in progress (p=0.004). \textbf{Conclusion:} High prevalence of zoonotic pathogens among Norway rats and its association with demographic, environmental risk factors and pest control treatment induce need, the same survey to be carried out in the rest 9 municipalities in Belgrade.
A SURVEY ON ABORTIONS CAUSED BY LEPTOSPIRA SPP. IN SHEEP FARMS LOCATED ON THE SUBURB OF TABRIZ

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Topic: 6. Epidemiology and Infectious Diseases / Zoonoses

Leptospirosis is an important infectious disease of animals and humans caused by the pathogenic leptospires which are classified into one species of Leptospira interrogans containing over 212 serovars. This study will conduct to determine the prevalence of Leptospira-induced abortions in Tabriz (north-west of Iran) sheep herds and to determine the pathogenic Leptospira serovars responsible. The purpose of this study is to determine of seroprevalence of leptospirosis among the aborted ewes and in the same time, detection of bacterial DNA in aborted fetal tissues by the PCR protocol. Peripheral blood samples will be taken from 135 ewes aborted in the farms of Tabriz (North-West of Iran) and their sera will be separated by centrifugation. Following, serum samples will be analyzed by ELISA (Pourquire-ELISA Kit manufactured by France). Consequently, tissue samples will be taken from the stomach (fluid), liver, kidney, spleen, lung, heart and placenta of aborted fetuses and will be tested by PCR. Statistical analysis will be done by SPSS software version 16 and McNemar between MAT and PCR tests results.

Key words: Abortion, Leptospirosis, Ewe, PCR, MAT
PROPERTIES OF SWINE INFLUENZA VIRUS DETECTED IN RUSSIA IN 2009-2012

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Topic: 6. Epidemiology and Infectious Diseases / Control, Prevention and Immunophylaxis

Serological monitoring demonstrated seropositive animals on above 90% of pig farms. In the majority of cases influenza A H1N1 virus antibodies were detected. In addition H3N2 virus antibodies were also detected and in a number of cases antibodies against both influenza A H1N1 and H3N2 viruses were identified. Sequencing of influenza A virus isolated from pigs samples allowed identification of avian-like H1N1 virus, classical swine subtype H1N1 virus (pandemic virus) and H2N3 virus. Herewith, it was demonstrated that pandemic subtype H1N1 virus strain prevailed on the RF farms. Sequence analysis of a A/Swine/Vladimir/VL/2009 H3N2 virus isolated in 2009 showed closest similarity with swine viruses isolated in China in 2003-2006 and human isolates from Hong Kong recovered in 1999, but the HA gene was greater than 5% divergent from any other isolate suggesting that the virus had been drifting as a unique lineage for an extended period. Experimental studies of pig infection with A/Swine/Vladimir/VL/2009 H3N2, A/swine/Tatarstan-SA/2011/H1N1(pandemic), A/swine/Tatarstan/2009/H1N1 avian-like isolates demonstrated that the pandemic strain induced subclinical infection while the avian-like H1N1 and H3N2 viruses induced acute respiratory disease.

The works was performed within the ISTC Project # 2800.
THE INFLUENCE OF DOG PARKS (ECO ZONES) ON THE REDUCTION OF DOG
FAECAL CONTAMINATION IN CENTRAL PARKS OF BELGRADE

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Topic: 6. Epidemiology and Infectious Diseases / Zoonoses

A permanent increase of dog population present a hygienic and ecological problem of urban environment because of a permanent contamination of public places. For this reason, from 1995, we have been performing permanent parasitological control of green areas and public places in Belgrade. During 2011, Belgrade City authorities adopted new measures that bound dog owners to remove dog excrement through the doggy-pot system and that introduced dog parks (Eco centers) within the parks themselves. Here we present the results of our examination performed in Belgrade area during 2012 after the introduction of dog parks. We examined 98 samples of soil in green areas and public places and 80 samples of dog faeces. The samples were collected from April to June. Each soil sample was collected in a chess-board manner at a distance of 1 – 1.5m according to the feces disposition on the study area, and up to 2 – 3cm in depth. They were examined by sedimentation and floatation methods. Parasites contamination was detected at 28.12 % soil samples. Eggs in Toxocaracanis was found in 26.56 % samples, Dipyllidiumcaninum in 23.43%, Ancylostomidae spp. and Trichurisvulpis in 10.93 %, Strongyloidesstercoralis and Toxascarisleonina in 7.81 %. The result of our control has established over 40% decrease in parasites eggs as compared to the period 2009 – 2011.
ID: 374

THE CURRENT EPIZOOTIC SITUATION OF ASF IN RUSSIA

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Topic: 6. Epidemiology and Infectious Diseases / Zoonoses

Since 2007, in certain regions of Russia outbreaks of ASF were detected in both wild and domestic pigs. In the period from 2008 to 2012 the annual average amount of outbreaks was about 80, and 2012 it was more then 400.

Biological properties of epizootic ASF virus isolates, isolated in 2012 from domestic pigs in Russia were investigated. Virus reproduction features was investigated using virus isolation technique on primary cell cultures. Infection of susceptible animals was performed.
EVIDENCE OF THE FIRST OCCURRENCE OF SCHMALLENBERG VIRUS INFECTION IN THE CZECH REPUBLIC – A RETROSPECTIVE STUDY

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Topic: 6. Epidemiology and Infectious Diseases / Vector-Borne Diseases

Schmallenberg virus (SBV) is a previously unknown Orthobunyavirus from the Simbu serogroup of the Bunyaviridae family. SBV is transmitted by Culicoides midges and affects domestic and wild ruminants. Infected cattle show symptoms of fever, milk yield drop and diarrhoea. Transplacental infection results in the birth of malformed lambs, calves and goat kids. Since its discovery in Germany in autumn 2011, the virus was detected in many European countries. In the Czech Republic, SBV was first identified in December 2012 in two sheep farms. In this retrospective study we tested a total of 184 serum samples obtained from seven different wild ruminant species between November 2011 and September 2012. The samples were collected in 12 different districts of the Czech Republic and were tested for anti-SBV antibodies by the commercial ELISA ID Screen Schmallenberg virus Indirect Multi-species Confirmation test (ID Vet, France). Four samples coming from four different regions (Brno – city, Havlíčkův Brod, Česká Lípa and Rychnov nad Kněžnou) and four animal species (mouflon - Ovis musimon, domestic yak - Bos mutus f. grunniens, red deer - Cervus elaphus and bezoar goat - Capra aegagrus aegagrus) tested positive. The first two positive samples were collected in February and March 2012, respectively. Our study thus gives an evidence for circulation of SBV in the Czech Republic in early 2012 and at the same time brings the first information on the occurrence of SBV infection in domestic yak and bezoar goat.
DETECTION OF ANTIBODIES AGAINST SCHMALLENBERG VIRUS IN SHEEP, GOATS AND CATTLE IN WINTER 2012-2013 IN THE CZECH REPUBLIC

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Topic: 6. Epidemiology and Infectious Diseases / Vector-Borne Diseases

Infections with Schmallenberg virus (SBV), a novel Orthobunyavirus (Simbu serogroup, Bunyaviridae) are associated with an arthrogryposis–hydranencephaly syndrome in lambs, calves and goat kids. Infected adult cattle show symptoms of short fever, milk yield drop and diarrhoea. Culicoides midges are considered for natural vectors of this virus. SBV was first discovered in Germany in autumn 2011 and since that the virus was detected in many European countries. In the Czech Republic, SBV was first identified in December 2012 in two sheep farms and to date, according to the State Veterinary Administration of the Czech Republic, the virus was identified in 14 malformed offspring in a total of 11 farms. In this study we tested serum or plasma samples of sheep, goats and cattle for the presence of anti-SBV antibodies by the commercial ELISA ID Screen Schmallenberg virus Indirect Multi-species Confirmation test (ID Vet, France). We tested a total of 129 samples (obtained from 97 sheep or lambs, 10 goats and 22 cows) collected since December 2012 to February 2013 in 10 farms located in western and eastern Bohemia, south and eastern Moravia. Of these, a total of 76 samples (56 sheep, 5 goat and 15 cow samples) tested positive for anti-SBV antibodies (ie. 59% positive samples) and one sheep sample was dubious. Positive and the dubious samples came from all tested farms. Our results suggest that SBV can be relatively widespread in our country. To confirm this assumption more extensive studies are needed.
The relationship between humans and animals is getting closer, but does not come with appropriate knowledge about responsible ownership and zoonoses. The aim of this study was to assess the problems of the dog population of the Sorocabano neighborhood, in Jaboticabal, São Paulo, Brazil. Questionnaires were applied in 263 residences in September 2012. The survey involved a total of 846 residents where 54.37% are adults and 25.87% are elderly. The number of houses containing dog, cat or both was 181. When asked about the transmission of zoonosis, 202 people admitted knowing that dogs transmit diseases to humans, 220 said the same for cats and 48 said there was no transmission. When asked if someone has been attacked by a dog, cat or both, 64 people answered yes, and within these, 25 were bitten in their own residence, showing that many times, the injuries are caused by the owners of the pets. The results of the chi-square test had shown that the Sorocabano’s population has knowledge about aggression and diseases transmitted by dogs (p<0.05) but there is not a significant association between aggression and transmitted diseases by cats (p>0.05). These results indicate that the population does not know how to prevent or unaware of diseases and disorders in the relationship with their pets, which makes use of data relevant to the contribution and improvement in the actions of the Office of the City Health Surveillance and educational campaigns.
EXPERIMENTAL STUDY OF BIOLOGICAL PROPERTIES OF H5N1 INFLUENZA VIRUS DURING EXPERIMENTAL INFECTION OF 18-DAY-OLD DOMESTIC GOSLINGS

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Influenza virus strains H5N1 A/duck/Novosibirsk/02/05 (clade 2.2.), A/chicken/Primorsky/85/08 (clade 2.3.2.), A/grebe/Tyva/100/09 (clade 2.3.2.) and A/grebe/Tyva/434/10 (clade 2.3.2.) were used for intranasal infection of 18-day-old domestic goslings at a dose of 6.0 lg EID₅₀/cm³.

The disease clinical signs were similar for all four influenza virus strains. Post-mortem examination results demonstrated inflammation (hyperemia) in pancreas, heart and brain.

Nervous signs (tremor, incoordination), conjunctivitis and occasionally cornea opacification were reported in the diseased birds.

Post-mortem examination of all died birds demonstrated hemorrhages, heart, duodenum and rectum, pancreas, lung edema and occasionally inflammatory and dystrophic lesions of kidney.

Infection with A/chicken/Primorsky/85/08 (clade 2.3.2.), A/grebe/Tyva/100/09 (clade 2.3.2.) strains resulted in death of goslings within 6 days; contact birds died within 7 days. Strain A/duck/Novosibirsk/02/05 (clade 2.2.) killed the infected ducks within 8 days and one of the three contact ducks – within 9 days. The incubation period of these three strains lasted for at least 2 days.

Course of infection induced in 18-day-old gosling by A/grebe/Tyva/434/10 H5N1 strain was less acute. Incubation period lasted for at least 3 days. All goslings became diseased after infection but within 9 days death of only 3 birds out of 5 was reported. Starting from day 8 of the experiment two gosling demonstrated recovery process (birds’ energy was steadily recovered; the birds eagerly consumed feed and water). Previous experimental results suggest that goslings are capable of recovery after infection with A/grebe/Tyva/434/10 H5N1.

The work was performed within the ISTC Project # 2800.
REDUCTION OF SANITARY INDICATOR MICROORGANISMS IN ANAEROBIC DIGESTION OF POULTRY LITTER IN COMBINATION WITH SILAGES

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Topic: 6. Epidemiology and Infectious Diseases / Biosecurity

In connection with experiments aimed at optimizing anaerobic digestion of organic fertilizers, microbiological tests were performed to assess the epizootiological safety of resulting finished product to fertilize the soil by quantities of sanitary indicator microorganisms. Mixtures of substrates were composed and tested in technological parameters established for other experiments: mesophilic temperature regime 33°C, dry matter content of 7%, residence time in the digester 15 days. Combinations were in the ratio 40:60 of mulch bed of broiler chickens with different silages: from beetroot, sugar and salad beets, corn and autumn leaves of Paulownia elongata. It was found that combinations used are effective for production biogas. At the same time is performed significant reduction of microorganisms in final products, which are assessed as safe for soil application. Microbial reduction is mostly in bio slime derived from the anaerobic digestion of the substrate with silage from P. elongata. In the silage from the three types of beets this indicator is best for that from salad beet, and at a lesser extent - for corn silage.

Key words: anaerobic digestion, poultry litter, Paulownia elongata, beet, silage, decontamination.
PHYLOGENETIC ANALYSIS OF GROUP A PORCINE ROTAVIRUS VP6 GENE SEQUENCES

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Topic: 6. Epidemiology and Infectious Diseases / Zoonoses

Porcine rotaviruses are important enteric pathogens not only because of their economic impact on swine production in terms of mortality and morbidity but also because they are a potential source of heterologous RV infections in humans and cattle. The VP6 protein is the major structural component of the virion and plays an important role by interacting with the outer capsid proteins VP4 and VP7 and the core protein VP2. It is hydrophobic in nature and carries antigens that have been described to induce protective immunity. According to VP6 group A rotavirus is classified as I genotypes. A total of ten fecal samples, were collected from pigs in farms from São Paulo and Minas Gerais Brazilian states in 2012. This study aimed at the phylogenetic analysis based on complete nucleotide sequences of VP6 encoding genes. Viral RNA was extracted and these stool were tested by RT-PCR, using primers development in this study. First round PCR products were subjected to bidirecional sequencing and analyzed with Neighbor-Joining algorithm, Maximum Composite Likelihood as substitution model, and 1,000 bootstrap replicates. Strains circulating in farms from Brazil are phylogenetically close to other homologous strains belonging to the I[5], already described in porcine, equine and human. The comparison ranging from swine sequences related to VP6 revealed a minimum nucleotide identity of 87.2% and a maximum nucleotide identity of 100%. These analyses showed variability intragenic. This data will provide a broader surveillance of circulating rotavirus, a key to a better understanding of epidemiology and prevention of the disease.
NUCLEOTIDE SEQUENCE ANALYSIS OF THE NSP1, NSP3, NSP4, VP4 AND VP7 GENES FROM PORCINE GROUP A ROTAVIRUSES IN BRAZIL

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Topic: 6. Epidemiology and Infectious Diseases / Zoonoses

Group A rotaviruses (RVAs) can infect both humans and animals and have been recognized as an important cause of diarrhea in porcine. Recently, RVAs have been classified on the basis of all 11 segments and this allows a more complete analysis of unusual rotavirus strains that might represent reassortment between strains of rotaviruses isolated from humans or among strains of human and animal origin. According to NSP1, NSP3, NSP4, VP4 and VP7, group A rotavirus is classified as A, T, E, P and G genotypes, respectively. It is attributed to NSP4, a role of enterotoxin-like causing secretory diarrhea and NSP1, role of interferon antagonist. Non structural protein NSP3, is a multifunctional protein and recognized translation enhancer. VP4 and VP7 are the rotavirus proteins confirmed to induce virus neutralizing antibodies. The A, T, E, P and G genotypes of a total of three rotavirus strains from pigs in farm from Mato Grosso Brazilian state were determined by complete nucleotide sequence and phylogenetic analysis. Viral RNA was extracted and these stool were tested by RT-PCR using primers development in this study and already related. First round PCR products were subjected to bidirectional sequencing and analyzed with Neighbor-Joining algorithm, Maximum Composite Likelihood as substitution model, and 1,000 bootstrap replicates. Strains circulating in farm from Brazil are phylogenetically close to other homologous strains belonging to A[8], T[7], E[1], P[22], P[13] and G[5], already describe in porcine, equine, human and bovine. The molecular epidemiology of RVAs is crucial for preventing or reducing the incidence of porcine RVA diarrhea.
Ancylostomiasis can be a zoonotic infection that do not use humans as a definitive host, the most common being *A. braziliense* and *A. caninum*. The normal definitive hosts for these species are dogs and cats; humans may also become infected when have been in contact with contaminated soil, that can act as reservoir or vector of this zoonotic parasite, and potentially can infect the host causing cutaneous larva migrans (also known as creeping eruption or sandworm eruption). The objective was determine the presence of *Ancylostoma spp* in soil of fishing village “El castillo” of Navolato, Sinaloa, México. **Methods:** The composite samples of soil of fishing village, were determined for representative samples described by the technique of Thrusfield (2005) was used: \( n = \frac{t \times SD \times L}{L^2} \). Where \( n \)=sample size, \( t \)=value of the normal distribution (Student t) for a 95% confidence level (\( t \approx 1.96 \)), \( L \)=accepted error or precision (5%), and \( SD \)=weighted disease prevalence (%); the total of composite sample of soil was 55, took surface moist soil scraping of 100 grams of soil for each sample; transferred to the laboratory of parasitology of the FMVZ-UAS to be analyzed by the sedimentation technique. **Results:** 11 (20 %) of the 55 composite samples of soil were positive to *Ancylostoma spp*. **Conclusion:** The contamination of soil with *Ancylostoma spp.* represent risk for the pets and public of these recreation place, is necessary implement control strategies and education for the prevention of the infections.
STUDIES ON EHRLICHIOSIS IN DOGS

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Topic: 6. Epidemiology and Infectious Diseases / Vector-Borne Diseases

Ehrlichiosis is a tick-borne infectious and zoonotic disease. Blood Samples and ticks were collected from 100 police dogs from different police stations in Cairo, Egypt during a period extending from 2007 to 2008. The most important clinical signs were observed in the form of fever, anorexia, epistaxis, emaciation, polyurea and conjunctivitis. Meanwhile, the remaining dogs were apparently healthy. Blood film examination revealed that 9/100 (9 %) was positive for ehrlichiosis by the finding A. phagocytophilum inclusions (morula-like structures) in circulating neutrophils. Meanwhile, the final overall prevalence of different Ehrlichia spp. detected by nested PCR was 80 % Ehrlichia spp.; from which 20 % were A. phagocytophila, 60 % were unidentified Ehrlichia spp. and 0 % E. canis. The obtained DNA sequence of the unidentified Ehrlichia spp. sample revealed three detected Ehrlichia spp.: Ehrlichia ruminantium, Ehrlichia chaffeensis and Ehrlichia muris with 92 % homologous. All ticks were identified as Rhipicephalus sanguineous, and DNA of Ehrlichia spp. was detected in 7 tick samples. Moreover, the highest infection rate was recorded in age groups of 3 to 6 years (90.0 %), females (91.3 %), Malino (90.0 %) and German Shepherd breed (82.6 %) and ticks-infested dogs (93.5 %). The drugs of choice for treatment of ehrlichiosis were Tetracyclines and doxycyclines. Finally, dogs play a critical role in the transmission of different Ehrlichia spp. rather than E. canis, and R. sanguineus is the main vector and reservoir of Ehrlichia spp. in dogs in Egypt.
IDENTIFICATION OF HYALOMMA MARGINATUM RUFIPES KOCH, 1844 (ACARI: IXODIDAE) FROM OTHER HYALOMMA SPECIES BASED ON IMMATURE STAGES


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**Topic:** 6. Epidemiology and Infectious Diseases / Vector-Borne Diseases

The genus *Hyalomma* comprises the most ixodid tick species those parasitize camels in Egypt. Although the immature stages of tick species play an important role in distribution of ticks and tick-borne diseases, the identification mainly depends on the adult stage. Therefore, this study tries to identify the specific characteristics of both nymphaal and larval stages of *Hyalomma marginatum rufipes* Koch, 1844 using scanning electron microscopy and morphometric analysis to be easy differentiate them from those of other *Hyalomma* spp described before in Egypt. Results showed that the nymph and larva of *H. marginatum rufipes* can be easy identified from those of *H. anatolicum excavatum* Koch, 1844, *H. dromedarii* Koch, 1844 and *H. impressum* Koch, 1844 butthey are strongly close to *H. marginatum marginatum* Koch, 1844. The nymph of *H. marginatum rufipes* can be distinguished from *H. marginatum marginatum* by the number and distribution of dorsal and ventral idiosomal setae and the distribution of sternal setae. All morphological characteristics of *H. marginatum rufipes* larva resemble those of *H. marginatum marginatum* larva. The measurements of nymphaal and larval structures of *H. marginatum rufipes* are significantly lower than those of *H. marginatum marginatum*. 
MYCOPLASMA BOVIS ANTIBODIES PREVALENCE IN CZECH CATTLE HERDS

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Topic: 6. Epidemiology and Infectious Diseases / Emerging Diseases

Mycoplasma bovis, a causative agent of pneumonia, mastitis, arthritis, otitis and other health disorders, is often overlooked pathogen in Czech cattle herds. This is the first report on seroprevalence of Mycoplasma bovis infection in the country performed in 2012.

A total of 25 herds were chosen and blood samples were collected from ten randomly selected animals in each herd. Dairy and beef cows, heifers and calves from large-scale and also smaller farms were included. The commercially available indirect enzyme-linked immuno sorbent assay kit (Bio X Diagnostics, Belgium) was used for serological examination. The overall seroprevalence of Mycoplasma bovis infection in cattle reached 18.8% (47/250), ranging from 0 to 80% in dairy cattle. Beef cattle herds never had more than 30% of positive animals. From 25 examined herds, 19 were tested positive.

The study could indicate the widespread of Mycoplasma bovis infection in cattle herds in the Czech Republic. Interestingly, the herd seroprevalence is four times higher than seroprevalence in individual animals. Further studies are needed to extend our knowledge about epidemiology of Mycoplasma bovis in Czech cattle herds.
PHOTODYNAMIC THERAPY: A NEW THERAPEUTIC APPROACH FOR VETERINARY MEDICINE?

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Topic: 6. Epidemiology and Infectious Diseases / Control of Diseases in Exotic Zoo Animals

Photodynamic therapy (PDT) is an emerging therapeutic approach that consists of combining harmless low-intensity light with non-toxic photosensitizers (PS), to promote the formation of reactive oxygen species and induce cellular death either for tumoral destruction or microbial inactivation. Although different microbes express variable sensibility to PDT, no resistance has been reported and different strategies for PS functionalization can target specific cells leading to minimal host tissue damage. The pioneer veterinary PDT was reported during 1980’s, when cats and dogs presenting a wide cancer variety, received an intravenous injection of hematoporphyrin derivative and were locally exposed to light. Partial tumor remission demonstrated the potential of this technique. Afterwards the effectiveness of PDT for animal neoplastic treatment was proved positive against solid tumors in esophagus, prostate, face, oral cavity, brain, skin and others. Marine animals have also been subjected to PDT with successful results. Penguin (Spheniscus magellanicus) podermatitis healed 5 weeks after treatment, green turtle (Chelonia mydas) carapace fracture and fibropapillomatosis recovered after the light (laser) mediated treatment alone. Phototherapies can also encompass small and large domestic animal. Canine oral papillomatosis and cattle hoof diseases, exhibited good clinical results and other possible applications are emerging. In summary, optical therapy is continuously conquering space in Veterinary Medicine over the past few decades and for the next years this pattern tend to be accelerated by the wide range of potential applications, development of new PS and affordable light sources in parallel with the enlightenment of the mechanisms involved.
FREE-RANGING FOXES AS SOURCE OF RICKETTSIA ANDEANAEE IN THE COQUIMBO REGION, CHILE

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Topic: 6. Epidemiology and Infectious Diseases / Vector-Borne Diseases

Rickettsia andeanae (Ra) it has been reported in Amblyomma ticks from South-American countries, and recently in Chile. Many rickettsial infections have their life cycle in wildlife. To determine the presence of Ra in ticks from dogs and to assess whether free-ranging foxes are a source of infection to dogs a transversal study in Coquimbo region in Chile was carried out. Sampling was conducted in the city of Coquimbo and nearby rural localities, in spring-summer 2011-2012. One dog per household was examined and ticks were collected when present; after taxonomic identification, gltA and ompA genes were amplified and sequenced. 226 dogs were examined in urban and rural sites and 25 foxes trapped in rural sites. 129 (57%) dogs and 23 (92%) foxes had ticks. In the city 53% (60/114) of dogs had ticks from Rhipicephalus sanguineus (Rs) and all ticks were negatives to Ra. In rural localities 62% (69/112) dogs had ticks, mainly Rs (84%), but also Amblyomma tigrinum (At) (16%). 92% (23/25) of foxes had ticks, of which all were At; of these Ra was found in 87% (20/23) of ticks and in 50% (8/16) of At in rural dogs. Non Rs was positive. The exclusivity of At and Ra in rural areas, the higher parasitism by At and the higher frequency of infection by Ra in foxes than in dogs suggest a likely direction of infection from foxes to dogs in the Coquimbo region. This study was funded by Fondecyt Nº 11100303, Nº1100809.
In spite of the reduction of swine brucellosis prevalence in Brazil, *B. suis* infection still occurs, posing a risk to animal and human health. An outbreak of swine brucellosis affecting a herd in Jaboticabal, State of São Paulo. *B. suis* biovar 1 was cultured from aborted fetuses and sows. The aim of this investigation was to evaluate the performance of the rose Bengal plate test after treatment of the serum with rivanol (RBPT-RIV) and of the fluorescence polarization assay (FPA) for the serological diagnosis of swine brucellosis, and compare them with the techniques recommended by Brazilian rules: rose Bengal (RBPT), complement fixation (CFT) and a combination of standard agglutination and 2-mercaptoethanol tests (SAT+ME). Serum samples of 333 animals from *Brucella suis* infected swine herd and 1,100 serum samples from pig herds free of infection collected at a slaughterhouse were analyzed. Sensitivity of the FPA ranged from 95.94% to 96.05%, and sensitivity of the RBPT-RIV ranged from 79.7% to 82.79%, depending on the condition. Specificity of the FPA ranged from 98.0% to 99.54%, and specificity of the RBPT-RIV was 100%. Adopting for the FPA a cut-off settled in 85.9 mP, the relative sensitivity and specificity was 98.9% and 98.6%, respectively. The RBPT had a combined value of sensitivity and specificity higher than did the CFT and the SAT+ME. Agreement among the tests was excellent. The highest agreement was observed between CFT and SAT+ME (kappa = 0.98), and the lowest was observed between FPA and RBPT-RIV (kappa = 0.87).
Q-FEVER: SEROPREVALENCE AND IDENTIFICATION OF CAUSATIVE AGENT IN DIARY COWS IN THE CZECH REPUBLIC

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Topic: 6. Epidemiology and Infectious Diseases / Zoonoses

Q fever is a highly contagious zoonosis caused by the strictly intracellular bacterium Coxiella burnetii (C. burnetii). Besides humans it affects many species of domesticated and free-living animals. Domestic ruminants are the main reservoirs of infection, and transmission to humans is mainly accomplished through inhalation of contaminated aerosols.

The objective of this study was to detect specific antibodies against C. burnetii in cattle herd, where abortions in dairy cows were reported and to detect the presence of the ethiological agent of Q fever in their milk and vaginal swabs.

Blood serum samples, individual milk samples and vaginal swabs were collected in October 2011 from 148 diary cows in cattle herd with reproductive disorders. Blood serum samples were examined for the presence of specific antibodies against phase I and II C. burnetii by the commercial ELISA kit. Demonstration of C. burnetii in milk samples and vaginal swabs was performed using real-time PCR.

The examination of 148 bovine serum samples revealed 36 samples (24,3%) positive and 9 samples (6,1%) were dubious. From a total of 148 individual milk samples examined by real time PCR 3 (2%) of them were positive. Three vaginal swabs (2%) from a total of 148 samples were tested positive. All positive milk samples and 2 positive vaginal swabs were obtained from seropositive animals. One positive vaginal swab came from animal with no specific antibodies against C. burnetii.
ID: 500

SEASONAL INFLUENZA IMMUNIZATION OF SWINE WORKERS – A PILOT PROJECT

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Topic: 6. Epidemiology and Infectious Diseases / Biosecurity

As a general biosecurity recommendation it is recommended workers sick with influenza not have direct contact with swine, and there is a widespread recommendation in North America that swine workers regularly be immunized for seasonal influenza. Nevertheless there remains a relatively low compliance rate among the general population. It was the intent of this project to determine if the vaccination rate of swine workers can be increased through making immunization more easily available.

Methods: A in 2011 and again in 2012 a Registered Nurse who was willing to travel to the farms was hired. Each participant received an Influenza Fact Sheet” from the provincial Department of Health and were offered a free seasonal influenza vaccine.

Results: In 2011 Out of 105 farm workers eligible for vaccination, the program succeeded in vaccinating 35 people in addition to the 15 that were already vaccinated prior to the program thus raising the vaccination rate from 14% to 47.6%. In 2012, out of a potential population of 86 personnel, 68 were vaccinated, 8 could not be reached and 10 declined leading to an immunization rate of 79%.

Conclusions: In 2011 The rate of vaccination reached approximately 50% of the target population, which is a higher compliance greater than the national rate of the general public. In 2012 when the program was repeated, significantly more workers were vaccinated. Making vaccination more convenient does enhance the rate of seasonal influenza vaccination and persisting over multiple years appeared increase the immunization rate in this pilot study.
PCR-RFLP FOR DETECTION OF FASCIOLA HEPATICA INFECTION IN SNAILS OF GALBA TRUNCATULA AND LYMNAEA STAGNALIS FROM NORTHWESTERN IRAN

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Topic: 6. Epidemiology and Infectious Diseases / Vector-Borne Diseases

The liver fluke, Fasciola hepatica, is considered as the most common cause of Fasciolosis in both domestic livestock and humans. This study was carried out to detect prevalence of F. hepatica larval stages in snails of Galba truncatula and Lymnaea stagnalis northwestern Iran. Snails’ collection was undertaken through searching 28 perennial and seasonal freshwater habitats in Nort West of Iran from May to December 2010. The snails were identified to the species level and subjected for molecular investigation. Polymerase chain reaction (PCR) was utilized to amplify 28s rRNA gene of Fasciola larval stages in the snails tissues. The PCR products were subjected to restriction fragment length polymorphism (RFLP) using AvaII and DraII enzymes. PCR amplification for detecting the prevalence of Fasciola larvae infection in the snails elucidated that 50% of G. truncatula from Dolatabad and 2.5% of L. stagnalis from Jabalkandi were infected. PCR-RFLP patterns revealed that 16.6% of G. truncatula and 1.1% of L. stagnalis were infected by F. hepatica larval stages. Molecular detection of F. hepatica in the snails, as intermediate hosts, was proven to be useful for establishment of suitable control programs against fasciolosis in livestock and humans in the region.
SEASONAL AND GEOGRAPHICAL DISTRIBUTION OF CERCARIAL INFECTION IN SNAILS OF LYMNAEA GEDROSIANA IN WEST AZARBAIJAN PROVINCE, IRAN

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Topic: 6. Epidemiology and Infectious Diseases / Vector-Borne Diseases

Trematodes are a diverse group of endoparasites which require molluscan and vertebrate animals as intermediate and definitive hosts in their life cycle. The present study was carried out to determine the diversity and spatio-temporal distribution of infection with trematodes’ cercariae in the snail Radix gedrosiana from northwest Iran. A total number of 6759 lymnaeid snails were collected from 28 snail habitats; of these R. gedrosiana was the prevalent pond snail (74.37%) which examined for cercarial infection by shedding method. Results indicated that the overall infection rate was 8.03%. The most frequent trematodes cercariae in the snail were xiphidiocercariae (81.98%), furcocercariae (32.26%), echinostome cercariae (5.19%), and monostome cercariae (1.24%). The highest infection rate in R. gedrosiana (100%) was with echinostome cercariae from Golestaneh in autumn. Due to the important role of pond snails in transmission of cercariae to fish as a source of zoonotic diseases, it is essential to estimate the distribution and abundance of the snails and the rate of their infection with different trematodes’ cercariae, and establish control programs in each region.