



## Opportunities and challenges of using diagnostic databases for monitoring livestock diseases in Denmark

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**8<sup>th</sup> Annual EPIZONE meeting**  
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# **Spatiotemporal analysis of the Porcine Reproductive and Respiratory Syndrome (PRRS) epidemic in Denmark using laboratory submission data**

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Porcine reproductive and respiratory syndrome (PRRS) virus infects domestic swine populations causing production losses in many European countries. The virus has two different strains designated as European (EU) and American (US) strain. It has been assumed that 30% of Danish swine herds are sero-positive with one or both PRRS strains. The PRRS surveillance program is based on serology tests performed in a monthly or annual basis for the breeding and intensive production herds respectively. The objective of this study was to characterize the PRRS epidemic in Denmark from 2007 to 2010 using retrospective spatiotemporal analysis of serological tests.

Records of PRRS serology submissions made from January 2007 to December 2010 stored in the DIANOVA Information Management System at the National Veterinary Institute (DTU Vet) were used in the analysis. Each submission consists in individual blood samples collected for surveillance purposes and its frequency depends on the herd type. The herds were classified as PRRS positive or negative based on the individual serology test results for both virus strains. The prevalence of both PRRS strains was calculated on a monthly basis for the breeding and intensive production herds. The herd numbers registered in the laboratory submissions were merged with the Danish Herd Identification System database, in order to obtain the geo-coordinates of the herds. Spatiotemporal analysis was performed, in order to characterize PRRS-EU and PRRS-US distributions for both control levels.

The analyses indicated a seasonal pattern in the between-herd prevalences of PRRS-EU and PRRS-US for both herds type. The prevalence of both PRRS strains was higher in the intensive production herds (mean=37%) when compared with the breeding herds (mean=17%). The spatiotemporal analysis detected a number of clusters of PRRS-EU and PRRS-US in both herd types. This suggests that PRRS still disperse between herds and that additional control efforts should be considered.

# Persistent spatial clusters of prescribed porcine antimicrobials

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Emergence of antimicrobial resistance has increased the public awareness of the use of veterinary antimicrobials. Antimicrobial treatment of production animals is, according to Danish legislation, restricted to clinical disease<sup>1</sup>. However, management practices vary substantially among swine farmers, including the choice of drug, dose, treatment time and the perception of metaphylaxis.

It is hypothesized that farms with a persistently high antimicrobial use are spatially clustered. Clustering of disease, demographic characteristics, management practices and medication habits in a given region are expected to explain persistent clusters. Therefore, the objectives of this study were to identify the geographical distribution of Danish indoor commercial pig farms prescribing significantly more antimicrobials during the two years of study period and to evaluate the effect of farm characteristics on the identified clusters.

Data on antimicrobials prescribed for swine during 2012-2013 were extracted from the Danish national database on veterinary prescriptions (VetStat) and merged with data on farm characteristics from the Central Husbandry Register. Amount of prescribed antimicrobials was measured as Animal Daily Doses/100 swine/day and analyzed on a six-monthly basis for each of the three age groups; sows (2,062 farms), weaners (2,886 farms) and finishers (5,417 farms). A total of 6,143 farms were included in the study.

A purely spatial multivariate cluster detection algorithm for continuous outcomes was run in SaTScan including all three age groups. The model was run four times, for each of the six-month time periods. Farms included in a significant cluster in all four consecutive time periods were identified. This resulted in 551 farms of where 172 held sows, 241 had weaners and 484 farms had finishers. The clusters were centered in three geographic regions; Southern Jutland, The Triangle Region and a region bordering the western part of the Limfjord outlet.

Whether this apparent clustering of farms with a persistently high use of antimicrobials is caused by certain farm characteristics, management practices or an actual higher disease frequency remains unknown. Results should be followed-up with additional investigations of potential risk factors, such as: herd size and type, herd density, health status, veterinary affiliation or management practices.

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<sup>1</sup> Legal order no. 543, 29/05/2013, in Danish: "Bekendtgørelse om dyrlægers anvendelse, udlevering og ordinerings af lægemidler til dyr"

# Optimizing infectious disease control through Bayesian modeling

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## Overview

Recently a PhD project in veterinary epidemiology with the title “Optimizing infectious disease control through Bayesian modeling” was started at DTU-VET. This poster will discuss the methodological aspects of the project.

## Background

The project is focused on limiting the spread of infectious diseases between cattle farms in Denmark. More specifically it has become apparent that there has been a rise in clinical cases of the contagious bacterium *Mycoplasma bovis* in Denmark. During 2013, bulk tank milk samples were collected in three different cross sectional studies and found that 21, 7 and 11% of the dairy herds were sero-positive, while the prevalence of PCR-positive herds was 1.6-2.0% in 2011-2013.

Infections with *M. bovis* can cause diseases in cattle, such as mastitis, pneumonia conjunctivitis and/or arthritis. During an outbreak in cattle herds many animals may become infected and mortality can be high, leading to both animal welfare problems and large economic losses. The costs of mycoplasma disease include milk production losses, increased treatment costs, death and culling losses, costs of diagnostics and control and extra costs due to non-pathogen-specific preventive measures.

The object of this PhD study is to establish a modeling framework that enables policy makers to execute well informed decisions, when the aim is to implement control measures against contagious pathogens such as *M. bovis*.

## Materials and Methods

Challenges in combining register based data such as information about movement of cattle between premises, spatial information and diagnostic information collected through research projects or directed control efforts will be assessed.

This project seeks to improve the understanding of the transmission mechanisms between farms, especially the importance of spatial proximity and movement of animals. A key point in the project is that this framework enables adaptive decision making. When for instance more information about a disease becomes available through, for example, on-going field projects, the information can be incorporated directly into the modelling framework. This provides an updated inference about the predicted outcome of the model and the suggested control strategies. A possible approach to meet the project objective is to apply a Bayesian Network analysis that takes the temporal dynamics of the problem into account.

# Opportunities and challenges of using diagnostic databases for monitoring livestock diseases in Denmark

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Several databases are being used in Denmark to record information at all stages and levels of modern livestock production. These databases are all developed for different purposes and gather large volumes of routinely collected data. Examples of existing databases for livestock are the Central Husbandry Register (CHR), Meat inspection database for cattle and swine, mortality database and movement database. These databases are owned by the Ministry of Food, Agriculture and Fisheries. Other databases, such as the Danish Cattle Database, are owned by the agricultural sector. In addition to the technical and political bottlenecks of gathering and combining data from the different databases, the questions remain on the sensitivity and timeliness of data for detecting unexpected animal health events. Thus, it is important to explore changes in data records over time from different databases in order to detect potential patterns. This includes describing trends, seasonality and the potential impact of covariates on the baseline patterns. The potential of these data as monitoring tools can be evaluated using performance indicators such as residuals, predictive positive values, sensitivity and specificity, and by comparing the predictions of models with previous diseases events in Denmark. A further challenge is to identify the most adequate surveillance timescale (i.e. daily, weekly or monthly basis) as well as suitable spatial distances, in order to identify outlier events when the features of the alarm (e.g. shape and amplitude) are unknown. Using rule-based anomaly detection will allow developing a spatio-temporal monitoring framework based on time-series analysis and statistical process control in order to optimize methods for detection of anomalies in data patterns and methods for early warning. It is expected that the use of such information in space and time, might provide information which can be used either directly as a syndromic surveillance tool or to aid in a more targeted or directed disease surveillance program. This will enable a timely and appropriate response to a disease outbreak, minimizing economic impacts through timely implementation of disease prevention and control measures.

# Evaluation of the risk of introduction of Rift Valley Fever into Spain

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In this work we present an assessment of the introduction of Rift Valley Fever (RVF) in Spain applied to surveillance in the human and veterinary fields. The risk of introduction of RVF in the countries of the Mediterranean neighbouring the European Union was assessed by the EFSA (2013) to be non-negligible via the unofficial introduction of infected animals in outbreak years. Spain has two autonomous cities located in North Africa where there is a large muslim community. During the Eid-al-Adha festival the demand of live animals is very high and it is possible that animals are introduced from neighbouring countries for immediate slaughter. While the Spanish Authorities have put measures in place to minimise the risk of transmission of RVF from a potentially infected animal to humans, there is a need to identify the locations and times at higher potential risk of transmission and spread of RVF from North Africa to Spain particularly via mosquitoes. Potential competent vector mosquitoes can be transported by the wind to the nearest cities of Ceuta and Melilla and to the Spanish mainland, separated only by the 14 km wide Gibraltar Strait. The conditions for the establishment in Spain have been evaluated by analysing the national database of vector surveillance since 2005 along with environmental and climatic variables as well as animal and human hosts. Joining both the evaluation of the introduction as well as the conditions for the establishment we aim to offer a support for the early detection of a zoonotic emerging disease like RVF.



# **Serological surveillance of Schmallenberg virus infection in local and import cattle in the Russian Federation**

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Schmallenberg virus (SBV) infection is an emergent vector-borne infection of ruminants. Schmallenberg virus falls in the Simbu serogroup of the genus *Orthobunyavirus*. When infected cattle, sheep and goats show clinical signs of fever, diarrhoea and reduced production of milk. Another profile of SBV infection includes stillbirths and congenital malformations if infection occurs during gestation.

To date, a total of 24 countries have reported SBV incursions, however, only nine countries have notified OIE about clinical signs in 2011-2012.

Russia is located in close proximity or shares land with countries that were affected by SBV, which necessitates close surveillance of this infection for epidemiological purposes. In 2013 Russia launched a national SBV surveillance of local (born and raised in Russia) and import (imported before 2012) ruminants. A total of 19960 serum samples from 41 regions were screened.

The target population included 93475 and 102787 animals imported in 2011 and 2012, respectively. The regions that accounted for the highest proportion of imported animals were: Bryanskaya oblast – 42072 (21.4%), Voronezhskaya oblast – 31300 (15.9%), Kaliningradskaya – 13720 (6.9%), Republic Tatarstan – 10332 (5.2%), Lipetskaya oblast – 10237 (5.2%), Vladimirskaya oblast – 8897 (4.5%), Tymenskaya oblast – 7099 (3.6%).

As expected, 9.5% of imported animals tested positive, with no positive local animals being identified. These findings suggest that SBV has not yet arrived in Russia and indicate potential risks of incursion and spread.

# Prevalence of Newcastle disease virus in Ukraine in the period 2011-April 2014

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Newcastle disease viruses (NDV) have been placed in the genus *Avulavirus*, sub-family *Paramyxovirinae*, family *Paramyxoviridae*. NDV, a virus that is able to infect over 240 species of birds and is included in List A of the Office International des Epizooties. The fast development of poultry industry and insufficient management are the most actual factors which can cause the fast spread of NDV in Ukraine.

The aim of our study was to find out the number of clinical manifestation in herds and study the prevalence of NDV in Ukraine. Our study was carried out in the period 2011-April 2014. Samples from 24 regions and Crimea Peninsula were sent for the research. We analyzed 1496 samples, which we collected in 92 flocks of Ukraine with a variable number of birds experiencing respiratory signs, egg production failures, enteritis or nervous signs. Samples were analyzed by histology method for detection of the characteristic for NDV lesion in trachea, lungs, brain, stomach, intestine, spleen, kidney and liver.

In the case of NDV infection epithelium of the mucous membrane of the trachea was absent. In the submucosal membrane of the trachea revealed significant hemorrhage, infiltration of lymphocytes and macrophages. We also observed signs of necrotic changes in submucosal glands membrane of the trachea. In the lung, liver and kidney was detected edema of the endothelial cells of vessels. In some cases we also detected necrosis of the endothelial cells of vessels in these organs. In case of nervous clinical signs we observed also perivascular lymphocytic infiltration in brain. In such cases we performed histopathology analysis one more time within 14 days to exclude Marek's disease as one of the possible causes of changes in brain. In 42% of NDV positive cases we also observed depletion of spleen. However, we need to note that this lesion is not specific and could occur due to the influence of other factors. The next step of our study was confirmation of the histology result by PCR.

The amount of NDV positive samples is 1051, which is nearly 70%. NDV was detected in farms from 21 regions of Ukraine and in Crimea Peninsula. The circulation of NDV wasn't detected only in Poltava, Chernihiv and Ternopil regions. We want to note that the number of samples that we examined in these regions was insufficient for analysis of the situation and therefore, more research is needed. We compared our data from 2012 (43% infected) with our results from 2013 (52% positive samples) and detected an increasing number of NDV outbreaks. During the last 5 years we used the same method and the same PCR test system for this research so we can make some deductions about the tendency of NDV to spread.

Also we need to add that in 372 samples the specific for NDV mild histopathological lesion were observed, but PCR was negative for NDV. In such cases we made PCR of smears from the walls and cages of poultry houses and the PCR was positive for NDV. Thus, it was shown the circulation of the pathogen in flocks and the necessity to control the situation.

# Animal Chlamydioses in China

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## **Background and Significance:**

It is very important for scientists abroad to understand the situation of animal chlamydioses in China.

## **Objectives:**

To introduce effective control measures for animal chlamydioses in China

## **Methods:**

Several methods were applied: culture in chicken embryos; indirect hemagglutination (IHA) developed at LVRI; detection of *ompA* and *pmp* DNA; vaccination using a formalin-inactivated, egg-grown vaccine developed at LVRI; antibiotics (tetracycline, doxycycline, and rarely terramycin)

## **Results:**

Using IHA, *Chlamydia psittaci* was prevalent in chicken, duck, pigeon, goose and other fowl in 10 provinces with seropositive rates of 10% to 50% in last three decades. Likewise, *Chlamydia abortus* was prevalent in cows, yaks, sheep, goats and pigs with seropositive rates in different regions of 2-40% in ovine, 5-53% in swine, and 3-35% in bovine. Effective control measures include chlortetracycline were used before. However, antibiotic treatment did not prevent the spread of infection and may conceivably affect the development of immunity. Yearly vaccination was effective in sheep, swine and cows in endemic areas.

## **Conclusions:**

Although formalin-inactivated *Chlamydia abortus* vaccines have been used in China since 1981, the disease will spread if vaccination is suspended. Sporadic outbreaks in different areas and different animals threaten heavily the livestock industry of China. Although some success was obtained for controlling disease in livestock, none was recorded for the control of avian chlamydiosis. So far, there is no commercialized vaccine for avian chlamydiosis. Each year, huge economic losses are reported in avian production. Moreover, the disease is a potential risk for human health.

# **A Serological survey of bovine ephemeral fever in china**

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Bovine ephemeral fever (BEF) is caused by the arthropod-borne rhabdovirus bovine ephemeral fever virus (BEFV). The virus is classified family *Rhabdoviridae*, genus *Ephemerovirus*, causing a debilitating febrile illness in cattle and water buffaloes. At present, there is no standard assay in the world except the micro-neutralization test (MNT) used for the serological investigation of BEFV (NO.NY/T 543-2002, published by Ministry of Agriculture of the People's Republic of China in 27 August 2002). However, this approach needs more strict requirements than other assays, therefore, it turns out to be difficult to carry out at some common laboratories. An indirect ELISA assay was established for the serological investigation of BEFV infection in cattle. In the assay, a protein of approximately 18 kDa (including the G1 antigenic site of BEFV glycoprotein) expressed in PET-30a was used as coating antigen. The ELISA is an inexpensive and rapid serological detection method that would be suitable for screening BEFV infection on a large scale. Presently, there is a lack of serological surveillance for BEFV in cattle in China. Therefore, we examined the seroprevalence of BEFV in cattle in China during January 2012 to May 2014. Cattle sera samples collected from 19 provinces in China. Among 1808 sera samples, the seropositivity rate of cattle BEFV ranged from 6.7% to 81% in 19 provinces. Our findings emphasize that the occurrence and prevalence for BEFV are in almost all tested regions in China, suggesting BEFV maybe span in the other areas in China. Hence, the study can provide a reliable reference to monitor and control BEFV in China, even use a well model to detect other arbovirus.

# Fish Viral Surveillance in Finland

Tuija Gadd

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The amount of food fish produced in Finland is on average about 13 thousand tons yearly, mainly rainbow trout. Also whitefish is important. Fish is also produced for restocking and export juveniles. Baltic salmon, landlock salmon, brown trout, sea trout, brook trout, char, pikeperch, grayling, pike cyprinids and sturgeon are farmed in Finland.

In general, fish viruses are monitored extensively since 70`s and the situation is considered to be good except from 1970`s until the year 2000: the sea trout rhabdovirus (STRV) isolation from farmed brown trout in 1987, some infectious pancreatic necrosis virus (IPNV) isolations at the coastal area yearly and isolation of an iridovirus from pike-perch fingerlings with no signs of disease in 1996.

Viral disease situation has been favorable until 2000 when viral haemorrhagic septicaemia virus (VHSV) genotype Id was diagnosed for the first time in 2000 from four rainbow trout farms showing clinical signs in Åland and Pyhtää in Finland. The same genotype had been isolated from rainbow trout farms in three separate locations: Åland in the Baltic Sea, and Uusikaupunki in the Gulf of Bothnia, and Pyhtää in the Gulf of Finland. The majority of isolations have been from Åland, and since 2009 have only been from there. From Pyhtää the VHSV genotype Id was isolated only in 2000 and 2001 and from Uusikaupunki only once in 2004 and 2008. VHSV genotype II, isolated from the lamprey in 2003 and herring in 2004-2006 from Baltic Sea, is thought to be independent of the VHSV Id epidemic in farmed rainbow trout in Finnish brackish waters. Eradication of VHSV has been successful except for Åland where it is still present.

Perch rhabdovirus (PRV) causing mortality to grayling fry in inland fish farms have been encountered for the first time in 2002. PRV has been isolated from one farm during 2002, 2009 and 2010 and from another farm in 2003. PRV was also occasionally isolated from wild perch and sea trout from the Baltic Sea. Both PRV and STRV belong to a new genus *Perhabdovirus*.

IPNV both genotype 2 and 5 is encountered yearly at the coastal area, continental Finland has been considered IPNV free until 2012 when IPNV genotype 2 was diagnosed at some inland fresh water farms yearly. Statistics over fish virus surveillance will be presented.

# Factors affecting the infectivity of tissues from pigs with classical swine fever: Thermal inactivation rates

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CSF outbreaks can originate from many sources. However, future introductions into the UK are highly likely to be linked to some form of human transport of infected material. Risk assessment of practices that could cause an introduction of CSF, such as the illegal importation of meat or the disposal of catering waste, have to consider viral properties such as, how much virus may be present, how long virus will survive under the conditions encountered, how likely a susceptible animal will encounter the product and the amount of virus that would need to be consumed to cause an infection.

To facilitate such assessment we have investigated the rate that CSFV becomes inactivated at temperatures of relevance for cold storage (4°C), ambient temperatures (25°C), composting of catering waste (56°C) and cooking (68°C) in serum and tissues that comprise pork products (muscle, fat and lymph node).

Decimal reduction or D values, (time taken to reduce the viral TCID<sub>50</sub> 10-fold) indicate that CSFV survives substantially longer in muscle and serum at lower temperatures compared to fat and lymph node tissues. For example, at 4°C the D values in fat and lymph node were 1.5 (SD=0.4) and 1.9 (SD=0.8) weeks, respectively, whereas in muscle 5.6 weeks (SD=1.3) were required for a 10-fold reduction in the viral titre. These data highlight the importance of considering the product matrix when assessing likely virus survival times during a process.

Together with data we have generated on the oral infectious dose required to initiate CSF these results can be utilise to assess the threat that products of porcine origin pose for introduction and subsequent spread of CSFV.

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## Case report of equine influenza in Italy, in 2014.

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In the last decade, outbreaks of equine influenza (EI) in the European horse population were due to the circulation of the H3N8 virus, American lineage and, more recently, clade 2 of the sublineage Florida. Although EI in Italy is endemic, diagnostic requests for this infection during respiratory syndromes are rare. The last isolations of EI virus in Italy were in 2005, with the Rome and Bari strains presenting a high identity to strain A/eq/SouthAfrica/4/2003 (successively included in the sublineage Florida clade 1). In January 2014, in a racetrack in Rome, an investigation was conducted by the National Reference centre for equine diseases, following the onset of respiratory distress in one of the stables. The episode was principally characterised by fever and dry cough involving eleven flu vaccinated foals that had arrived at the end of 2013. From the preliminary tests conducted on the nasal swabs of these animals, using a panel of Real Time (RT) PCR's for the detection of equine respiratory viruses, only that for the detection of the protein M gene of the influenza virus type A resulted positive. To genetically characterize the viral strain, the PCR positive swabs (4/11) were inoculated in chicken embryos. RNA was extracted from the allantoic liquid, collected after three sub-passages of the isolates, and subsequently used for the reverse transcription RT-PCR performed with a pair of primers specific for the HA1 region of the segment IV of the viral genome. The first amplicon of 1080bp was used in the second round PCR, amplifying a sub-region of 220bp. On sequencing, this fragment presented a homology of 99.5% with the strain "North Rhine Westphalia" (GenBank acc.num. KJ538149), isolated, at around the same time in Germany, and the strain "Yokohama" (GenBank acc.num. AB761396.1), previously isolated in Japan, in 2012, both pertaining to clade 2 of the sublineage Florida. The present case study confirms what was reported in 2013, by the Panel of experts of the OIE, for the surveillance of EI and vaccine composition, relative to the exclusive circulation in Europe, in 2012, of clade 2, sublineage Florida. In this situation, because the affected animals were young, it was not possible to establish if the vaccine protection could have been compromised due to the limited number of vaccinations received or, the incomplete efficacy of the ones used. Starting from 2004, of the vaccines commercially available, only the composition of some has been updated with the recommended strains, A/eq/SouthAfrica/2003 ed A/eq/Ohio/2003, both belonging to clade 1 but not with the most recent circulating strains for which protection is apparently incomplete. Surveillance for EI is highly recommended especially to verify the appropriateness of the vaccine composition.

# **The First Detection of Lymphocystis Disease in Turbot (*Psetta maxima*) and Sturgeon (*Acipenser stellatus*) in Turkey**

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Lymphocystivirus is one of five genera of viruses within the viral family *Iridoviridae* which infects the fish. We have faced an unusual syndrome in our hatchery in spawning fishes. Virus isolation on BF-2 cell line had been achieved. A polymerase chain reaction was conducted to detection. After the identification of the agent sequence analysis was performed by using same primers used in the detection. This is the first isolation and detection of lymphocystis disease in Turkey.



# **Altitudinal waves of rabies in red foxes in response to oral vaccination in the Italian Alps during the 2008-2011 rabies epidemic.**

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After more than ten years of absence, rabies re-appeared in Italy in 2008 and rapidly spread in red fox population in the north-eastern regions, despite three manual vaccination campaigns. Since winter 2009, in order to contain a further westward spread of the infection and to minimize the risk of human exposure, a series of emergency oral rabies vaccination (ORV) was performed in the affected areas starting from winter 2009-2010, through aerial distribution of vaccine baits. The last rabid fox was detected in February 2011 and Italy has been officially declared free from rabies from February 2013. Due to climatic constraints, the winter vaccination campaign in 2009 was only performed below a threshold altitude of 1000 m above the sea level (asl), coinciding with the average freezing point in that winter season. We compared the spread of the disease above and below the threshold altitude to evaluate the disease dynamics parameters using field data. The study was focussed only on the first ORV, to better catch the effect of vaccination on a fully susceptible fox population. Using the doubling time method, the basic reproductive number for rabies in foxes was estimated before ( $R_0$ ) and after the vaccination campaign ( $R_V$ ) in a limited study area that accounted 164 rabies cases out of 170 detected in the whole vaccination area during the entire study period (23 October 2009 - 10 May 2010). The ORV had a positive effect in reducing fox rabies cases below 1000 m asl where vaccine baits were distributed (42,3% vs 19,4% of the total cases before and after ORV, respectively). On the contrary, rabies cases increased above 1000 m asl (57,7% vs 80,6% of the total cases before and after ORV, respectively). Nevertheless, analysis did not detect any marked difference in  $R_0$  vs.  $R_V$  (being respectively 2.17 and 2.13, before and after the first ORV). Despite a partially immune fox population, rabies could persist taking advantage of the unvaccinated fox population at altitudes higher than 1000 m asl where the susceptible fox population was large enough to allow the virus persistence even during winter.

# Phylogenetic Analyses of Bovine Viral Diarrhea Virus (BVDV) Detected in Cattle in Turkey

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**Abstract:** Bovine viral diarrhea virus (BVDV) has been reported in many countries and causes economic losses. Rapid detection and culling of persistently infected animals and efficacious vaccination are key factors to control bovine viral diarrhea virus (BVDV) infections in cattle. The aim of this study was to investigate frequency and detection of persistently infected cattle and analyse the diversity of bovine viral diarrhea virus in cattle in Turkey. For this, 1600 blood samples were collected from animals from 4 different regions in Turkey and analysed by antigen capture ELISA. BVDV was detected in samples collected from 14 of out of 20 farms surveyed. Of the 30 animals testing positive, 22 were available for testing one month after the original samples were collected. Samples were collected from these animals and tested by ELISA and real-time RT-PCR. Upon retesting 8 out of 22 samples were found to be positive by both assays. Genomic regions, coding from the viral glycoprotein E2 and the 5'UTR of BVDV, from 19 BVDV strains were amplified and sequenced. Phylogenetic analysis indicated that 17 Turkish BVDVs belonged to the BVDV-1 genotype and two belonged to the BVDV-2 genotype. Based on comparison of 5' UTR sequences, 8 strains (5, 6, 10, 11, 12, 13, 17 and 19) belonged to the BVDV1f sub-genotype, 1 strain (strain 8) belonged to the BVDV1i subgenotype and one strain (14) belonged to the BVDV1d subgenotype. One strain (4) was closer to the BVDV1f sub-genotype than to other sub-genotypes, but relatively distant from other BVDV1f sub-genotype strains. The remaining 6 strains (1, 2, 3, 7, 9, 18) belong to a novel sub-genotype. Similar results were obtained on the bases of comparison of E2 sequences with one exception. Strain 5 grouped with BVDV1f strains based on 5' UTR comparison but was grouped with the novel sub-genotype based on E2 sequence comparison. In conclusion, the results of phylogenetic analyses indicate that diverse strains were found in this study and this point should be considered in the design of diagnostic and preventive measures to be used in Turkey.

# Molecular Epidemiology of Bovine Norovirus in Turkey

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In this study, we performed a survey to detect BNoV in Turkey between 2009 and 2011 using 235 fecal samples from neonatal calves with diarrhea that were analyzed by nested reverse transcription RT-PCR using primers located in the consensus sequences of the reported BNoV RNA dependent RNA polymerase (Rdrp) gene. BNoV Rdrp gene was detected 1.7% (4/235) of samples by nested-RT-PCR, respectively. The nucleotide sequence of partial Rdrp fragments from the BNoV isolates including the newly identified Turkish isolates showed more than 86.3% nt and 96.8% aa identity each other. Interestingly, a comparison of the nt and aa sequences, one of Turkish BNoV strains have been found to be 100% aa identical with some of the Italian and Tunisian strains. As a result BNoV is one of the pathogens which contribute neonatal calf diarrhea cases in Turkey.

# Detection of bovine torovirus in Turkey

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Bovine torovirus (BToV), a member of the family Coronaviridae, is an established gastrointestinal infectious agent in cattle. In this study, we performed a survey to detect BToV in Turkey between 2009 and 2011 using 235 fecal samples from neonatal calves with diarrhea that were analyzed by the nested reverse transcription (RT) PCR method using primers located in the consensus sequences of the BToV membrane (M) gene. The BToV M gene was detected in 4.7 % (11/235) of the samples using the nested RT-PCR method. The nucleotide sequences of partial M fragments from the BToV isolates, including the newly identified Turkish isolates, showed more than 96 % identity. The result indicates that BToV is one of the pathogens that contribute to neonatal calf diarrhea cases in Turkey.

# Isolation and Genotyping of Bovine Rotaviruses in Diarrheic Calves in Turkey

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The study has designed for isolation and genotyping of bovine rotaviruses collected from newborn calves in Turkey. A total of 226 feces samples between 2009 and 2011 taken from 0 to 2 months old calves with diarrhea from Agricultural Enterprises country. As a result, 39 (17.25%) of feces were found positive by ELISA antigen. The positive samples were inoculated in MA-104 cell cultures, and virus identification was carried out by neutralization test. RT-PCR assay was performed with specific primers in the way of VP4/VP7 and 28 of them were found positive. The 15 samples, 1 from each city, were detected. In the way of G as a result of heminested PCR 10 of them determined as G10 and 5 of them as G6, in the way of P as a result of heminested PCR 14 of them determined as P and 1 of it as P. The samples from VP4/VP7 were sent to the sequence and exactly genotyped. As a consequence of sequence the most common VP4/VP7 combinations were found in 9 samples G10 P (60%), 5 samples G6 P (33.3%), 1 sample G10 P (6.6%) in Turkey.

# Isolation, Genetic Characterization and Prevalence of Canine Distemper Virus in İstanbul

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In this research, prevalence of distemper virus, virus isolation in owned dogs and stray dogs, in İstanbul, and comparison of the local strains with isolates in the gene bank by sequence analysis either from samples or from canine virus isolates is planned. For this purpose, by visiting the animal shelter provincial-wide of İstanbul blood samples were collected in 200 EDTA and EDTA-free and 300 EDTA-free tubes, and from disease suspected animals 77 of tracheal swabs, 81 of rectal swabs, 15 organs (7 lungs, 2 tracheas, 3 livers, 3 intestines) samples were taken. These samples were isolated in VERO and MDCK cell cultures. Isolated 21 of 81 rectal swap samples, 5 tracheal swaps were found positive by Reverse Transcriptase Polymerase Chain Reaction Test (RT-PCR). 56 leukocytes from 200 blood samples were found positive by Reverse Transcriptase Polymerase Chain Reaction Test (RT-PCR). 52 serum samples of 500 were vaccinated and only 34 of 52 (65%) were found positive. 70 of unvaccinated animals (15,6%) were found positive.

# **A retrospective clinical and epidemiological study on feline coronavirus (FCoV) in cats in Istanbul, Turkey**

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The presence of antibodies to Feline Coronavirus (FCoV) and Feline Immunodeficiency Virus (FIV), together with Feline Leukemia Virus (FeLV) antigen was, investigated in 169 ill cats attending a veterinary surgery in Istanbul between 2009-14. FCoV and FIV seroprevalence was 37% (63/169) and 11% (19/163), respectively and 1% (2/169) of cats were FeLV-antigen positive. Antibodies against both FCoV and FIV were only detected in one cat, and similarly only one cat had FCoV antibodies and FeLV antigen. FCoV seroprevalence increased with age until 3 years old and decreased thereafter, and was highest in 2014 and among household cats living with other cats and with outdoor access. Symptoms typically associated with wet Feline Infectious Peritonitis (FIP) including ascites, abdominal distention or pleural effusion, coupled in many cases with non-antibiotic responsive fever, were observed in 19% (32/169) of cats, and 75% of these cats were FCoV seropositive. FCoV seropositivity was also associated with a high white blood cell count, high plasma globulin, low plasma albumin and low blood urea nitrogen. The percentage of FCoV seropositive and seronegative cats that died in spite of supportive veterinary treatment was 33% and 12%, respectively. In conclusion; these results indicate that FCoV is widespread and has a severe clinical impact in cats from Istanbul. Moreover, the incidence of FCoV infections could be rising, and in the absence of effective vaccination cat owners need to be made aware of ways to minimize the spread of this virus.

# BITING DOGS: A DATA COLLECTION MODEL

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Dogs' aggressions are an important public health issue: dog bite can transmit rabies infection from dog to man and represents a source of other infections, it may result in severe damages requiring hospitalization, surgery, rehabilitation. Therefore biting dogs can cause significant financial and legal burdens for their owners. In Italy, each dog bite must be reported to the public Veterinary Service (VS), which assesses the level of the risk of the dog. The veterinary authorities of the Veneto Region (North-eastern Italy) identified this issue as a priority and a specific working group produced a protocol for evaluating the biting dogs into a "0-3" scale of dangerousness and set specific measures for safekeeping of the animal depending on the severity of the identified risk (i.e. re-education of the dog, training course for its owner).

Moreover, a computerized data collection system has been activated from 2009, when fox-mediated rabies re-emerged in the Veneto Region. In order to obtain an archive of biting dogs and dogs with uncontrolled aggression, new tools were added to the Data Bank of the Regional Canine Registry (BAC) in which the data and the movements of dogs, together with the personal data of the owner and the keeper of the animal, are stored.

The new packages allowed to:

- link the bite to the dog and its owner and keeper;
- highlight the biting/aggressive dog and disclose all its history about aggressions;
- collect relevant data on the bite event such as context, date, victims, reporting authority;
- record main information about the dog evaluation (e.g. veterinarian, risk category attributed, date);
- register training course/s attended by the owner/keeper .

A total of 8,642 aggressions were recorded in BAC from 2010 to 2013, with no significant differences in the years. The biting dogs were 71% males and 28% females, whereas the dog population recorded in BAC is 53% males and 47% females. Most of the biting dogs, at the time of the event, were under 8 years old (71%) with an average of 5.4 years ( $\sigma$  3.5) and the most frequent biting events regard 2 years old dogs. Fifty-six% of dogs which bit for the first time, were evaluated "trouble-free", while 31% were placed in the first risk category, 6% in the second and 2% in the third, 5% weren't evaluated. Ninety-three.8% of biting dogs did not bite again, whereas 66% of re-aggressions occurred within 12 months from the first event.

The software allows the VS to collect and organize data directly and indirectly related to the biting event and verify the compliance to the mandatory provisions.

The information recorded in BAC can be processed to evaluate the incidence and distribution of biting dogs and are useful to improve knowledge of the occurrence of aggressions in order to improve a correct approach to the problem.

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