1st INTERNATIONAL CONFERENCE OF THE EUROPEAN COLLEGE OF VETERINARY MICROBIOLOGY

# Abstract Book

**Abstract Book of the**
**1ST INTERNATIONAL CONFERENCE OF THE EUROPEAN COLLEGE OF VETERINARY MICROBIOLOGY**
Athens, Greece, 26th-27th September, 2019

**Organiser:** European College of Veterinary Microbiology (ECVM)

**Co-organiser:** Study Group for Veterinary Microbiology (ESGVM) from the European Society for Clinical Microbiology and Infectious Diseases (ESCMID)

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# Abstract Book

# Programme

## Thursday 26th September 2019

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| 9.00-11.00 | Registration | | |
| 10.00-11.00 | Welcome and opening of the Conference | Chair of the 1st ICECVM: **Dorina Timofte**<br>President of the ECVM: **John Ikonomopoulos**<br>ESGVM representative: **Patrick Butaye** | |
| **11.00-13.00** | **Veterinary Bacteriology and Mycology** | | |
| 11.00-13.00 | Chair: Jaap A. Wagenaar / Co-chair: Patrick Butaye | | |
| 11.00-11.30 | Keynote lecture | **Bryan Markey**<br>University College Dublin, Ireland | Chlamydiae and enzootic abortion of ewes (EAE): personal reflections from 30 years of research |
| 11.30-11.50 | Invited lecture | **Els Broens**<br>Utrecht University, the Netherlands | Import of *Brucella canis* positive dogs into the Netherlands –implications for animal and human health |
| 11.50-12.20 | Invited lecture | **Urs Giger**<br>University of Pennsylvania, U.S.A. | Hereditary immunodeficiencies and infectious diseases in dogs |
| 12.20-12.40 | Invited lecture | **Mihai Mares**<br>School of Veterinary Medicine, Romania | New insights into pathogenic cryptococci affecting animals |
| 12.40-13.00 | Invited lecture | **Alicia Aranaz**<br>Universidad Complutense, Spain | Tuberculosis (*Mycobacterium bovis*) in animals: targets, tools and tasks. |
| 13.00-14.00 | Lunch break / Poster viewing | | |
| **14.00-17.10** | **Veterinary Virology** | | |
| 14.00-15.40 | Chair: Niciola Decaro / Co-chair: George Filiousis | | |
| 14.00-14.30 | Keynote lecture | **Vito Martella**<br>University Aldo Moro, Italy | Noroviruses in humans and animals |
| 14.30-14.50 | Invited lecture | **Charalambos Billinis**<br>Veterinary School of Thessaly, Greece | West Nile virus surveillance in wild birds in Greece |
| 14.50-15.10 | Invited lecture | **Spyridon Kritas**<br>Veterinary School of Thessaloniky, Greece | Control of respiratory infections and decision making in pig farms in Greece |
| 15.10-15.25 | Short talk<br>(selected from abstract submissions) | **Alessio Bartolami** | A reverse zoonotic transmission event: A/H1N1/pdm (09) in a turkey breeder flock |

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| 15.25-15.40 | Short talk (selected from abstract submissions) | **Herman Egberink** | Serological screening for coronavirus infections in cats |
| 15.40-16.10 | Coffee break / Poster viewing | | |
| 16.10-17.10 | Chair: Spyridon Kritas / Co-Chair: Evanthia Petridou | | |
| 16.10-16.25 | Short talk (selected from abstract submissions) | **Niels Dekker** | Evaluation of the efficacy of bacteriophages-derived lytic enzymes (lysins) to reduce colonization and transmission of *Streptococcus suis* in pigs |
| 16.25-16.40 | Short talk (selected from abstract submissions) | **Georgia Diakoudi** | Identification of a novel picornavirus from common pipistrelle bat (*Pipistrellus pipistrellus*) |
| 16.40-16.55 | Short talk (selected from abstract submissions) | **Constantinos Kyriakis** | Susceptibility of swine to human Influenza A Viruses and the emergence of zoonotic viruses |
| 16.55-17.10 | Short talk (selected from abstract submissions) | **Filomena Fiorito** | MG-132 interferes with iron cellular homeostasis and counteracts bovine herpesvirus 1 productive infection |

## Friday 27th September 2019

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| **9.00-11.00** | **Diagnostic Microbiology, MALDI-TOF, Genomics & Metagenomics** | | |
| 9.00-11.00 | Chair: Els Broens / Co-Chair: Dorina Timofte | | |
| 9.00-9.20 | Invited lecture | **Georgios Oikonomou**<br>University of Liverpool, U.K. | Studying the role of foot skin microbiome and host genetics in cattle lameness |
| 9.20-9.40 | Invited lecture | **Prof Joachim Spergser**<br>University of Veterinary Medicine, Austria | Matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-ToF MS) is a powerful tool for species identification and taxonomic resolution of mycoplasmas isolated from animals |
| 9.40-9.55 | Short talk (selected from abstract submissions) | **Panagiotis Ballas** | Elucidating the role of *Streptococcus uberis* in bovine endometritis |
| 9.55-10.10 | Short talk (selected from abstract submissions) | **Antonia Mataragka** | Investigation to determine variations in faecal shedding of *Mycobacterium avium* subspecies paratuberculosis (MAP) in sheep, with regards to parturition |
| 10.10-10.35 | Short talk (selected from abstract submissions) | **Dimitrios Papadopoulos** | *Campylobacter coli* and *Campylobacter jejuni* prevalence and antibiotic resistance in Greek swine farms |
| 10.35-11.00 | Coffee break / Poster viewing | | |

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| **11.00-17.05** | **Antimicrobial Resistance, One Health and Food microbiology** | | |
| 11.00-12.35 | Chair: Bryan Markey / Co-Chair: Patricia Poeta | | |
| 11.00-11.30 | Keynote lecture | **Jaap A. Wagenaar**<br>Utrecht University, the Netherlands | The Dutch approach to reducing the use of antimicrobials in livestock |
| 11.30-11.50 | Invited lecture | **Patrick Butaye**<br>Ross University School of Veterinary medicine, St. Kitts and Nevis | Transfer of AMR: what have we learned from the past? |
| 11.50-12.05 | Short talk<br>(selected from abstract submissions) | **Adriana Belas** | ESBLs/pAmpC- producing *Escherichia coli* causing urinary tract infections in companion animals and humans in Portugal: antimicrobial resistance, pathogenicity and clonal diversity |
| 12.05-12.20 | Short talk<br>(selected from abstract submissions) | **Eva Cunha** | Fighting antimicrobial resistance dissemination in veterinary medicine using the antimicrobial peptide nisin |
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| 14.30-15.00 | Keynote lecture | **Luca Guardabassi**<br>University of Copenhagen, Denmark and Royal Veterinary College, UK | Veterinary research on AMR: need for a change? |
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| 16.00-17.05 | Chair: Constanca Pomba / Co-Chair: Alicia Aranaz | | |
| 16.00-16.20 | Invited lecture | **Patricia Poeta**<br>University of Trás-os-Montes and Alto Douro, Portugal | "One Health" approach to tackle antimicrobial resistance in Portugal |

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| 16.20-16.35 | Short talk (selected from abstract submissions) | **Geoffrey Foster** | *Corynebacterium ulcerans* an emerging zoonosis in Scottish dogs |
| 16.35-16.50 | Short talk (selected from abstract submissions) | **Lukasz Grzeskowiak** | Recent findings on *Clostridioides difficile* in pigs: succession, infection and microbial colonisation resistance |
| 16.50-17.05 | Short talk (selected from abstract submissions) | **Sonja Kittl** | Increasing MRSA carriage in Swiss pigs - a risk for veterinarians and farmers? |
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# Veterinary Bacteriology and Mycology

# Chlamydiae and enzootic abortion of ewes (EAE): personal reflections from 30 years of research

**Bryan Markey**[1]

[1]Associate Professor, School of Veterinary Medicine, University College Dublin, Ireland

In 1907 Ludwig Halberstaedter and Stanislaus von Prowazek published drawings of Giemsa-stained smears of conjunctival scrapings, they called the particles 'Chlamydozoa' from the Greek word for a cloak or short mantle [1]. These infectious agents were initially mistaken for viruses because they passed through bacterial filters and could not be grown on agar or in medium. In 1957 T'ang and others reported successful cultivation in the chicken embryo yolk sac [2]. Cultivation in several cell lines susceptible to infection by these microorgansims soon followed.

Chlamydiae are obligate intracellular bacteria with an unusual, biphasic developmental cycle. They replicate within cytoplasmic vacuoles in host cells. Chlamydiae have a relatively small genome of approx. 1Mbp and rely on the host cell for most of their metabolic needs, hence the term 'energy parasites'. In the developmental cycle of chlamydiae, infectious and reproductive forms are morphologically distinct. Infectious extracellular forms, called elementary bodies (EBs) are small (200 to 300 nm), metabolically inert and osmotically stable. The reticulate body (RB), about 1 µm in diameter, is metabolically active, osmotically fragile and replicates by binary fission within the endosome. About 20 hours after infection, the developmental cycle becomes asynchronous with some RBs continuing to divide while others condense and mature, forming EBs. In general, replication continues for up to 72 hours after infection when the host cell lyses.

The order *Chlamydiales* consists of eight families but by far the most significant species are found in the family *Chlamydiaceae*. For many years only a single genus comprising two species, *C. trachomatis* (human isolates) and *C. psittaci* (animal isolates) was recognized. Largely based on differences demonstrated by nucleic acid sequencing studies of the 16S and 23S rRNA genes, two genera, *Chlamydia* and *Chlamydophila*, were validly published by Everett et al. in 1999 [3]. Many scientists were unhappy with this division and in 2015 the two genera were merged into the single genus *Chlamydia*, comprising eleven official species [4].

Chlamydiae infect over 450 species of birds and a large number of mammalian species including humans. In recent years, isolations have also been reported from invertebrate species. The gastrointestinal tract appears to be the usual site of infection in animals. Infections are often subclinical and persistent. Both the severity and the type of disease produced by chlamydiae are highly variable, ranging from clinically inapparent infections and local infections of epithelial surfaces to severe systemic infections. Diseases associated with chlamydial infections include conjunctivitis, arthritis, abortion, urethritis, enteritis, pneumonia and encephalomyelitis.

Methods used for the diagnosis of chlamydial infections may involve demonstration of organisms in stained impression smears, immunohistochemistry, detection of chlamydial DNA by the polymerase chain reaction and isolation in susceptible cell lines or embryonated hens eggs. Animal infections can be confirmed by serology. However, interpretation of results is complicated by the fact that many of the available serological procedures detect antibodies against the genus-specific chlamydial LPS and therefore do not differentiate between chlamydial species.

In Ireland, the two most frequently diagnosed infectious causes of ovine abortion are *Toxoplasma*

*gondii* and *Chlamydia abortus*. These infectious agents are also important from a public health point of view, particularly with regard to pregnant women. Enzootic abortion of ewes (EAE) caused by *C. abortus* is primarily a disease of intensively-managed flocks. The disease is economically significant in most sheep-producing countries. Infection is usually introduced into clean flocks when infected replacement ewes abort. Large numbers of chlamydiae are shed in placentas and uterine discharges from affected ewes. EBs can remain viable in the environment for several days at low temperatures. The oral route has been considered the principal route of infection for many years but the natural course of the infection was only fully reproduced by Gutierrez *et al.* in 2011 [5]. Typically ewe lambs acquire infection during the neonatal period and abort during their first pregnancy. As a result, the most dramatic outbreaks of EAE often occur in the year following the introduction of infection into a flock. Although up to 30% of animals in a fully susceptible flock may abort, a rate of 5 to 10% is more usual in flocks in which the disease is enzootic. The site of persistence in non-pregnant ewes is unknown. The first signs of chlamydial infection of the placenta are detectable at about day 90 of gestation.The organism targets the trophoblast layer giving rise to inflammation, thrombotic vasculitis and tissue necrosis in the placenta. Dissemination to foetal tissues occurs but the pathological changes are mild. Abortion is considered to result from a combination of factors including reduced efficiency of foetal-maternal exchange, disruption of placental endocrine function and disruption of the immunological balance ('immune expulsion') between foetus and dam [6]. The Th1:Th2 paradigm described in mice does not seem to fit the ruminant model of pregnancy particularly well but then the ovine cotyledonary synepitheliochorial placenta differs substantially in structure from the haemochorial placenta of mice and undergoes limited trophoblast invasion of maternal tissue (found only in the placentomes). Interferon gamma produced by infected trophoblast cells may restrict chlamydial replication through the enzyme indoleamine 2,3-dioxygenase (IDO) and the resulting 'tryptophan starvation', but alternatively it may trigger an inflammatory response that may compound the damage caused by the infection [7].

The advent of molecular diagnostic methods has revolutionized the detection of *C. abortus*. Polymerase chain reaction techniques are available and can be carried out using species-specific primers to distinguish *C. abortus* and *C. pecorum* [8]. A real time PCR protocol for *C. abortus* is available [9]. A multiplex assays for detection of *T. gondii* and *C. abortus* has been developed [10]. However, the exquisite sensitivity of these assays has highlighted a new issue, the widespread detection of *C. abortus* in sheep flocks. Fortunately quantitative values obtained using real time assays can be very helpful in assigning pathological significance to chlamydial detection results. A number of different serological tests are available for the detection of chlamydial antibodies including the complement fixation test, ELISA and indirect immunofluorescence. *Chlamydophila abortus* shares some common antigens with *C. pecorum* and a number of Gram-negative bacteria. The use of recombinant antigens specific for *C. abortus* helps to improve the specificity of serological tests [11], but the serological tests currently available do not distinguish between vaccinated and infected animals. Chlamydiae are susceptible to a number of antibiotics which can be used during an outbreak. Administration of long-acting oxytetracycline to in-contact pregnant ewes has been shown to increase the number of live-born lambs. However, antibiotic treatment does not eliminate the infection and treated ewes may shed chlamydiae at parturition. A live attenuated vaccine, containing a chemically-induced temperature sensitive mutant strain, is available and must be administered to ewes prior to breeding. An inactivated vaccine is also available which can be used in pregnant animals. The abortion rate and level of shedding of the organism is significantly reduced in vaccinated animals. There are concerns over the use of the live attenuated vaccine as it has been shown to produce abortion in some flocks. It is possible that the immunity it evokes may simply be a matter of dose and route of administration [12]. There has been some success in identifying immunogenic proteins that may be useful both diagnostically and in eliciting a protective immune response [13]. A panel of selected recombinant proteins appears to offer a safe and efficacious way forward in developing new vaccines [14].

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# Import of *Brucella canis* positive dogs into the Netherlands–implications for animal and human health

**Els M. Broens[1], Marloes A.M. van Dijk[1], Marjolijn Holtslag[4], Nicole Willems[2], Vanessa X.N. Visser[3], Ingrid Keur[3], Marc Engelsma[4], Jaap A. Wagenaar[1,4], Hendrik Jan Roest[4,5]**

[1]Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands
[2]Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands
[3]NVWA Incident Crisiscentre (NVIC), Netherlands Food and Consumer Product Safety Authority (NVWA), Utrecht, the Netherlands
[4]Wageningen Bioveterinary Research, Lelystad, the Netherlands
[5]Ministry of Agriculture, Nature and Food Quality

## INTRODUCTION

*Brucella canis* had never been isolated from dogs in the Netherlands until November 2016 when the first case was detected. This first case was a 1 year old, castrated, female mixed breed dog which was imported from a rescue centre in Romania in February 2016. The dog showed intermittent lameness and back pain. A CT-scan showed a multifocal discospondylitis. Bacteriological culture from the affected intervertebral discs was found positive for *Brucella canis*.

## FOLLOW-UP

To raise awareness among veterinarians, information was disseminated. This resulted in over 30 notifications to the Incident and Crisiscentre of the Netherlands Food and Consumer Product Safety Authority in 2017 and 2018. Track and trace investigations confirmed 20 *B. canis* serologically positive dogs, all imported from Eastern Europe. In 11 of these serological positive dogs, *B. canis* was cultured from blood, urine and/or biopsies. The most prominent clinical sign in the dogs were intermittent lameness and back pain.

## IMPLICATIONS FOR ANIMAL AND HUMAN HEALTH

Import of dogs from Eastern Europe occurs frequently and poses a potential threat of importing *B. canis* into the Netherlands. Infected dogs may show reproductive problems, but in castrated dogs, lameness and back pain are most prominent. Transmission of *B. canis* between dogs and from dogs to humans occurs by direct contact. *B. canis* can be shed in reproductive excreta, urine, saliva and nasal secretions. Antimicrobial treatment of dogs is controversial as it can lead to clinical improvement, but it will not eliminate the bacterium. Therefore, clinically recovered dogs may still spread *B. canis*. To prevent further spread of the infection, castration or euthanasia may be justifiable.

The prevalence of human *B. canis* infections is probably underestimated as the diagnosis might be missed due to unawareness, nonspecific clinical signs and absence of accurate serological tests for *B. canis* antibodies in humans.

# Hereditary immunodeficiencies and infectious diseases in dogs

**Urs Giger**[1]

[1]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, USA

## INTRODUCTION

Animals with recurring or persistent, antimicrobial-unresponsive and unusual infections likely suffer from a hereditary (also called primary) immunodeficiency disorder. Immunodeficiencies represent a large heterogeneous group of dysfunctions of host immunity increasing the risk for infections. They can arise through disturbances in antigen-specific defense mechanisms mediated by lymphocytes, the nonspecific defense system (which includes phagocytes, plasma proteins, and physical barriers), or both. Many genetically determined immune defects have been described in the dog, whereas only a few are known in cats. A definitive diagnosis often requires specific immune testing in addition to routine laboratory tests, and successful therapeutic interventions are limited. The molecular defects for several primary immunodeficiencies have been elucidated allowing for DNA screening. A few hereditary immunodeficiency disorders are or were prevalent within certain breeds of dogs, whereas others occurred in isolated families/cases.

The nonspecific immune system, also known as *innate* or *natural immunity*, should be functional at birth and available on short notice to protect the host from invasion by all sorts of organisms. It includes physicochemical barriers, phagocytes, complement and other plasma proteins, and natural killer cells. Congenital barrier defects particularly involve the skin and mucous membrane surfaces and are associated with infections of specific organs. A variety of hereditary skin diseases are being further defined. The Ehlers-Danlos syndrome, causing fragile, hyperextendable skin and frequently also joint laxities in many dogs and cats as well as the myxedematous skin and immunodeficiencies of Shar-peis, predisposes the animals to pyoderma, whereas ciliary dyskinesia in dogs increases the susceptibility to rhinosinusitis and pneumonia. Similarly, x-chromosomal ectodermal dysplasia in German Shepherds is associated with skin as well as other immunodeficiencies.

Disorders of the phagocytic system involve defects of neutrophils and monocytes as well as the complement system and can lead to pyogenic and granulomatous infections. The granulomatous reaction can occur when neutrophils malfunction and mononuclear cells are recruited. A wide variety of pyogenic bacteria (e.g., staphylococci, *Escherichia coli*, *Klebsiella*, *Enterobacter*) are usually involved, most of which represent normal microflora or pathogens of relatively low virulence. Recurrent infections of the skin, respiratory tract, and oral cavity are common, and intermittent bacteremia and overwhelming sepsis are also seen. Multisystemic amyloidosis, vasculitis, and immune complex disease are complications that can occur because of chronic recurrent or persistent infection. Cyclic hematopoiesis and leukocyte adhesion deficiency (LAD) are examples of serious quantitative and qualitative phagocytic defects, respectively. A unique immunodeficiency causing a predisposition to avian tuberculosis in Miniature schnauzers (and also Basset hounds) has recently been elucidated at the molecular level.

The specific immune system can be divided into humoral and cell-mediated immune systems and includes B and T lymphocytes, immunoglobulins, and cytokines. Deficiencies of B lymphocytes or humoral immunity affect the production of immunoglobulins and lead to increased susceptibility to pyogenic bacterial infections. Deficiencies of T lymphocytes or cell-mediated immunity (CMI) are associated with viral and fungal infections, but intracellular bacterial infections may also occur. Animals with cellular immunodeficiencies may have smaller thymic and tonsillar tissues as well as intestinal and

peripheral lymph nodes and decreased numbers of circulating lymphocytes.

The degree of immunodeficiency varies greatly between defects. Infections may be systemic or restricted to a particular organ system like the skin or respiratory tract. Some immunodeficiencies lead to overwhelming infections and death within the first few days to weeks of life, whereas others, such as morphologic leukocyte changes, are not consistently associated with any noticeable predisposition to infection. Chédiak-Higashi syndrome in smoke-colored Persian cats is characterized by abnormally large eosinophilic granules in polymorphonuclear leukocytes. It causes no immunodeficiencies but does cause a bleeding tendency resulting from a platelet storage pool disease. Similarly, Birman cats with acidophilic granulation of neutrophils and dogs and cats with various lysosomal storage diseases (e.g., mucopolysaccharidosis, gangliosidosis, mannosidosis) have granulation or vacuolation of leukocytes without being immunocompromised; they also exhibit frequently a lymphocytosis. The Pelger-Huët anomaly, which is characterized by hyposegmentation of granulocytes, causes no immunodeficiency in animals, even though the leukograms of affected dogs and cats reveal the most severe left shift with a normal leukocyte count.

Although an increased susceptibility to opportunistic infections develops, the type of infection varies depending on the type of defect within the immune system. A few immunodeficiency disorders predispose animals to a restricted group of unusual infectious agents. Some male dachshunds appear predisposed to *Pneumocystis* pneumonia, and German shepherd dogs may be prone to systemic aspergillosis or rickettsiosis. Doberman pinschers and Rottweiler dogs are more likely to develop parvoviral disease. Golden and Labrador retrievers and Bernese mountain dogs that had high serum antibody titers to *Borrelia burgdorferi* were more likely to have glomerulonephritis. Basset hounds and miniature schnauzers have an increased susceptibility to systemic avian mycobacteriosis, and possibly candidiasis, toxoplasmosis, and neosporosis. American and English foxhounds appear to be predisposed to developing leishmaniasis. Great Danes and Dobermans may be more susceptible to cryptococcal infections. Furthermore, a genetic predisposition to demodicosis has been proposed in various canine breeds and families. Feline infectious peritonitis has also been suggested to have a genetic basis. The mechanisms predisposing particular animals to specific infections still remain unknown in many breeds but was recently discovered in Miniature Schnauzers with increased susceptibility to systemic fatal avian tuberculosis.

MAJOR CLINICAL SIGNS OF PRIMARY IMMUNODEFICIENCY DISORDERS:

1. Recurrent infections, chronic and protracted course of infection, or both
2. Infection with common non-pathogenic (opportunistic) or aberrant infectious agents
3. Severe and often atypical infectious disease manifestations
4. Delayed, incomplete, or lack of response to antimicrobial therapy
5. Adverse reactions to modified-live virus vaccines

The above-mentioned key signs of infection develop in animals with a primary immunodeficiency generally early in life. Despite receiving colostrum, clinically affected animals may have illness during the neonatal to juvenile period and may develop recurrent and overwhelming infections that lead to severe debilitation and death before 1 year of age. Several animals, but typically not all, in a litter may be affected, whereas the parents are usually healthy. A genetic predisposition to infection is rarely noted after 1 year of age (e.g., avian tuberculosis in Miniature Schnauzers). Furthermore, animals with primary immunodeficiencies may have other special clinical manifestations. Hypersensitivity reactions

may occur and reflect an overall dysregulation of the immune system caused by a lack of one or more components or a chronic antigen stimulation from inadequate clearance of infections. Chronic systemic infections may also hamper the growth rate. Characteristic coat color dilutions and increased tendency for surface bleeding are seen e.g. in collies with cyclic hematopoiesis, Persian cats with Chédiak-Higashi syndrome, and Weimaraners with an incompletely defined immunodeficiency. Nude Birman kittens are athymic and severely immunocompromised like nude mice, and animals with ectodermal dysplasia are associated with a complete lack or loss of hairs are not necessarily causing immunodeficiencies.

The mode of inheritance of primary immunodeficiencies has not yet been determined in all cases. Autosomal recessive transmission, with affected males and females born to healthy parents, is usual, but a few exceptions exist. The Pelger-Huët anomaly is inherited as an autosomal dominant trait and is clinically not associated with immunodeficiency in animals. Severe combined immunodeficiency caused by two different mutations in the common γ-chain interleukin-2 (IL-2) receptor in Basset hounds and Cardigan Welsh corgis are X-chromosomal recessive disorders; so only males are affected, and the dams and half of her female littermates are carriers. Thus, the breed, gender, age of onset, type of infections, and other special characteristics may clinically suggest a specific immunodeficiency. Furthermore, it follows that within a breed the immunodeficiency is typically caused by the same defect and mutation, while different breeds may have mutations in the same or different genes.

## DIAGNOSTIC STUDIES

Although an immunodeficiency may be suspected on the basis of clinical evidence, specific laboratory tests are generally required to reach a definitive diagnosis. A minimum database of information, including results of a complete blood count, serum chemistry screen, and urinalysis, should always be obtained and may suggest a specific disorder. The differential leukocyte count and microscopic evaluation of a blood smear are the most important test results. Leukopenia in the presence of an active bacterial infection is by far the most feared condition. It should be noted that generally, some breeds have normally low white blood cell counts such as greyhounds. Neutropenia may be transient, as it occurs with cyclic hematopoiesis every 12 to 14 days or parvovirus infection, or persistent, as it is seen in animals with cobalamin malabsorption or overwhelming infections (sepsis). Lymphopenia may be observed in dogs with a T-cell or severe combined immunodeficiency. Although leukocytosis is expected during periods of infection, defects in leukocyte adhesion and egress from blood circulation at sites of infection may be associated with disproportionately high leukocytosis for the degree of infection as seen with hereditary LAD and glucocorticoid usage. Dachshunds with *Pneumocystis* pneumonia also have very marked leukocytosis. Anemia of chronic disease is often observed in infected animals caused by several factors, but the erythrocyte count may be in the normal range even if the animals have active infections and during periods of treatment and remission. Careful microscopic review of a blood smear may reveal leukocyte abnormalities such as granulation and vacuolation resulting from lysosomal storage diseases or Chédiak-Higashi syndrome, acidophilic granulation of leukocytes in Birmans, phagocytized microorganisms, or toxic leukocyte changes that suggest overwhelming bacterial infections.

Serum globulin concentrations are generally higher during chronic infections. Low or normal globulin levels in infected animals may suggest major external losses or diminished production from a humoral (B-cell) immune defect. Indeed, specific immunoglobulin deficiencies have been recognized in dogs. Serum protein electrophoresis may identify a γ-globulin deficiency, but immunoelectrophoresis is required to detect the class and degree of immunoglobulin deficiency. Maternal immunoglobulins can only be absorbed during the first day of life and influence the values during the first few weeks. IgM can be synthesized very early in life, whereas the development of IgA may be delayed for months. Thus, it is important to compare values with data from age-matched controls. Titers against specific antigens can be measured, followed by evaluation of the antibody response to vaccination against particular agents.

T-cell or combined immunodeficiencies cause defective CMI responses. The animal may have prolonged allograft rejection times and decreased delayed-type hypersensitivity to skin testing with viral vaccines, tuberculin, or dinitrochlorobenzene (DNCB). Reduced in vitro lymphocyte stimulation results may also be caused by a primary lymphocyte defect or the infection.

The identification of the agents infecting an animal is important for diagnostic as well as therapeutic reasons. Appropriate cultures of tissues, body fluids, and excretions for microorganisms and antigen and serologic blood tests are needed. Antibody titers may also be used to assess a response to vaccines and humoral immunity.

Gross and microscopic histopathology and cytology may reveal certain microorganisms, but are most helpful in characterizing the architecture, morphology, maturation, and function of the immune system, such as of the leukocytes, bone marrow, lymph nodes, thymus, and spleen, as well as other barrier systems. In ciliary dyskinesia, morphologic abnormalities of cilia may be identified by electron microscopy, but functional studies by imaging techniques or on respiratory epithelial biopsy specimens are also indicated.

For additional characterization of the immunodeficiencies, special leukocyte studies are often required. Surface marker studies by fluorescent-assisted cell sorters or flow cytometers can differentiate between T- and B-cells, determine T- and B-cell ratios, and determine the presence or absence of leukocyte adhesion proteins (CD11/18) or IL-2 receptors. Lymphocyte function studies include lymphocyte stimulation and plaque-forming assays for in vitro immunoglobulin production. Phagocyte function studies assess leukocyte adhesion, migration, chemotaxis, phagocytosis, "respiratory burst," and bactericidal activity. All functional assays should be performed on fresh blood cells (<1 day) and compared simultaneously with an age- and breed-matched control. Furthermore, in vitro lymphocyte functions are generally impaired and phagocyte functions are enhanced during periods of active infection. Whenever possible, it is advisable to control the infection before studying leukocyte function.

Finally, mutation- and breed-specific tests are offered for several hereditary immunodeficiencies in dogs and cats by special laboratories like Laboklin and PennGen.

## TREATMENT AND PREVENTION

Successful control of infection in immunodeficient animals depends on the underlying disease as well as the type and severity of the immune defect. In immunocompromised patients, early and aggressive antimicrobial therapy is indicated even for mild infections with nonpathogenic agents. Because of the immunodeficient host's potential inability to kill bacteria, bactericidal antibiotics are recommended until bacterial infections are controlled.

No practical treatments for primary immunodeficiencies exist to be curative (except for parenteral cobalamin administration to animals with cobalamin malabsorption). Thus, immunocompromised animals with infection generally have a guarded to poor prognosis. Despite aggressive antimicrobial therapy, their infections are difficult to control, leading to overwhelming infections, protracted courses, and recurrences. Some leukocyte defects cause death before 1 year of age, whereas others may not lead to a markedly increased predisposition to infection. In experimental studies, bone marrow transplantation and gene therapy corrected several canine leukocyte defects. Indeed, dogs with hereditary immunodeficiencies and other genetic defects have served as intermediate between experiments in murine models and its application in humans to test safety and efficacy of novel therapies.

Examples of Primary Immunodeficiencies

| **Disease (Syndrome)** | **Inheritance** | **Breeds** | **Characterization** |
|---|---|---|---|
| Ciliary dyskinesia (immotile cilia syndrome) | AR | Many dog breeds | Rhinosinusitis, bronchopneumonia with bronchiectasis, situs inversus |
| Complement component 3 (C3 deficiency) | AR | Brittany Spaniel | Pyogenic infections, lack of complement-mediated phagocytosis |
| Bactericidal neutrophil defect | U | Doberman Pinscher | Upper respiratory infections, reduced bactericidal activity |
| Cyclic hematopoiesis (cyclic neutropenia) | AR | Collie (gray) | Severe neutropenia every 12-14 days, reactive amyloidosis |
| Leukocyte adhesion deficiency (LAD or CD18 deficiency) | AR | Irish Setter, Red and White Setter, cat | Severe leukocytosis, infection with limited pus formation, lack of neutrophil adhesion |
| Pelger-Huët anomaly | AD | Aust. Shepherd, Fox-hound, others, cats | Hyposegmented granulocytes, no immunodeficiency |
| Selective cobalamin malabsorption (Cubulin or Amnionless deficiency) | AR | Giant Schnauzer, Border Collie, Beagle, A. Shepherd, Komondor | Weight loss, inappetence, leukopenia with hypersegmentation megaloblastic bone marrow, methylmalonic aciduria |
| Increased susceptibility to avian mycobacteriosis | U | Miniature Schnauzers, Basset Hound | Systemic avian tuberculosis and few other unusual infections |
| Increased susceptibility to *Pneumocystis* pneumonia | AR | Dachshund, Miniature Schnauzers | *Pneumocystis* pneumonia |
| Susceptibility to fungal and rickettsial infections | U | German Shepherd | Severe ehrlichiosis, Rocky Mountain spotted fever, disseminated aspergillosis |
| X-linked severe combined immunodeficiency (X-SCID) | XR | Basset Hound, Cardigan Welsh Corgi | Severe bacterial and viral infections, no IgG and IgA, deficient lymphocyte blastogenesis |
| Severe combined immunodeficiency (SCID) | AR | Jack Russell terrier, Friesian Water dog | Severe serum immunoglobulin deficiency, hypoplasia of lymphoid tissues |
| Thymic abnormalities and dwarfism | U | Weimaraner | Reduced growth, thymosin responsive |
| Recurrent infections/ inflammation | U | Weimaraner | Pyoderma, severe abscess, bleeding tendency |
| Selective IgA deficiency | U | Beagle, Shar-pei, German Shepherd | Respiratory and GI infections |

| | | | |
|---|---|---|---|
| Hypotrichosis congenital and thymic atrophy | AR | Birman | Nude kittens, neonatal death, no thymus |
| Chédiak-Higashi syndrome | AR | Persian | No immunodeficiency, large granules in phagocytes, bleeding tendency |

Owners must consider the potential zoonotic risks involved with keeping an immunodeficient animal with infections that may be contagious to humans, particularly immunosuppressed humans exposed to foxhounds with leishmaniasis and Miniature schnauzers and basset hounds with avian mycobacteriosis.

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# New insights into pathogenic cryptococci affecting animals

**Mihai Mareș**[1]

[1]School of Veterinary Medicine, *Ion Ionescu de la Brad* University of Agricultural Sciences and Veterinary Medicine, Iași – Romania

Cryptococci are capsulated, melanin-producing budding yeasts belonging to *Phylum Basidiomycota*. They have a worldwide distribution and some species are primary or opportunistic pathogens for humans and both wild and domestic animals (rare cases occur in birds, reptiles, and amphibians). In the last decade, the molecular taxonomy of the *Cryptococcus neoformans / Cryptococcus gattii* species complex has been refined and seven species were recognized as new taxa in the complex – namely *C. neoformans*, *C. deneoformans*, *C. gattii*, *C. bacillisporus*, *C. deuterogattii*, *C. tetragattii*, and *C. decagattii*. Cryptococci exhibit numerous virulence factors that are linked with the invasive character of infection and their persistence in the host tissues. Thus, the polysaccharide capsule, melanin, urease, phenotypic switching, presence of "titan cells", and inositol utilization are among the most effective virulence factors.

Cryptococci are successful pathogens because of their ability to evade the host defense mechanisms and to persist in environments containing antifungals.

# Tuberculosis (*Mycobacterium bovis*) in animals: targets, tools and tasks.

**Aranaz A.[1], Santos S.[2,3], Fernández-Novo A.[2], Calahorra F.[3], Ruiz-Santa Quiteria J.A.[1]**

[1]Dept Animal Health, Faculty of Veterinary Medicine, Universidad Complutense de Madrid
[2]Bovitecnia, Madrid
[3]Dept Animal Production, Faculty of Veterinary Medicine, UCM

Tuberculosis is an infectious disease caused by microorganisms of the *Mycobacterium tuberculosis* complex, *M. bovis* and *M. caprae* are the most relevant in animal health. This mycobacterial infection represents a concern worldwide because of its high economic impact and zoonotic potential. Tuberculosis transmitted between livestock, wildlife, and humans challenges health protection, the economic sustainability of agriculture, and the conservation of wildlife.

For almost a century, organised eradication of bovine tuberculosis has been a major objective of farming communities and public authorities. Despite the use of vast economical and human resources, infection remains endemic in some areas, while in others sporadic outbreaks are still detected, posing significant challenges to disease elimination.

Work is being carried out to understand underlying causes: (1) contribution of cattle-to-cattle transmission; (2) role of other domestic animals, (3) performance of official diagnostic tests, and (4) effect of interferences in the diagnostic tests. The weight of these causes may also differ depending on the farming system and ecological factors.

Recent studies have renewed the interest in serological assays as diagnostic tests, alone and in combination with skin test. Currently there is growing information in terms of number of animals and conditions of use, and it seems to be robust regardless the antibody detection assay (ELISA test, multi-antigen print immunoassay, dual-path platform assay) used for antibody detection.

The aim is to improve the toolbox of diagnostic tests for tuberculosis and to develop a strategy for implementation of antibody detection after the anamnestic response evoked by the intradermal test. This strategy can be variable depending on the epidemiological situation of the affected herd. This potential use would need to be supported with a cost-benefits assessment that would take into account costs of additional handling and laboratory test *vs* benefit of prompt removal of infected animals.

# Veterinary Virology

# Noroviruses in humans and animals

**Vito Martella**[1]

[1]Department of veterinary Medicine, University of Bari Aldo Moro, Italy

Noroviruses are major human enteric pathogens, associated with acute gastroenteritis in patients of all ages. Transmission of norovirus occurs by either direct or indirect modalities. Contaminated water, seafood, fruits and vegetables are a frequent source of infection [1]. Norovirus positive-sense, single-stranded RNA genome is subjected to impressive genetic diversification, via accumulation of punctate mutations and recombination, thus posing a challenge for their classification, for diagnostics and for the development of vaccines. Noroviruses can be classified into different genogroups and further into genotypes through a dual nomenclature system based on partial ORF1 (encompassing the RNA-dependent RNA polymerase) nucleotide sequence and on the complete VP1 (capsid protein, ORF2) amino acid sequence [2]. Based on the VP1, noroviruses can be classified into 10 (GI-GX) genogroups, which can be further divided into 46 genotypes [2]. Host susceptibility to norovirus infection is restricted genetically and the histo-blood group antigens (HBGAs) have been recognized as important receptors for virus binding [1]. Cultivation in vitro of noroviruses has long been impossible, until the development of organoid (enteroid) systems [3] and this has hampered the understanding of several viral biological properties. Noroviruses have been identified in several animal species, including livestock animals, pets and other terrestrial and aquatic animals, although their pathogenic role in those animal species is unclear [4, 5]. Several pieces of evidence suggest a possible inter-species circulation of noroviruses among closely related host species, and between humans and animals, thus raising concern for possible zoonotic risks.

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# West Nile virus surveillance in wild birds in Greece

**Charalambos Billinis**[1]

[1]Laboratory of Microbiology & Parasitology, Faculty of Veterinary Medicine, University of Thessaly

## BACKGROUND

West Nile Virus (WNV) is a mosquito-borne flavivirus. Wild birds play an important role as reservoir hosts and in the introduction of the virus to new unaffected areas. The largest European outbreak took place in Greece, with more than 624 confirmed cases of human infection and 79 deaths reported from 2010 to 2014.

## OBJECTIVES

Evaluate exposure of various species of wild birds to West Nile virus and correlate results with human cases data. Discover environmental factors associated with the disease spread. Create possible spatial patterns of future dispersion using Geographical Information System (GIS).

## MATERIALS AND METHODS

A serological and molecular surveillance was performed, in more than 1500 wild birds, belonged in 36 different species, that were hunted, found dead or trapped since 2009 in mainland Greece.

## RESULTS

WNV seropositive birds were hunted eight months before the outbreak of human cases in 2010 [1]. Migratory birds were found to be exposed to WNV prior to their time of arrival in Greece during autumn migration. Many corvid samples were found to be positive, indicating an extended exposure of resident wild birds to the virus. Results showed association of human cases with wild birds' exposure to the virus; no avian sera were found positive in prefectures not affected by the WNV outbreak. A similar WNV strain to the one detected in humans was also detected in hunter-harvested Eurasian magpies [2]. Distance from permanent water bodies and altitude were identified using Geographic Information Systems (GIS) and statistical analysis, (two-step cluster analysis) as environmental factors associated with the presence of positive birds indicating high-risk areas [3]. After a two-year hiatus, in 2017, a total of 48 human cases were reported in various areas of the Peloponnese region [4]. One month before human cases occur, dead wild birds (Eurasian magpies and Hooded crows) and wild birds with neurological signs were reported in the area of the epicentre. The virus was detected in Eurasian magpies. In 2018, a total of 316 human cases were reported, 243 of which showed neurological signs; a total of 50 human deaths cases were reported. Wild bird deaths showing neurological signs were reported from various areas of mainland Greece, and especially Attica region; positive wild birds were detected in the same region. In 2019, until mid-August, a total of 96 human cases have been reported, 59 of which showed neurological signs and 10 deaths [5].

## CONCLUSIONS

These findings underscore the importance of surveillance of wild birds for zoonotic diseases such as WNV and that pre-emergence surveillance of wildlife can be a powerful tool as part of an effective warning system to prevent or reduce the impact of emerging zoonoses.

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# Control of respiratory infections and decision making in pig farms in Greece

**Spyridon K. Kritas**[1]

[1]Department of Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece

The presence of significant infectious diseases in farms, depends not only on the pathogens themselves but also on several other factors, either known or unknown. Decision making is usually focused to controlling the known causative agent e.g. by administering a vaccine or an antibiotic, and very rarely considers the problem through its multifactorial perspective. This presentation makes an attempt to examine common respiratory infectious problems found in Greek pig farms through a simultaneous approach of dominant and most substantial tendency of many known and unknown factors by using Multiple Correspondence Analysis (MCA).

Significant infectious respiratory diseases such as pseudorabies (PRV or Aujeszky's disease), swine influenza (H1N1 and H3N2), pleuropneumonia due to Actinobacillus pleuropneumoniae-App and Porcine reproductive and Respiratory Syndrome (PRRS) often observed in Greek pig farms, are simultaneously viewed in association with factors such as farm size, stocking density, distance from the nearest pig farm, supply of breeding animals from other sources, quarantine of newly purchased animals, the implementation of basic hygienic-biosecurity measures, the implementation of the all-in all-out system (AIAO), the existence of financial problems, the presence of nervous, respiratory, digestive and reproductive symptoms, the production stage in which the symptoms may have been observed (eg newborn, weaned, fattened animals) as well as the existence of increased mortality. The results will be discussed under the view of decision making.

# A reverse zoonotic transmission event: A/H1N1/pdm (09) in a turkey breeder flock

**Bortolami A.[1], Gobbo F.[1], Zecchin B.[1], Fusaro A.[2], Leardini S.[1], Cecchettin K.[1], Pastori A.[2], Zamperin G. [2], Monne I.[2], Bonfante F.[1], Terregino C.[1,2]**

[1]Istituto Zooprofilattico Sperimentale delle Venezie, SCS6 Laboratorio di Virologia Speciale e Sperimentazione
[2]Istituto Zooprofilattico Sperimentale delle Venezie, SCS5 Ricerca e Innovazione

Research regarding zoonotic diseases often focuses on infectious diseases that have the potential to be transmitted from animals to humans, while less attention is given to transmission events occurring from humans to animals [1]. However, reverse zoonotic transmission events are increasingly reported and the aim of this work was to better understand what happened in an episode of an Influenza A (IA) virus transmission from humans to turkeys. In April 2019 a turkey breeder company contacted the Italian Official Veterinary Authority to complain about a sudden and drastic drop in egg production in mature hens (38 weeks old) from a holding comprising 4 barns. Tracheal swabs and blood sera from birds of each barn were collected for virological and serological testing.

Serology shown positive samples for IA virus in hens barns, the tracheal swabs from hens and stags tested positive by M gene rtRT-PCR for IA and negative for H5, H7 and H9 subtypes by specific RT-PCR. Sequencing of the HA gene showed that clinical specimens of hens and stags had a high similarity (99-100%) with human A/H1N1pdm(09) strains of the 2018-2019 flu season, revealing a possible human source of the virus. Full-length genome sequences analysis showed clustering of all the genes with contemporary human A/H1N1pdm viruses. Intra- and between-barn viral diversity was detected and mutations associated with possible mechanisms of adaption to the avian host were identified.

Previous research shown that turkeys are relatively resistant to human IA viruses when inoculated via respiratory route [2], however transmission can occur via intrauterine exposure [3]. Timely detection, high biosecurity measures and the implementation of a continuous surveillance are necessary to avoid further spread of human viruses in animals and to reduce the risk of the emergence of reassortant viruses posing a risk for human and animal health.

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# Serological screening for coronavirus infections in cats

**Herman Egberink[1], Shan Zhao[2], Wentao Li[2], Nancy Schuurman[2], Frank van Kuppeveld[2], Berend Jan Bosch[2]**

[1]Virology and Clinical Infectiology Division, Department of Infectious Diseases & Immunology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584CL Utrecht, the Netherlands
[2]Virology Division, Department of Infectious Diseases & Immunology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584CL Utrecht, the Netherlands

## OBJECTIVE

Coronaviruses (CoVs) are known for their high mutation rate and potential for cross-species transmission. Previous studies have demonstrated that some non-feline coronaviruses can infect feline cells as well as cats after experimental infection. Thus, cats might become naturally infected with CoVs of other species. The S1 receptor binding subunit of the CoV spike protein is immunogenic and possess low amino acid identity, making it a potential tool for serologically monitoring cross-species transmission.

## MATERIALS AND METHODS

In total 137 field cat sera and 25 FCoV type-1 or type-2 specific antisera were screened by ELISA for antibodies against S1 of 12 different CoVs, including human CoVs (MERS, SARS, HCoV-229E, HKU1, OC43 and NL63), porcine CoVs (PEDV, TGEV, PDCoV), bovine CoV and Feline CoV type 1 and 2.

## RESULTS

Antibodies were detected against FCoV type 1 (54,7%) and type 2 (19,0%), PEDV (19.7%), TGEV(10.9%), PDCoV(5.8%) and HCoV-229E(11.7%). The PEDV and TGEV positive sera were also positive for FCoV. Also some FCoV-specific antisera showed reactivity against PEDV and TGEV, suggesting the existence of cross-reactive antibodies. Domain mapping of antibody epitopes indicated the presence of conserved epitope(s) particularly in the CD domains of S1. Some sera exclusively reacted with human coronavirus HCoV-229E or the porcine coronavirus PDCoV. Also the FCoV specific sera did not show cross-reactivity against S1 of HCoV-229E and PDCoV.

## CONCLUSIONS

Despite the low amino acid identity, cross-reactivity among S1 proteins of some of the coronaviruses could be observed. The existence of cross-reactive antibodies against TGEV and PEDV makes it difficult to draw any conclusions about potential cross-species transmission. A few cats had antibodies exclusively against HCoV-229E and PDCoV, which might be elicited by an infection with these or related viruses. The potential role of cats in cross-species transmission of coronaviruses cannot be excluded and remains to be further elucidated.

# Evaluation of the efficacy of bacteriophages-derived lytic enzymes (lysins) to reduce colonization and transmission of *Streptococcus suis* in pigs

**Niels Dekker[1], Annemarie Bouma[2], Ineke Daemen[3], Hans Vernooij[3], Leo van Leengoed[3], Daniel B. Gilmer[4], Jonathan E. Schmitz[4], Vincent A. Fischetti[4], Arjan Stegeman[3] and Jaap A. Wagenaar[1]**

[1]Faculty of Veterinary Medicine, Department of Infectious Diseases and Immunology, Utrecht University, Utrecht, The Netherlands
[2] Ministry of Agriculture, Nature and Food Quality, The Hague, The Netherlands
[3] Faculty of Veterinary Medicine, Department of Farm Animal Health, Utrecht University, Utrecht, The Netherlands
[4] Laboratory of Bacterial Pathogenesis and Immunology, The Rockefeller University, New York, USA

## OBJECTIVE

*Streptococcus suis* causes severe infections in pigs, and occasionally in humans. To control disease in pigs, large amounts of antimicrobials are used. An alternative, more pathogen specific approach could be the therapeutic use of bacteriophage lysins. Our objective was to study the effect of nasal and oral application of lysins ΔPlySs1 and PlySs2 [1] on S. *suis* serotype 9 colonization and transmission, and on clinical signs.

## MATERIALS AND METHODS

Two experiments that only differed in lysins doses were performed. Each consisted of one lysins- and one placebo-treated group. In each group 5 pigs were inoculated intranasally with S. *suis*, and 6 contact-pigs were added. Pigs were monitored for two weeks, in which treatment was given to both inoculated (days 3-4 and 8-10) and contact pigs (days 1-4 and 8-10). Per treatment a pig received a combination of ΔPlySs1 and PlySs2, in low (0.8 and 0.4 mg) or high doses (1.1 and 3.5 mg) in the two experiments respectively. Saliva and nose swab samples, and tonsillar tissue samples were tested for S. *suis* by quantitative bacteriological culture.

## RESULTS

Lysin-treated pigs showed a significant reduction in S. *suis* loads in saliva (1.27-1.81 $^{10}$LogCFU) and nose samples (1.67 $^{10}$LogCFU) on one day (high-dose group) or two days (low-dose group). Transmission rates did not differ between lysin-treated and control groups ($P_{low\text{-}dose}$=0.530; $P_{high\text{-}dose}$=0.487), and clinical signs and mortality were comparable.

## CONCLUSION

Although phage lysins ΔPlySs1 and PlySs2 show a clear lytic activity against S. *suis in vitro* and strongly reduce S. *suis* colonization in a mouse model [1,2], they appeared not to be effective in pigs with the current formulation. Application did not reduce S. *suis* transmission between animals or protect against clinical signs and mortality. Reduction of mucosal colonization was only observed on some days of lysins administration.

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# Identification of a novel picornavirus from common pipistrelle bat (*Pipistrellus pipistrellus*)

**Georgia Diakoudi[1], Urška Jamnikar Ciglenečki[2], Vito Martella[1], Urška Kuhar[2]**

[1] Department of Veterinary Medicine, University of Bari, Valenzano, Italy
[2] Faculty of Veterinary Medicine, University of Ljubljana, Ljubljana, Slovenia

## OBJECTIVE

Picornaviruses are important pathogens with zoonotic potential, causing a wide range of diseases in humans and animals [1]. Bats have been recognized as natural hosts for various viral agents, with some viruses being regarded as a possible zoonotic threat to humans [2]. Here, we report the identification of a novel picornavirus found in a common pipistrelle bat in Italy, using metagenomic viral discovery approach.

## MATERIALS AND METHODS

We used next-generation sequencing (NGS) with metagenomic approach to determine the virome of a common pipistrelle bat found in the north of Italy. Libraries were constructed from both DNA and RNA extracted from a pool of organs (encephalon, intestine and viscera) and were sequenced on the Ion Torrent PGM platform, producing ~1.1 million reads. The complete polyprotein region of the bat picornavirus was aligned with picornavirus strains obtained from GenBank and sequence and phylogenetic analysis were performed.

## RESULTS AND DISCUSSION

Bioinformatic analysis of the NGS data detected the full genome of a novel picornavirus. Sequence analysis of the new picornavirus revealed a single ORF encoding a large polyprotein of 2462 amino acids, that displayed the highest nucleotide (65.4%) and amino acid (70.7%) identity to a partial genome sequence of bat picornavirus (GenBank accession no. KJ641686), found in 2010 in China. Upon phylogenetic analysis, the bat virus clustered tightly with kobuviruses, sharing 51.4% nucleotide and 44.5% amino acid identity with Aichivirus A. Human kobuviruses (Aichivirus) are involved in 0.9–4.1% of sporadic cases of pediatric gastroenteritis. Novel kobuviruses genetically closely related to human aichiviruses have been found in domestic and wild carnivores and in livestock animals. The identification of kobuvirus-like pricornaviruses in bats is therefore interesting in terms of understanding the evolution of kobuviruses across the various mammalian hosts.

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# Susceptibility of swine to human Influenza A Viruses and the emergence of zoonotic viruses

**J. Fletcher North[1], Peter J. Neasham[1], Kirklin L. McWhorter[1], Scott D. Silvis[1], Virginia Aida[1], Paul H. Walz[1], S. Mark Tompkins[2] and Constantinos S. Kyriakis[1,2]**

[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA
[2]Center for Vaccines and Immunology, University of Georgia, Athens, GA, USA

## OBJECTIVES

Swine are natural hosts of influenza A viruses (IAVs) with similar pathogenesis and immune responses compared to humans. Occasionally, human IAVs (huIAVs) infect pigs, reassort with swine IAVs (swIAVs) and contribute gene segments to emerging viruses. However, the initial spill-over of huIAVs to pigs is rarely detected, raising questions about the competency and persistence of human viruses in pigs and the risk of reassortment.

## MATERIALS AND METHODS

In this study we compared the replication kinetics and immune responses of swine and human IAVs in pigs. Groups of 8-week-old influenza seronegative piglets were infected intranasally with $10^{7.0}TCID_{50}$ of one of the following viruses: (a) sw/NC/154076/2015, a gamma H1N1 swIAV, (b) CA/07/2009, the prototype 2009 H1N1 pandemic virus, (c) sw/NC/151671/2015, a clade IV H3N2 swine virus and (d) TX/50/2012, a seasonal H3N2 huIAV. Nasal swabs were collected daily from challenge to day 14 post challenge (PC). Virus titers were determined by virus isolation in MDCK cells and M-gene qPCR. On days -2, 2 and 4 PC bronchoalveolar lavage (BAL) fluids were collected for cytology and multiplexing for porcine cytokines and chemokines.

## RESULTS

swIAVs replicated at significantly higher titers compared to the huIAVs, while they were detectable in nasal swabs for up to 10 days PC, compared to the huIAVs that shed for up to 6 days. swIAVs also induced more robust cell-mediated responses.

## CONCLUSIONS

Infection with swIAVs resulted in respiratory disease, increased virus replication and shedding compared to huIAVs. The lack of disease following huIAV infection may explain why wholly huIAVs are not isolated from swine. In-depth research is required to better understand how reassortant viruses with zoonotic potential emerge in pigs.

## ACKNOWLEDGEMENTS

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# MG-132 interferes with iron cellular homeostasis and counteracts bovine herpesvirus 1 productive infection

**Filomena Fiorito[1], Carlo Irace[2], Marialuisa Piccolo[2], Francesca Paola Nocera[1], Rita Santamaria[2], Luisa De Martino[1]**

[1]Department of Veterinary Medicine and Animal Production
[2]Department of Pharmacy, University of Naples Federico II, 80137 Naples, Italy.

## OBJECTIVES

Bovine herpesvirus 1 (BoHV-1) may provoke rhinotracheitis, conjunctivitis, abortions and shipping fever in cattle. To efficiently reproduce, BoHV-1 requires an iron-replete cell host. However, bovine cells (MDBK) response to viral infection determines an increase in ferritin levels and a decrease of transferrin receptor 1 (TfR-1) expression, finally lowering labile iron pool extent. Hence, cells could limit iron availability for virus spread [1, 2]. MG-132, a proteasome inhibitor, reduces the efficient BoHV-1 release inhibiting apoptosis and stimulating autophagy [3]. Ferritin, the major iron storage protein in mammalian cells, is proteasome-mediated degradated [4]. Herein, the influence of MG-132 on iron metabolism during BoHV-1 infection was examined.

## MATERIALS AND METHODS

Cell viability, virus titration and Western blot were carried.

## RESULTS

At no-cytotoxic level, MG-132 up-regulated cellular levels of ferritin. Following BoHV-1 infection, by maintaining sustained ferritin expression levels, MG-132 diminished cytotoxicity and viral replication. Ferritin accumulation observed after infection, which was almost doubled in the presence of MG-132, might be caused by the inhibition of proteasome-mediated degradation pathway. A concomitant down-regulation of TfR-1 expression has been also observed, which could contribute to limit cellular iron availability.

## CONCLUSIONS

Inhibition of cellular proteasome pathway could further limit iron availability for virus production. Indeed, the inhibitory effect on virus replication was accompanied by a decreased cell death. Thus, proteasome inhibitor may result in a marked antiviral ferritin-iron accumulation.

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# Diagnostic Microbiology: MALDI-TOF, genomics and metagenomics

# Studying the role of foot skin microbiome and host genetics in cattle lameness

**Georgios Oikonomou**[1]

[1]University of Liverpool, U.K.

Lameness is a major health and welfare issue facing the global dairy cattle industry and causing detrimental economic losses. An epidemiological study conducted recently by our research group showed that one third of the dairy cows in more than 60 farms visited across the UK suffered from impaired mobility on the day of visit [1]. Lameness in dairy cattle has been associated with foot lesions of both infectious and non-infectious aetiology. Digital dermatitis (DD), one of the main infectious disorders, is a painful foot skin disease affecting ruminants worldwide, compromising the welfare of hundreds of millions of production animals annually. More than 40% of UK dairy cows are affected and it has been estimated to cost the US and EU dairy industry over one billion dollars per year [2]. Bacteria of the genus *Treponema* are considered the main pathogen associated with the disease; however, its aetiopathogenesis and transmission patterns have not yet been fully elucidated. The main non-infectious lameness causing lesions are claw horn disruption lesions (CHDLs); namely, sole ulcers (SU), white line disease (WLD), and toe necrosis (TN) lesions. These non-infectious lesions can be secondarily infected.

We have recently examined the role of the foot skin microbiota in the development of DD lesions, explored the microbiota profile of complicated lameness causing lesions, and investigated host genomic regions that are linked to lameness associated traits and foot skin microbiota profiles. A total number of 554 cows from three different farms in the UK were genotyped; foot skin swabs were collected from 259 of these cows approximately two months before their expected calving. These animals were then examined again at calving and two months after calving. Furthermore, 51 cows from ten different farms were used to examine the microbiota profiles of complicated lameness causing lesions [3]. Foot skin microbiota profiles of lameness associated lesions were determined using 16S rRNA gene amplicon sequencing and the data were analysed using multivariate analysis approaches. A shotgun metagenomics approach has also been employed for a subset of these 259 samples. Cattle genomic DNA samples were genotyped using a 50K single nucleotide polymorphism (SNP) chip, and genome-wide association (GWA) and regional heritability mapping approaches (RHM) were performed in order to identify genomic regions associated with lameness associated traits.

Cows that did not acquire DD lesions during the study had different foot skin microbiota profiles from those which did acquire DD lesions. A farm/ management effect on the foot skin microbial profiles was also described. DD, complicated CHDLs and interdigital phlegmon (IP) lesions were shown to have polymicrobial profiles consisting of similar anaerobes, such as *Treponema* spp., and *Porphyromonas* spp. Fastidiosipila spp. were shown to be associated with lameness causing lesions for the first time. In addition, lameness associated traits were found to have significant genomic variation, moderate heritability and partially oligogenic architecture. Interestingly, some significant genomic regions were found to be potentially associated with the relative abundance of DD associated bacteria, namely, *Treponema* spp. and *Peptoclostridium* spp.

In conclusion, our recent studies showed that changes in the foot skin microbiota profiles were detectable before the presence of clinically detectable DD lesions and that more research targeting bacteria that may open the way to *Treponema* spp. may be warranted. We were also able to show that susceptibility to lameness causing lesions and colonization of the foot skin by bacteria of the genus *Treponema* is affected by the host genetics.

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# Matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-ToF MS) is a powerful tool for species identification and taxonomic resolution of mycoplasmas isolated from animals

**Joachim Spergser[1], Claudia Hess[2], Igor Loncaric[1], Ana S. Ramírez[3]**

[1]Institute of Microbiology, University of Veterinary Medicine Vienna, Austria
[2]University Clinic for Poultry and Fish Medicine, University of Veterinary Medicine Vienna, Austria
[3]Unidad de Epidemiología y Medicina Preventiva, Facultad de Veterinaria, Universitad de Las Palmas de Gran Canaria, Spain

In veterinary diagnostic laboratories identification of mycoplasmas is mainly achieved by demanding, cost-intensive and time-consuming methods that rely on antigenic or genetic identification. Since MALDI-ToF MS seems to represent a promising alternative to the currently practiced cumbersome diagnostics we assessed its applicability for the identification of almost all mycoplasma species isolated from vertebrate animals so far. For generating main spectrum profiles (MSPs) the type strains of 98 *Mycoplasma*, 11 *Acholeplasma*, and 5 *Ureaplasma* species, and in case of 69 species, one up to 7 clinical isolates were used. To complete the database, 1-7 representatives of 95 undescribed *Mycoplasma* species isolated from livestock, companion animals and wildlife were also analyzed. A large in-house library containing 755 MSPs was generated and the diversity of spectra within a species was assessed by constructing dendrograms based on a similarity matrix deduced from comparison of MSPs. All strains of a given species formed cohesive clusters clearly distinct from all other species and phylogenetically closely related species clustered closely but were separated accurately, overall indicating that the established database was highly robust. Further validation of the in-house mycoplasma library using 335 independent clinical isolates of 32 mycoplasma species most frequently recovered from animals in veterinary diagnostic laboratories confirmed the robustness of the established database by achieving reliable species identification with log scores ≥ 1.80 [1]. In conclusion, using this extended in-house reference database, MALDI-ToF MS proved to be an excellent method for the identification and differentiation of animal mycoplasmas, combining convenience, ease, speed, precision and low running costs. Furthermore, this method is a powerful and supportive tool for the taxonomic resolution of animal mycoplasmas.

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# Elucidating the role of *Streptococcus uberis* in bovine endometritis

**Panagiotis Ballas[1], Karen Wagener[2], Christoph Gabler[3], Marc Drillich[2], Monika Ehling-Schulz[1]**

[1]Functional Microbiology Unit, Institute for Microbiology, Department of Pathobiology, University of Veterinary Medicine Vienna, Vienna, Austria
[2] Clinical Unit for Herd Health Management in Ruminants, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria
[3] Institute of Veterinary Biochemistry, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

## OBJECTIVE

S. *uberis* is a pathogen well known for its involvement in bovine mastitis, but in the recent past, information about its role in the development of bovine endometritis is emerging [1]9, 15, and 21 after parturition, and to investigate the associations of selected species with clinical endometritis (CE. This study aimed to elucidate the role of S. *uberis* in bovine endometritis, using a primary cell culture model.

## MATERIALS AND METHODS

Isolates of S. *uberis* collected in previous studies [1]9, 15, and 21 after parturition, and to investigate the associations of selected species with clinical endometritis (CE, were screened for a variety of virulence factor genes. Strains harboring different virulence factor genes (n=8) were co-cultured with endometrial primary cells by using different multiplicity of infection (MOI) concentrations, and cellular viability was assessed in pre-defined intervals. Out of these strains, three strains were selected for evaluating the mRNA expression of cellular pro-inflammatory factors (n=5).

## RESULTS

High prevalence of virulence factor genes was found. Genes encoding for virulence factors, such as streptokinase, and surface lipoprotein, were found in all strains, while hyaluron capsule gene *has*C, and S. *uberis* adhesion molecule gene *sua*, were found in 83 % and 93 % of the isolates, respectively. Co-culture of the cells with S. *uberis* resulted in a significant decrease of cellular viability after 24 hours at MOI 1, with some strains being able to destroy up to 60 % of the cells. After 48 h post-infection, almost 80 % of the cells were dead. When the pro-inflammatory factors expression was measured, it was found that infection with S. *uberis* provokes a significant upregulation (3-fold change) of Toll-like receptor genes (*TLR4*), Chemokine ligands genes (*CXCL2*) and also Prostaglandin-Endoperoxide Synthase 2 (*PTGS-2*), and Interleukin (*IL-8*) genes.

## CONCLUSION

The current study provides the first information about the effect of S. *uberis* on uterine cells. Decreased cellular viability and upregulation of pro-inflammatory factor genes was observed, supporting the hypothesis that S. *uberis* is a potential endometritis pathogen.

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# Investigation to determine variations in faecal shedding of *Mycobacterium avium* subspecies paratuberculosis (MAP) in sheep, with regards to parturition

**Antonia Mataragka[1], Kyriaki Sotirakoglou[2], John Ikonomopoulos[1]**

[1]Department of Anatomy and Physiology of Farm Animals, Faculty of Animal Science and Aquaculture, Agricultural University of Athens, 75 Iera Odos, Athens, 11855, Greece
[2]Department of Plant Breeding and Biometry, Faculty of Crop Science, Agricultural University of Athens, 75 Iera Odos, Athens, 11855, Greece

## OBJECTIVES

In analyzing the parameters that could be used to improve the effectiveness of test-and-removal for the control of ovine paratuberculosis in practice, we conducted an investigation consisting of a preliminary [1] and a final stage [2], to assess whether test-positivity of sheep varies between specific stages of breeding.

## MATERIALS AND METHODS

During the preliminary stage, samples of blood and faeces were collected from 42 adult female animals, 1-2 weeks antepartum (PP1), postpartum (PP2) and before mating (PP3), and they were tested with ELISA and real-time polymerase chain reaction (qPCR) for the detection of MAP antibody and DNA, respectively. In the final stage, the analysis was conducted using qPCR on 85 samples of feaces collected from an equal number of animals; samples were collected 4–15/1–3 days antepartum (FP1/FP2), postpartum (FP3/FP4), and before mating (FP5).

## RESULTS

The percentage of animals that react positively to faecal qPCR was statistically significantly lower in PP1, compared to PP2 and PP3. A similar finding was recorded in the final stage, with qualitative (positive/negative) qPCR results indicating a tendency of increase in the level of positivity, from FP1 to FP4, which peaked in the latter, reaching its maximum value that was statistically significantly higher compared to FP5. The quantitative qPCR results indicated a trend that was similar in both stages of the analysis, with higher levels of positivity postpartum (PP2 and FP4) with regards both to single, and multi-reactors.

With regards to ELISA, only one of the animals tested reacted positively (2.38%, 1 of 42, PP1-3), which corresponded to 2.8% of qPCR-reactors.

## CONCLUSION

In sheep with no record of paratuberculosis, the percentage of animals that react positively to the detection of MAP DNA using faecal qPCR, as well as that of the animals reacting strongly positively to the specific test, are statistically significantly higher in the period of 4-15 days postpartum, compared to those antepartum and before mating. In this respect, the specific period is more suitable for the application of a test-and-removal scheme aiming to the control of disease, using qPCR. The use of ELISA for the same purpose is not recommended due to low sensitivity.

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# *Campylobacter coli* and *Campylobacter jejuni* prevalence and antibiotic resistance in Greek swine farms

**Dimitrios Papadopoulos[1], Evanthia Petridou[1], Georgios Filioussis[1], Theofilos Papadopoulos[1,2], Konstantinos Papageorgiou[1], Maria Chatzistilianou[3], Spyridon K. Kritas[1]**

[1]Department of Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece
[2]Department of Epidemiology and Public Health, Sciensano, Brussels Belgium
[3]Cinic of Pediatrics-Immunology and Infectious Disease, Faculty of Medicine, School of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece

The objective of this study was to determine the prevalence of C.*coli* and C.*jejuni* in Greek commercial swine farms, as well as their resistance to certain antibiotics. For this reason, 20 swine farms had been selected throughout the Greek territory, and from each farm, 10 rectal samples had been randomly collected from each of the following five age groups: group 1-suckling pigs (age 0-28 days), group 2-nursery pigs (age 29-70 days), group 3-grower pigs (age 71-105 days), group 4-finisher pigs (age 106 days to slaughter), group 5-sows (over 9 months of age), e.g. 50 samples per farm; 1,000 samples in total.

The ISO 10272-1:2017 [1] method was used for the detection and isolation of Campylobacter spp,. Speciation of Campylobacter strains was made according to the 2nd version of the protocol for PCR amplification of C.*jejuni* and C.*coli* [2,4].

The agar dilution method was used for the antimicrobial susceptibility testing. All isolates were tested for their susceptibility to 5 antimicrobials (gentamycin, erythromycin, ciprofloxacin, tetracycline, meropenem). The EUCAST breakpoint tables version 8.1 were used for the interpretation of the results [3]. The C.*jejuni* ATCC 33560 and C.*coli* ATCC 33559 were used for the Quality Control.

The results have shown that 80% of farms were positive for C.*coli* or/and C.*jejuni*. In total, 48,6 % (95% CI 36,7-60.6) of the samples were positive for both *Campylobacter* species (380 C.*coli*, 109 C.*jejuni*, 2 later sequenced as C. *lari*). Prevalence for C.*coli* was 38.1% (95% CI 28.2-49.2) and for C.*jejuni* 10.3% (95% CI 6,7-15.4). Significant differentiations in prevalence was recorded between different age groups. Concerning antimicrobial resistance, high rates of resistance (67,2%) were recorded for tetracycline, while 18,1% and 7,3% of all isolates were resistant in ciprofloxacin and erythromycin, respectively. Low rates of resistance were recorded for gentamycin (3,9%). All isolates were susceptible to meropenem. Thirty-two of the isolates (6,52%) were classified as Multi Drug Resistant.

The above findings indicate high prevalence of C.*coli* and C.*jejuni* in pig farms with significant resistant rates in tetracycline and ciprofloxacin, that constitutes a potential reservoir for resistance genes spread.

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# Antimicrobial Resistance, One Health and Food microbiology

# The Dutch approach to reducing the use of antimicrobials in livestock

**Jaap A. Wagenaar[1,2] and David Speksnijder[1,3]**

[1]Department of Infectious Diseases & Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands
[2]Wageningen Bioveterinary Research (WBVR), Lelystad, the Netherlands
[3]University Farm Animal Practice, Harmelen, the Netherlands

Use of antimicrobials in animals poses a potential risk for public health as it contributes to the selection and spread of antimicrobial resistance (AMR). In the Netherlands the total therapeutic antimicrobial use (AMU) (in mass sold) in farm animals doubled between 1990 and 2007. A series of measures and initiatives were triggered by the findings of widespread presence of multi-resistant bacteria (MRSA, ESBLs) and discussions in the general public regarding public health risks associated with agricultural practices after the largest Q-fever outbreak in humans occurred which was associated with large scale goat farming.

From 2008 onwards, a series of joint initiatives by the Dutch Government, livestock sectors and the Royal Dutch Veterinary Association (KNMvD) resulted in a covenant describing measures for prudent veterinary use of antimicrobials. One of the decisions was to establish the Netherlands Veterinary Medicines Institute (SDa). This authority was founded with the tasks to i) collect the antimicrobial usage data of Dutch livestock, initially pig, poultry, veal calves and dairy farms (approximately 42,000 units) and define benchmark targets for AMU in these sectors (in defined daily dosages per animal year); ii) report annual trends; iii) identify high users/prescribers; iv) assess the effect of improvement programs developed by the livestock sectors. Reduction targets were set by the government at 20%, 50% and 70% reduction in 2010, 2013 and 2015, respectively, with reference to 2009. Next to the founding of the SDa, several other actions were performed at different levels (e.g. advices of the Dutch Health Council and the Antibiotics Policy Working Group of the Royal Dutch Veterinary Association (KNMvD), the development of treatment guidelines by the KNMvD) and implementation of new regulations by the Ministry of Agriculture, Nature and Food Qualitysuch as the ban on all preventive use of antimicrobials in livestock. Parallel to these actions, continuous monitoring of resistance in commensal *E. coli* had already been set up in livestock from 1998 onwards, enabling measurement of trends in resistance.

The total reduction of AMU (in mass sold) between 2009 and 2018 was 63.8%. Compared to 2007, the year with the highest veterinary usage there was a reduction of 68.3%. The use of antimicrobials defined as ‘’critically important for human health” (fluoroquinolones and 3rd and 4th generation cephalosporins) in livestock were strongly reduced; for 3rd and 4th generation cephalosporins to almost zero. These achievements have been made by Dutch farmers and their veterinarians through improved infection and health control measures, combined with the replacement of group treatments by individual treatments where possible. As a result of an enforced 1-to-1 relationship of farmers and veterinarians, it was possible to develop the Veterinary Benchmark Indicator allowing comparison of prescription levels between veterinarians. The SDa has played a crucial role by making the reporting of AMU transparent for all farms, by benchmarking farms (action, signaling and target level) and by benchmarking veterinarians. The government played a crucial role by setting targets, implementation of new legislation and strong enforcement of these regulations. Parallel to reduction of AMU there was a reduction of AMR in livestock observed.

The critical success factors were: massive public concerns and clear targets defined by the government (created a sense of urgency), measures initiated by private animal production sectors and veterinary

association (need for collaboration), having fully transparent usage data and the founding of an independent Netherlands Veterinary Medicines Institute (accepted by all parties involved). The reduction of AMU appears to be effective in reducing AMR in livestock. The results show that in a country with a very large and intensive animal production, reductions in AMU are feasible. The Netherlands is, after the US, the 2nd largest exporter of agricultural products in the world.

# Transfer of AMR: what have we learned from the past?

**Patrick Butaye**[1]

[1]Ross University School of Veterinary Medicine, St. Kitts and Nevis

Since the 1960, animal husbandry has been brought into relationship with antimicrobial resistance in bacteria from human origin. Indeed, it is clear that resistance in zoonotic pathogens has been selected in animals and has a direct impact on the treatment options in humans. However, the transfer of resistance genes has been a lager matter of debate and is still a debatable problem as measuring the potential transfer is problematic as in most cases, these resistances are also already present in human commensal and pathogenic bacterial, where they can be selected by human usage of antimicrobials. There were several episodes in which the discussion was actual and where conclusions were drawn, based on the technical potential at that time. This was the case for glycopeptide resistant enterococci, extended-spectrum beta-lactamase carrying Enterobacteriaceae, methicillin-resistant *Staphlylococcus aureus* and more recently, plasmid mediated colistin resistance in gram-negative bacteria. These examples will be discussed as to demonstrate what we have learned from the past and show the dilemmas and shortcomings of our understanding of the transfer of antimicrobial resistance between animals and humans.

# ESBLs/pAmpC- producing *Escherichia coli* causing urinary tract infections in companion animals and humans in Portugal: antimicrobial resistance, pathogenicity and clonal diversity

**Adriana Belas[1], Cátia Marques[1], Juliana Menezes[1], Luís Telo da Gama [1], Patrícia Cavaco-Silva[2,3] Constança Pomba[1]**

[1]CIISA - Centre of Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Portugal, Av. da Universidade Tecnica, 1300-477 Lisbon, Portugal
[2] Centro de Investigação Interdisciplinar Egas Moniz, Instituto Universitário Egas Moniz, Caparica, Portugal
[3]Technophage, Lisboa, Portugal

## OBJECTIVES

This study aimed to characterize third-generation cephalosporin (3GC)-resistant *E. coli* causing urinary tract infections (UTI) in companion animals (CA) and humans in the community (H).

## MATERIAL AND METHODS

3GC-resistant *E. coli* (CA n=35; H n=85) isolated from patients with UTI were tested against 14 antimicrobials. PCR-based assays were used to detect the major *E. coli* phylogenetic groups, Pathogenicity associated-islands (PAIs) (n=8), urovirulence genes (n=8), ESBLs/pAmpC) resistance genes. ESBL/pAmpC-producing *E. coli* isolates were typed by MLST. The ST131 clonal group and subclade C2 (H30-Rx) were identified by PCR

## RESULTS

Considering phylogenetic group 3GC-resistant *E. coli* isolates from CA and H mainly belonged to group-D and B2 (48.6%, 67.1%, respectively). The most frequent PAIs and virulence genes among isolates from CA and H were:PAI $IV_{536}$ (CA=72%, H=91.8%, p=0.017) and PAI $I_{CFT073}$ (CA=54.3%, H=78.8%, $p$=0.013); *ecp*A (CA=100%, H=100%) and *iuc*D (CA=48.6%, H=83.5%, $p$=0.0002). CA and H *E. coli* strains shared two MDR high-risk clonal lineages: ST131, previously described[1] and ST648, an emergent virulent lineage[2]. The $bla_{CTX\text{-}M\text{-}15}$ and the $bla_{CMY\text{-}2}$ were the most frequently ESBL/pAmpC detected genes in CA and H isolates. ST131 strains from CA and H mostly belonged to subclade C2.

## CONCLUSION

The cross-species sharing of important multidrug-resistant high-risk clones is a public health concern[3]. Considering that companion animals with UTI are generally treated at home by the owners, measures should be implemented to avoid the spread of these bacteria to the environment.

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# Fighting antimicrobial resistance dissemination in veterinary medicine using the antimicrobial peptide nisin

**Eva Cunha[1] and Manuela Oliveira[1]**

[1]CIISA- Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon

Antimicrobial resistance (AMR) is a major global health problem. Antibiotics misuse in human, animal, and environmental settings, together with the decreased investment in new antimicrobial compounds, are responsible for AMR dissemination. A control possible strategy involves the use of antimicrobial peptides, such as nisin [1]. These molecules, produced by most living organisms, act mainly by physically disrupting the bacterial cell membrane, leading to fast killing with low resistance development [1]. We evaluated nisin's potential for controlling periodontal disease (PD) in dogs, a high prevalent inflammatory disease [2]. The inhibitory activity of nisin incorporated in two delivery sytems (guar-gum gel – biogel - and toothpaste) was assessed using a collection of oral enterococci (n=20) obtained from dogs with PD [3], and the influence of canine saliva in nisin's action was also determined. *In vitro* susceptibility profile to nisin diluted in sterile water or incorporated in the biogel was assessed by evaluating Minimum Inhibitory(MIC) and Bactericidal Concentrations(MBC), as well as Minimum Biofilm Eradication(MBEC) and Inhibitory Concentrations(MBIC) [2]. Nisin incorporated in a toothpaste was evaluated using an agar-well diffusion assay [2]. The influence of canine saliva was tested as described elsewhere [4].

Nisin diluted in sterile water or incorporated in the biogel was effective against all isolates. The biogel presented lower MBC, MBIC and MBEC values, revealing a bactericidal effect against 95% of the isolates and antibiofilm potential. The nisin-toothpaste formulation showed antimicrobial activity against 95% of the enterococci tested. In the presence of canine saliva, nisin diluted in sterile water or incorporated in the biogel showed inhibitory activity against 95 and 85% of the isolates, respectively. However, the biogel produced more consistent inhibition halos, stabilizing nisin diffusion.

Nisin represents an appropriate candidate for PD control in dogs. Both delivery systems were effective, but the biogel showed higher bactericidal ability and diffusion capacity in the presence of saliva.

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# Extended Spectrum β Lactamase-producing *Enterobacteriaceae* (ESBL-E) shedding in race horses in Ontario, Canada

**Anat Shnaiderman-Torban[1], Shiri Navon-Venezia[2], Yossi Paitan[3,4], Holly Archer[5], Darryl Bonder[6], Scott J. Weese[5*], Amir Steinman[1*]**

[1]Koret School of Veterinary Medicine (KSVM), The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel
[2]Department of Molecular Biology, Faculty of Natural Science, Ariel University, Ariel, Israel
[3]Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Israel
[4]Clinical Microbiology Lab, Meir Medical Center, Israel
[5]Department of Pathobiology, Ontario Veterinary College, University of Guelph, Canada
[6]Ontario Equine Hospital, Ontario, Canada.
[*]equal contribution

## OBJECTIVES

To investigate the prevalence, molecular epidemiology and risk factors for ESBL-E shedding in race-horses, since this unique equine population is found under intensive management and medical care.

## MATERIALS AND METHODS

A prospective cross-sectional study was performed involving fecal samples collected from Thoroughbred horses that were housed at one big racetrack in Ontario, Canada. Samples were enriched in Luria-Bertani broth, plated onto CHROMagarESBL plates and sub-cultured to obtain pure cultures. ESBL production was confirmed using combination disc assay. Bacterial species were identified via MALDI-TOF and antibiotic susceptibility profiles were assessed using Vitek-2. *E. coli* sequence types were determined using Multi Locus Sequence Type (MLST) analysis. Medical records were reviewed and assessment of risk for individual variables was performed (SPSS).

## RESULTS

Overall, 169 adult Thoroughbred horses, originating from 16 different barns, were sampled. ESBL-E shedding rate was 12% (n=21/169); 22 isolates ESBL-E were molecularly studied (one horses had two isolates). The main species was *E. coli* (91%) and the major ESBL gene group was CTX-M-1 (59%). Other ESBL-E species were *Proteus hauseri* and *Enterobacter cloacae* (one isolate each). Nine different *E. coli* sequence types were identified: ST1730, ST10, ST1250, ST1403, ST1462, ST4527, ST7870, ST2008, and ST86. Two new *E. coli* sequence types were identified. Sixty-four percent of total isolates were defined as multidrug resistant. Resistance rates to antibiotics of ESBL-E were 71% for trimethoprim-sulfa, 62% for tetracycline, 62% for gentamicin and no resistance was identified for quinolones, amikacin and carbapenems. Antibiotic treatment in the previous month was found as a risk factor for shedding ($P<0.05$).

## CONCLUSIONS

Our findings demonstrate the potential diverse reservoir of ESBL-E in race-horses. Multidrug resistant bacteria should be further investigated to improve antibiotic treatment regimens and equine welfare.

## ACKNOWLEDGEMENTS

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# Antibiotic resistance; A one health and food security issue

**Roberto La Ragione**[1]

[1]Department of Pathology and Infectious Diseases, School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, GU2 7AL, UK

Antibiotics are critical for treating infections in human and veterinary medicine and the continued emergence of resistance in bacteria is considered a major global health, and food security threat. Importantly, antibiotics are not only used to treat infections in animals and humans, but in some countries they are still used extensively to enhance livestock production. Furthermore, antibiotics are commonly used in horticulture, where their global use is widespread and less controlled.

Antibiotic resistant bacteria from animals and humans can transmit in both directions, through human contact with farm, wildlife or companion animals or their environments, through ingestion of contaminated food (both imported and local produced animal and vegetable or fruit items) and through contact with effluent waste from humans, animals and industry. Furthermore, the acquisition of antimicrobial resistance (AMR) can influence the pathobiology of many pathogens including virulence, metabolism and persistence in the host and the environment.

Therefore, alternatives to antibiotics are urgently sought in order to reduce global antibiotic use and the emergence of AMR. Minimising the unnecessary and inappropriate use of antibiotics can reduce the selective pressure that favours the emergence and spread of resistant bacteria, and is an essential component of strategies to safeguard antibiotics critical for treatment of serious human and animal infections. Moreover, understanding the transmission dynamics of AMR is essential if suitable alternatives are to be developed.

This presentation will focus on the current issues surrounding antimicrobial resistance (AMR) including the drivers of AMR, dosing regimens, food security implications and the development of novel alternatives to antimicrobials, including pre and probiotics, and novel antimicrobials for important zoonotic bacterial pathogens.

# Veterinary research on AMR: need for a change?

**Luca Guardabassi**[1,2]

[1]Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark
[2]Department of Pathobiology & Population Sciences, The Royal Veterinary College, UK

The question posed by this lecture is whether the current research on antimicrobial resistance (AMR) in the veterinary sector meets the needs for combating AMR. Every year over 1,000 peer-reviewed articles on AMR in animals are published in scientific literature. Most studies report data on occurrence/prevalence of known resistant bacteria and/or resistance genes in specific animal, food and/or environmental samples at the local level (region, farm, clinic, etc.), and only few of them are integrated with highly discriminatory genotypic data to assess AMR transmission between reservoirs. Such studies are observational by nature, contribute only marginally to a better understanding of AMR and above all do not contribute to the solution of the problem. Besides clearly lacking originality, many of them are published in low impact journals and suffer design, sampling, measurement or reporting bias. On the contrary, relatively few papers are based on solution-driven research aimed at identifying, developing, or demonstrating new solutions to AMR. This research can be either fundamental or applied but usually implies a higher degree of originality and innovation. I think we can all agree that there is need for more research of this kind but the next question is: what is the solution-driven research that we need for combating AMR? This is not an easy question to answer.

Estimating the burden of a problem is essential to take appropriate policy decisions and design sustainable interventions to tackle the problem. However, the public health burden associated with antimicrobial use in animals is extremely difficult to quantify due to numerous data gaps. Although there is a clear association between antimicrobial use and AMR in animals, it remains unclear whether a decrease of antimicrobial use may lead to a reduction in the impact of AMR in human medicine. Modelling studies suggest that restricting antimicrobial consumption in the veterinary sector may prevent the introduction of new resistant bacteria but has in most cases no effects on the levels of AMR in bacteria that are already present in the human population [1]. Moreover, it appears that reducing the rate of AMR transmission between animals and humans is generally more effective than curtailing the volume of antimicrobials used [2]. Assuming that the conclusions of these studies are correct, early detection of new AMR genotypes of potential clinical impact in animals and development of new strategies to prevent AMR transmission should be prioritized in future research. Other examples of solution-driven topics include but are not limited to:

- Alternatives to antibiotics, including dietary interventions, immunostimulants and alternative therapies.
- Optimisation of antimicrobial use, including timing, dose and duration.
- 'On farm' rapid and cheap diagnostic tests and technologies for early detection of disease, discrimination between viral and bacterial infection, and confirmation of pathogen's susceptibility to first line antimicrobials.
- Reliable clinical breakpoints to predict in vivo antibiotic efficacy by antimicrobial susceptibility testing.

It is generally claimed that funds allocated to AMR research are insufficient but it is rarely debated how the allocated funds should be used. In the EU alone, research on AMR accounted for a total public investment of over EUR 1.3 billion from 2007 to 2013 [3], which is not an irrelevant amount. It is important that national and international funding agencies will prioritize solution-driven research in the future.

With specific reference to the veterinary sector, there is an urgent need for research targeting solutions for diagnosis, treatment and prevention of veterinary diseases, especially those diseases that account for most antimicrobial use in livestock production, like enteric and respiratory infections in pigs and cattle.

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# Risk of companion animal to human transmission of antimicrobial resistance

**Constança Pomba**[1]

[1]CIISA - Centre of Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Portugal, Av. da Universidade Tecnica, 1300-477 Lisbon, Portugal

Antimicrobials are important tools for the therapy of infectious bacterial diseases in companion animals. Loss of efficacy of antimicrobial substances can seriously compromise animal health and welfare. A need for the development of new antimicrobials for the therapy of multiresistant infections, particularly those caused by Gram-negative bacteria, has been acknowledged in human medicine and a future corresponding need in veterinary medicine is expected. During the last fifty years, the number of companion animals has substantially increased, and companion animals are often considered as "family members" enjoying close contact to their owners. Thus, humans may acquire antimicrobial resistance via direct contact from their pets. Problems of resistance development and of infection control in companion animal hospitals are mimicking those in human hospitals (microbiological hazards are identified in Table 1) [1].

Several studies have reported the colonization and sharing of *Escherichia coli* strains between companion animals and humans and very recently a first report of the fecal colonization and sharing of *K. pneumoniae* clonal lineages between healthy humans and dogs living in close contact has been reported [2]. In China, the detection of mcr-1 in colistin-resistant CTX-M-15-producing *E. coli* strains isolated from companion animals and the possible transmission of mcr-1-harbouring *E. coli* between companion animals and a person was reported [1]. Also, the transmission of NDM-5 ST167 and CTX-M-9 ST69 *E. coli* between dogs and humans in a family was described in Finland [3].

Table 1. Selected microbiological hazards identified [1].

**Table 2.** Selected microbiological hazards identified in this study

| Antimicrobial-resistant bacteria in companion animals | Type of hazard | Sources |
|---|---|---|
| MRSA | direct hazard | dogs, cats and horses |
| MRSP | direct hazard[a] | dogs, cats and horses |
| VRE | indirect hazard[b] | dogs and horses |
| ESBL-producing Enterobacteriaceae | indirect hazard | dogs, cats and horses |
| Carbapenem-resistant Gram-negative bacteria | indirect hazard[b] | dogs and cats |
| Colistin-resistant *E. coli* | indirect hazard | dogs and cats |

[a]Low number of cases of human infections originating from companion animals.
[b]No human infections originating from companion animals have been reported.

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# "One Health" approach to tackle antimicrobial resistance in Portugal

**Patrícia Poeta[1,2], Vanessa Silva[1-4], Gilberto Igrejas[2-4]**

[1]Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal
[2]Laboratory Associated for Green Chemistry (LAQV-REQUIMTE), New University of Lisbon, Monte da Caparica, Portugal
[3]Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal
[4]Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

According to the World Health Organization, infections with antibiotic resistant bacteria can cause a total of 700,000 deaths annually globally. The use of antibiotics promotes the development of resistance and influences co-selection processes in bacterial communities leading to the spread of antibiotics, resistant bacteria and resistance genes among humans, companion animals, livestock, wildlife and the environment. Considering the promiscuity of gene transfer systems among bacteria, the presence of resistance genes in the environment is considered an ecological problem. Thus, there is an urgent need to understand the dynamics of antimicrobial resistance (AMR), being the One Health approach essential to assess the origin, spread and flow of AMR mechanisms and to define new strategies to combat the problem.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important multidrug-resistant nosocomial pathogens worldwide and can cause high morbidity and mortality. S. *aureus* is naturally present in the skin, with lesions and dermatological diseases being a risk factor for MRSA infection. In addition to hospital concerns, strains of MRSA are increasingly common in infections associated with the human and animal community. Portugal remains one of the European countries with the highest prevalence of MRSA. However, information on the incidence of MRSA in animal populations is scarce, as is the dissemination of resistance determinants by anthropogenic sources. Therefore, it is necessary to address the problem on a number of fronts by promoting studies to elucidate AMR mechanisms in MRSA and other resistant bacteria, and to contribute to an emerging view of the extent of the AMR problem in the context of One Health.

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# *Corynebacterium ulcerans* an emerging zoonosis in Scottish dogs

**Geoffrey Foster[1], Steven Murray[1], Norman Fry[2], Kate Mark[3], Michelle Etherson[3], Peter Harrison[3], Pota Kalima[3], Richard Othieno[3]**

[1]SRUC Veterinary Services, Inverness, UK
[2]National Health Services, NHS Lothian, Edinburgh UK
[3]Public Health England, London, UK

*Corynebacterium ulcerans* can produce diphtheria toxin and has become the predominant cause of toxigenic diphtheria infection in the UK, making the organism of great clinical and public health importance [1]. Although rare, the frequency and severity of infections associated with C. *ulcerans* appears to be increasing [2]. Since 2017, three cases of C. *ulcerans* infection in people in Scotland were followed up, with sampling of known contacts, including pets.

## MATERIALS AND METHODS

Throat swabs were collected from five companion dogs and infected wounds from two of those connected to cases. Cultures were made on Columbia sheep blood agar and Hoyles medium, incubated aerobically at 37°C. Toxigenicity testing was performed at the HPE, Colindale. Infected animals received treatment for wound infections and to remove carrier status.

## RESULTS

*Corynebacterium ulcerans* was recovered from throat swabs from four dogs and a wound swab from one (see table). All were toxigenic.

## CONCLUSION

The recovery of C. *ulcerans* from dogs connected to three separate cases suggests a significant association between dogs and humans with cutaneous diphtheria. Furthermore, the recent isolation of C. *ulcerans* from dog wound diagnostic samples, submitted by veterinary practitioners, suggests that dog licking may be a potential means of transmission. Carriage of C. *ulcerans* was not detected in any of the infected dogs up to one month post-treatment, however, it is not known whether re-infection may appear over longer periods. This work demonstrates the importance of human and veterinary professionals working together in One Health investigations for the benefit of public health.

Table 1.

| Case | Animal | Sample | C. *ulcerans* result |
|---|---|---|---|
| 1 | A | Throat | Positive |
| | B | Wound | Positive |
| | B | Wound | Positive |
| 2 | C | Throat | Positive |
| | D | Throat | Negative |
| | D | Wound | Negative |
| 3 | E | Throat | Positive |

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## ACKNOWLEDGEMENTS

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# Recent findings on *Clostridioides difficile* in pigs: succession, infection and microbial colonisation resistance

**Łukasz Grześkowiak[1], Wilfried Vahjen[1], Jürgen Zentek[1]**

[1]Institute of Animal Nutrition, Freie Universität Berlin, Berlin, Germany

*Clostridioides difficile* is a spore-forming and opportunistic pathogen in animal husbandry and clinical settings. Shedding of this bacterium by farm animals is of concern regarding zoonotic transmission to farm workers and food. C. *difficile* has been documented as a major cause of uncontrolled enteritis outbreaks in neonatal piglets [1,2]. The reasons why only neonatal piglets develop infection are largely not known. This report demonstrates our recent published and ongoing findings on C. *difficile* colonisation, infection and interactions with the microbiota in piglets.

In our recent studies, suckling piglets harboured C. *difficile* and toxins A and B at high concentrations, which gradually decreased as piglets aged. Despite the high prevalence of C. *difficile* and toxins, piglets showed no clinical signs of infection suggesting that there must be predisposing factors facilitating the onset of the disease [3]. Enrichment of the faecal samples revealed the presence of this bacterium in previously C. *difficile*-negative sow and offspring faeces. We found that C. *difficile* is detected in faeces of sows during the periparturient period, however at low concentrations. Therefore, it seems that a low level of C. *difficile* in sows is sufficient to colonise piglet gut successfully, however environmental dissemination of C. *difficile* cannot be overruled. Therefore, pigs could be potential reservoirs of this bacterium and a spread of multi-resistance genes in the environment. The reasons why some piglets get sick and others are just asymptomatic carriers of C. *difficile* are still not clear. We found that microbial diversity indices were negatively associated with C. *difficile* counts in suckling piglets, supporting the phenomenon termed “microbial colonisation resistance”. Our data indicate the importance of passive protection from sow to offspring. Neonatal piglets that were separated from their mothers and fed formula, developed C. *difficile* infection in their colon [4], however without clinical symptoms, making the diagnosis of the disease more difficult. Further, our *in vitro* studies demonstrated that sow milk which contained antibodies against C. *difficile* toxins [5] could protect porcine intestinal cell lines from intoxication in a time-dependent manner.

There is a short “time window” for C. *dificile* colonisation and infection in pigs. The gut microbiota seems to set conditions for colonisation resistance against C. *difficile* in the offspring. The disease etiology is multifactorial and host, microbial and environmental factors may be involved. These should be considered when establishing successful strategies to combat C. *difficile* spread and infection in animal farming and elsewhere.

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# Increasing MRSA carriage in Swiss pigs - a risk for veterinarians and farmers?

**Kittl S.[1], Brodard I.[1], Heim D.[2], Andina-Pfister P.[3], Overesch G.[1]**

[1]Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland
[2]Federal Food Safety and Veterinary Office, Bern, Switzerland
[3]Gesellschaft Schweizer Tierärztinnen und Tierärzte GST, Bern, Switzerland

## OBJECTIVE

Over the last decade, prevalence of MRSA in Swiss pigs showed a dramatic increase from 2% in 2009 to 44% in 2017. The isolates almost uniformly belong to CC398, can however be split between *spa*-type t011 and t034. The higher prevalence is linked with an increase in t011, which used to be rare in Swiss pigs until 2015, but now almost equals t034. In light of this development, we wanted to analyze if and how these changes translate to MRSA detected in Swiss veterinarians and farmers.

## MATERIALS AND METHODS

A total of 212 veterinarians as well as 156 farmers were screened for the presence of MRSA by nose and hand (veterinarians only) swabbing. The obtained strains (15 from veterinarians and 8 from farmers) as well as selected isolates from pig noses (n=12), pork (n=2), poultry meat (n=3) and horses (n=3) were characterized by whole genome sequencing (Illumina NextSeq, v2, 2x150 bp). The phylogeny was assessed applying core genome MLST and core genome SNP analyses.

## RESULTS

In total, eight of 15 MRSA isolates from veterinarians and six of eight strains from farmers belonged to the livestock associated CC398; all of these veterinarians reported treating large animals. In small animal veterinarians only non-CC398 MRSA were found - indicating a non-animal derived colonization. Clustering according to t011 and t034 was confirmed by cgMLST. t034 strains from farmers were found to be closely associated to t034 strains from pigs. The same could be shown for t011 strains from horses and veterinarians. However, most of the pig t011 strains clustered in a separate group.

## CONCLUSION

Pig t034 strains appear to be the main MRSA in Swiss farmers while in veterinarians mainly horse-associated t011 strains can be found. The majority of pig t011 strains cluster separately and seem to be less frequently transferred to humans.

# Abstracts selected for poster presentation

# Pantropic canine coronavirus and concurrent viral coinfections in dogs

**Flora Alfano[1], Viviana Mari[2], Gianvito Lanave[2], Lorena Cardillo[1], Gianluca Miletti[1], Giorgio Galiero[1], Nicola Decaro[2], Giovanna Fusco[1]**

[1]Istituto Zooprofilattico Sperimentale del Mezzogiorno, Via Salute 2, 80055 Portici, Napoli
[2] Dipartimento di Medicina Veterinaria, Università degli Studi di Bari, Valenzano, Bari

Canine coronavirus (CCoV) strains with the ability to spread to internal organs, also known as pantropic CCoVs (pCCoVs), have been detected in domestic dogs [2] and wild carnivores [1]. Our study focused on the characterization of circulating pCCoV strains in the period 2014-2017 in Italy. Samples from the gut and internal organs of 352 dogs were screened for CCoV [2] and the potential pCCoV strains were subjected to sequence and phylogenetic analyses. The pCCoV positive samples were also tested for other canine viruses.

CCoV RNA was detected in the gut of 76 dogs, while 35 animals showed the presence of putative pCCoV strains in internal organs. Fifteen pCCoV strains were sequenced: 6 were from dogs of southern Italy and 9 from animals imported from other European countries. Only 3 dogs showed single pCCoV infections, while in the other cases there were coinfections with other viruses, mainly with canine parvovirus.

The phylogenetic tree, generated from partial ORF2 gene sequences, showed 5 different clustering groups. A strain felt in a cluster quite distant from all the others; 8 strains clustered with the Italia pCCoV strain 120/10; 2 strains were intermingled with enteric CCoVs; 3 other strains clustered with the wolf pCCoV strain and 3 Asian enteric strains. Another strain clustered with pCCoV strains identified in Italy and Greece. The detection of pCCoV strains related to Asian viruses is of particular interest and could be related to the importation or illegal trade of animals through eastern Europe. In conclusion, the present study demonstrates an increasing circulation of pCCoV in Italy, which reinforces the need for a continuous surveillance on both autochthonous and imported dogs.

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# *Glutamicibacter creatinolyticus* strain LGCM 259 infection in a horse: first genetic characterization

**Attili Anna-Rita[1], Gonçalves dos Santos Roselane[2], Hurtado Castillo Raquel[2], Gabriel Lucas[2], Profeta Rodrigo[2], Rifici Claudia[3], Mazzullo Giuseppe[3], Spier Sharon J.[4], Pinto Gomide Anne Cybelle[1], Brenig Bertram[5], Gala-García Alfonso[6], de Paula Castro Thiago Luiz[7], Cuteri Vincenzo[1], Azevedo Vasco[2], Seyffert Núbia[8]**

[1]School of Biosciences and Veterinary Medicine, University of Camerino, Italy
[2]Cellular and Molecular Genetics Laboratory, Institute of Biological Sciences, Federal University of Minas Gerais, Brazil
[3]Department of Veterinary Science, University of Messina, Italy
[4]Department of Veterinary Medicine and Epidemiology, University of California, Davis, California, USA
[5]Institute of Veterinary Medicine, University of Göttingen, Germany
[6]Institute of Biological Sciences, Federal University of Para, PA, Brazil
[7]Institute of Health Sciences, Federal University of Bahia, Brazil
[8]Institute of Biology, Federal University of Bahia, Brazil

## OBJECTIVES

*Glutamicibacter creatinolyticus* (formerly *Arthrobacter creatinolyticus*) belonged to the family *Micrococcaceae*. It plays a significant role in many ecosystems: soil, water, air, cheese, plant, and it was associated to urinary tract infections and bacteremia in humans [1]. Recently, *G. creatinolyticus* LGCM 259 (LGCM-259) was isolated from diffuse subcutaneous nodules adherent to muscular tissues from a mare in Italy. Since the identification is only possible using 16S rRNA and MALDI- TOF sequence analyses, this study was carried out to: -characterize LGCM-259 with a complete DNA sequence and annotation; -identify genes encoding virulence factors.

## MATERIALS AND METHODS

By comparative analyses among four isolated species of different habitats, available in the NCBI database, chromosomal sequencing using Hiseq technology (Illumina, USA) was conducted. The genome of LGCM-259 was automatically annotated using PROKKA. A phylogenomic tree and the presence of virulence genes were generated by Phylogenomic Tree Tool in Pathosystems Resource Integration Center (version-3.5.17), and BLASTp against the Virulence Factor Database (VFDB), respectively.

## RESULTS

LGCM-259 strain was sequenced and assembled in a circular chromosome, which exhibits a length of 3.3 Mb, with a G+C content of 66.4%, and a total of 2882 CDSs, 4 clusters of rRNAS (5S, 16S, and 23S), and 61 tRNA genes, respectively. The locus tag LGCM259_1698, LGCM259_0905 and LGCM259_1698 may be involved in multiple drug resistance to Rifampin, Elfamycin, and Fluoroquinolone (rpoB, tufA, tufB). The genome also displayed copper tolerance genes (copZ, csoR_1, cutC, aniA, pcoC), resistance to heavy metals such as arsenic, cobalt-zinc-cadmium (cobT, cobS, cadA), and chrome composts which are serious environmental contaminants.

## CONCLUSION

The LGCM 259 strain's genome was first characterize and its chromosome sequence has been deposited in the NCBI database under accession number CP034412. It affects animals and carries important bacterial virulence factors that are essential in cell viability and pathogenicity.

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# Differentiation between *Brucella melitensis* Rev1 vaccine strain, *B.melitensis* field strains and cross reactive bacteria by periplasmic protein analysis

**Babetsa Maria[1,2], Boukouvala Evridiki[2] , Ekateriniadou Loukia[2], Papadopoulos Athanasios[1]**

[1]Laboratory of Animal Physiology, Department of Zoology, School of Biology, Faculty of Sciences, Aristotle University of Thessaloniki, Greece
[2]HAO-DEMETER, Veterinary Research Institute, Campus of Thermi 57001, Thermi, Thessaloniki, Greece

## OBJECTIVE

Identification of immunogenic proteins for the differentiation of *B.melitensis* infected animals from vaccinated or infected with cross reactive bacteria.

## MATERIALS AND METHODS

Periplasmic proteins were extracted from bacterial cultures of *B.melitensis Rev1*, four *B.melitensis* field strains and two cross reactive bacteria (*Y.enterocolitica* O9 *and E.coli* O:157) by the chloroform method [1]. 0.2μg of the extracted proteins from each strain, were analyzed in 10% SDS PAGE. The periplasmic proteins of the vaccine strain and one representative from the field strains were also analyzed by 2D electrophoresis using ZOOM®IPG Runner™ (Life Technologies) (IEF-Zoom® Strip pH 4-7, 10%,SDS PAGE) and the proteins were electrotransferred to nitrocellulose membranes (NCP) for Western Blot analysis. As primary antibodies were used positive serums from vaccinated sheep (60 days post inoculation) and as secondary antibody was used the HRP conjugated Protein G.

## RESULTS

Immunogenic protein bands were detected in the *B.melitensis* bacteria but not in the cross reactive. The pattern of the bands varies between the vaccine strain and the examined field strains. More specific, a 62kDa band was detected only in the vaccine strain and a 42kDa band was detected only in all field strains. After the 2D analysis, 6 immunogenic spots (MW 48-75kDa, Ip 6,5-4,5) were detected only in the vaccine strain and 5 (MW 28-75kDa, Ip 6,5-4,5) only in the field strain.

## CONCLUSIONS

The immunogenic proteins detected only in the *B.melitensis* examined strains, but not in the cross reactive could be candidates for the confirmation of the animal infection with *B.melitensis* while the different immunogenic proteins between the vaccine strain and the field strain, could be candidates for the differentiation of vaccinated from the infected animals.

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## ACKNOWLEDGEMENTS

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# Development of a SYBR Green real-time PCR for the simultaneous detection of canine circovirus infecting dogs and wild carnivores

**Balboni Andrea[1], De Arcangeli Stefano[1], Urbani Lorenza[1], Battilani Mara[1]**

[1]Department of Veterinary Medical Sciences, Alma Mater Studiorum - University of Bologna, Via Tolara di Sopra 50, 40064, Ozzano dell'Emilia, BO, Italy

Canine circovirus (CanineCV) strains identified in dogs and in wild carnivores, in particular in foxes, show different genome sequences [1]. The study aimed to develop a real-time PCR (qPCR) assay able to detect all the CanineCV and to use the new assay to test field samples. A SYBR Green qPCR assay targeting a fragment of 132 nucleotides in the intergenic region (IR) between the 3' ends of the two major open reading frames was developed. The IR was chosen as molecular target because, on the basis of an alignment of 32 nucleotide sequences retrieved from the GenBank database, it resulted highly conserved among all CanineCV infecting dogs and wild carnivores. Serial 10-fold dilutions of a recombinant plasmid containing one copy of the target sequence were used as external standards for the construction of the standard curve and the sensitivity determination. Melting experiments were performed after the last extension step. After optimisation of the assay, the DNA extracted from faecal samples of 79 dogs and 32 red foxes were tested. Samples showing target DNA amount greater than or equal to the LOD and a specific melting peak in both replicates were considered as positive. The assay showed high sensitivity and reproducibility. The specific melting temperature ranged from 93.2 °C to 93.6 °C and the limit of detection corresponded to 5 copies of the target DNA. Eight out of 79 (10.1%) dogs and 1/ 32 (3.1%) red foxes faecal samples were positive. The qPCR assay which was developed is a reliable, specific, and sensitive tool with potential utility to detect all CanineCVs and it should be used for surveillance and diagnostic activities in both dogs and wild carnivores.

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# Survey of ESBL/Carbapenemase-Producing Enterobacteriaceae and MRSA in Companion Animals in close contact with humans, Portugal

**Adriana Belas[1], Juliana Menezes[1], Constança Pomba[1] and the PET-Risk Consortium**

[1]CIISA - Centre of Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Portugal, Av. da Universidade Tecnica, 1300-477 Lisbon, Portugal

## OBJECTIVE

The purpose of this study was to evaluate the presence of extended-spectrum β-lactamases (ESBL) producing Enterobacteriaceae and Methicillin-Resistant *Staphylococcus aureus* (MRSA) faecal carriage in healthy companion animals (CA) in close contact with humans.

## MATERIAL AND METHODS

Between January 2016 and July 2019, 72 healthy companion animals (26 cats, 46 dogs) living in close contact with humans were enrolled, an epidemiological questionnaire was performed to the owners and an informed consent was obtained. Fecal samples were inoculated on MacConkey agar plates containing 1.5µg/mL cefotaxime, 1.0 **µg/mL meropenem, MRSA ID and CHROMagar Acinetobacter plates.**

## RESULTS

Third generation cephalosporin (3GC)-resistant Enterobacteriaceae were detected in nine dogs (19.6 %) and in two cats (7.7 %). Carbapenem-resistant Enterobacteriacea were not observed. Only one cat was positive for MRSA gastrointestinal colonization. Among the dogs that harbored 3GC-resistant Enterobacteriaceae, in 44.4 % (n=4/9) of the dogs, the owners worked in veterinary healthcare, 22.2% of the dogs (n=2/9) had antimicrobial treatment and hospitalization in the last year and 22.2% (n=2/9) dogs had shelter access.

## CONCLUSION

The isolation of multidrug-resistant Enterobacteriaceae in CA is an emerging problem and dissemination of resistant bacteria through fecal contamination into the environment shouldn't be neglected.[1] The knowledge of risk factors may help to limit the impact of resistance.[2]

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# Development of an Edible Bait Vaccine to Control Rabbit Haemorrhagic Disease Virus 2 (RHDV2) in Wild Rabbits

**Carina L. Carvalho[1,2], Madalena Monteiro[1], Paulo Carvalho[1], Paula Mendonça[1], Jorge Correia[3], Berta São Brás[3], Conceição Peleteiro[3], Elsa Duarte[2], António Mira[2], Sandra Branco[2], António Roldão[4], Margarida D. Duarte[1,3]**

[1]Instituto Nacional de Investigação Agrária e Veterinária (INIAV I.P.), Av. da República, Quinta do Marquês 2780-157 Oeiras
[2]Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM), Instituto de Formação e Investigação Avançada (IIFA), Universidade de Évora, (UÉ) Pólo da Mitra, Ap. 94, 7006-554 Évora
[3]Centro de Investigação Interdisciplinar em Sanidade Animal (CIISA), Faculdade de Medicina Veterinária (FMV), Universidade de Lisboa, Avenida da Universidade Técnica, 1300-477 Lisboa
[4]Instituto de Biologia Experimental e Tecnológica (iBET), Avenida da República, Estação Agronómica, 2780-157 Oeiras

## OBJECTIVES

FIGHT-TWO aims the development and production of an edible pathogen-free Rabbit haemorrhagic disease virus 2 (RHDV2) vaccine, based in Virus-Like Particles (VLPs), to be distributed in the field as bait or in dry feed. RHDV2 causes an often-lethal systemic infection in the European rabbit (*Oryctolagus cuniculus*) and, since its emergence in 2010 in France, is one main factor underlying the species' decline, indirectly impacting on several endangered predator species [1]. This recombinant VP60 (major capsid protein) based-VLPs, will be updated according to the virus evolution in an *open system*, to protect a broader proportion of the wild rabbit populations, crucial to reduce RHDV2 transmission and to control the infection. This oral vaccine overcomes the need capture and manipulation of the animals, unfeasible in wild populations. VP60-VLPs are protein cages that mimic the overall structure of the native virions harbouring no genetic material, although able to induce a protective immune response when administered parenterally [2] or orally [3]. The project partnership includes INIAV, the Nacional Reference Laboratory for animal diseases, two Portuguese Veterinary Universities (Évora and Lisbon) and iBET, a private institute with vast experience in the vaccines production field.

## MATERIALS AND METHODS

The insect cells-baculovirus expression vector system (IC-BEVS) will be used to produce this novel vaccine. Experimental oral vaccination of domestic and wild rabbits with the prototype will be carry out.

## RESULTS

A bank of *vp60* sequences is being obtained to support the selection of a subset of representative strains to be included in the vaccine. The *vp60* gene of those strains will be cloned and used to construct the recombinant baculoviruses.

## CONCLUSION

FIGHT-TWO will allow to proceed with one of the 12 measures specified in an Action Plan for the Control of Rabbit Haemorrhagic Viral disease in Rabbits (Dispatch 4757/17 of 31 May, Ministry of Agriculture), supporting more generalist management policies towards the recovery of wild rabbit populations and RHD control, the recovery of ecosystems where the rabbit is keystone.

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# Effect of olive mill wastewaters (OMW) on *Campylobacter* spp. shedding in broiler chickens

**Patrizia Casagrande-Proietti[1], Sara Bellucci[1], Raffaella Branciari[1], Massimo Trabalza-Marinucci[1], Laura Musa[1], Maria Pia Franciosini[1]**

[1]Dipartimento di Medicina Veterinaria, University of Perugia, Perugia, Italy

## OBJECTIVE

Food wastes are sources of different compounds that can be used as natural additives in the food and feed industry. The olive oil industry produces aqueous waste (olive mill wastewater, OMW), rich in phenols with antioxidant and antimicrobials properties, able to inhibit or delay the growth rate of several bacteria *in vitro*. In this trial the dietary effect of OMW on *Campylobacter* spp. shedding in broiler chickens was investigated.

## MATERIALS AND METHODS

A commercial basal feed was supplemented with OMW (tyrosol to verbascoside ratio 1:1), to reach a total polyphenol concentration in the feed (125 µg/g), and administered to chicks from the 22$^{nd}$ to the 45$^{nd}$ day of age, when the animals were slaughtered. A negative (CTR) and a positive control group (α tocopherol added at 245 mg/kg feed, VIT-E) were also included in the experimental design. A total of 117 22-day-old male chicks were randomly assigned to the three experimental grower diets with three replicates of 13 birds each. *Campylobacter* spp. isolation and enumeration were performed in mCCD agar plates at 21 and 45 days of age collecting fecal samples from each subgroup's five animals. Presumptive *Campylobacter* spp. Colonies, showing the typical morphology, were counted.Multiplex PCR was carried out for identification of *Campylobacter* spp.

## RESULTS

OMW group showed at 45 d a lower (P<0.001) *Campylobacter* load compared to the CTR and the VIT-E groups (table 1). All *Campylobacter* were identified as *C. coli* .

## CONCLUSIONS

These results highlight the potential use of OMW by-products against *Campylobacter* spp. in poultry and it could represent an alternative treatment to the use of antimicrobials in order to reduce the antibiotic resistance problem .

Table 1. *Campylobacter* spp. shedding expressed in CFUx log/ g in the three experimental groups

| AGE | CTR | OMW | VIT. E | P value |
|---|---|---|---|---|
| 21 days | 0.69 | 0.92 | 1.22 | 0.769 |
| 45 days | 2.18$^{b}$ | 1.26 $^{a}$ | 1.90$^{b}$ | <0.001 |

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# Multidrug-resistant ESBL-producing *Salmonella Infantis* in food chain of broiler meat production in Italy from 2016 to 2019

**Patrizia Casagrande Proietti[1], Sara Bellucci[1], Alessia Zicavo[2], Stefania Scuota[2], Laura Musa[1], Laura Menchetti[1], Alberto Giannone[1], Maria Pia Franciosini[1]**

[1]Dipartimento di Medicina Veterinaria, University of Perugia, Perugia, Italy
[2] Istituto Zooprofilattico dell' Umbria e delle Marche, Perugia, Italy

## OBJECTIVE

The study was carried out to detect phenotypically the susceptibility to different classes of antimicrobials and to investigate the presence of ESBL gene in *Salmonella Infantis* from broilers in Italy.

## MATERIALS AND METHODS:

From 2016 to 2019 a study was performed in 106 *Salmonella Infantis* isolated from fecal and environmental samples in farms, from carcass skin samples at slaughter and from processed chicken meat products. Susceptibilities to antimicrobials (penicillins, tetracyclines, quinolones, fluoroquinolones, trimethoprim/sulfamethoxazole, beta-lactam and carbapenems) were evaluated by the agar diffusion method. Susceptibility to colistin was evaluated by broth microdilution method. Screening for ESBL-phenotypes was carried out by double-disk synergy test (DDST) with cefotaxime, ceftazidime and amoxicillin -clavulanic acid disks. The beta-lactamase $bla_{CTX\text{-}M\text{-}1}$ and the plasmid-mediated *mcr* genes responsible for colistin resistance were investigated by PCR.

## RESULTS

Fifty-nine S. *infantis* isolates (56%) showed a multi-resistance to 4 classes of antimicrobials, 36 (33%) had a resistance to 5 and 6 classes and 11(11%) showed resistance to 1,2 and 3 classes. Eleven isolates (11%) were carbapenem-resistant. Over the study period we observed a reduction of ESBL S. *Infantis* isolates, although 63(59%) isolates exhibited ESBL phenotype carrying $bla_{CTX\text{-}M\text{-}1.}$ There were no statistically significant differences among the matrices. Moreover a reduction of ciprofloxacin-resistant isolates (P=0.001) and an increase of gentamicin-resistant (P=0.003) isolates were found. The 41% of isolates was colistin-resistant (MIC 4-32 µg/mL), one colistin-susceptible isolate (MIC 2 µg/mL) was *mcr*-1 positive.

## CONCLUSIONS

The high prevalence of ESBL S. *Infantis* through the food chicken meat chain, is becoming a global problem in veterinary and in human medicine [1]. In this scenario it is important to adopt sanitary measures in order to decrease the presence in the chickens farms of this multiresistant bacterium which has a great ability to adapt also in unfavourable environments.

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# Improving the hemagglutination inhibition test for canine parvovirus type 2 antibodies detection

**Alessandra Cavalli[1], Costantina Desario[1], Michele Camero[1], Vito Martella[1], Nicola Decaro[1], Canio Buonavogli[1]**

[1]Department of Veterinary Medicine, University of Bari.

Canine parvovirus type-2 (CPV-2) is associated with severe gastroenteritis in pups. Testing the antibody levels in pups at the time of vaccination is the best practice to ensure the effectiveness of vaccination. The gold standard serological test for CPV-2, inhibition of hemagglutination (HI), is affected by some limitations, such as the presence in canine sera of non-specific agglutinins and the production of a "false" sedimentation button, due to an excess of swine red blood cells (RBCs) used in the HI assay, according to the method commonly used. These issues can lead to incorrect interpretation of the test. In this study, we report a modified HI test implemented to determine CPV-2 specific antibodies more precisely. Pre-treatment with concentrated RBCs was effective to remove the non-specific agglutinins from the sera. Non-specific agglutinins were identified in most sera collected from pups younger than 4 month old, whilst were observed more rarely in the sera of older dogs. Also, decreasing from 0.8% to 0.1% of the RBC concentration helped avoiding self-precipitation of RBCs in the HI assay.

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# Biofilm formation in the swine pathogen *Streptococcus suis*

**Monia Cocchi[1], Silvia Deotto[1], Martina Ustulin[3], E. Schiavon[2], Gabrita De Zan[1], Denis Vio[3]**

[1]Istituto Zooprofilattico Sperimentale delle Venezie, (UD)
[2]Istituto Zooprofilattico Sperimentale delle Venezie, (PD)
[3]Istituto Zooprofilattico Sperimentale delle Venezie, (PN)

In swine industry, *Streptococcus* (S.) *suis* infection is responsible of different pathologies such as arthritis, pneumonia, septicaemia and endocarditis. Moreover, the bacterium is an emerging zoonotic agent worldwide. The pathogenetic mechanism is not completely understood and S. *suis* infections are known to be multi-factorial. The ability to form biofilm is considered a virulence factor, enabling bacteria to persist and colonize tissues and resist from host immune system and antimicrobials [2, 4]. S. *suis* produces biofilm, but different factors (capsule, fibrinogen, e.g.) affect its formation [1, 3]. This study aimed at defining the biofilm formation in S. *suis* strains isolated from different organs in cases of swine streptococcosis. Forty-six S. *suis* strains collected during the period 2017-2019 (joints, n=5; lung and pericardium, n=30, brain=9, kidney=1, liver=1) were submitted to the microtiter plate test, as previously described, without adding fibrinogen [5]. Results showed that 33/46 (72%) were no biofilm producers, 12/46 (26%) were weak to moderate producers, while only one tested strain resulted a strong biofilm producer (2%). According to the literature, the most of the strains of S. *suis* do not produce biofilm in usual culture conditions [1]. Noteworthy, the strong biofilm producer strain was identified from the pericardium in a weaned pig affected by septicaemia, while the other isolates from liver and kidney were moderate and negative biofilm producers, respectively. Since S. *suis* is a heterogeneous bacterial species, both genetically and phenotypically, the analysis of the biofilm formation from isolates recovered from different organs may add information about the virulence and the pathogenetic mechanism of the strains.

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# Abscence of MRSA in livestock and farm environment located near a hospital centre

**Susana Correia[1-4], Vanessa Silva[1-4], Juan García-Díez[5], Paula Teixeira[6], Kevin Pimenta[1-4], María Teresa Tejedor-Junco[7], Gilberto Igrejas[2-4], Patrícia Poeta[1,4]**

[1]Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[2]Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal
[3]Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[4]Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Lisboa, Caparica, Portugal
[5]Animal and Veterinary Research Centre (CECAV), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[6]Associação de Criadores do Maronês (ACM), Cooperativa Agricola de Vila Real, Vila Real, Portugal
[7]Research Institute of Biomedical and Health Sciences, University of Las Palmas de Gran Canaria, Canary Islands, Spain

In order to follow a One Health approach to determine the prevalence and transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) in cattle, oral and nasal swabs of 49 healthy cows (11 Friesians and 28 cross-breeds) were recovered together with 19 human, 13 water and 20 soil samples from the animals' handlers and environments. Although the closeness to the main hospital centre of the region, samples were collected in higher mountain rural areas from extensive production systems that mainly use natural resources and do not routinely use antimicrobials in subtherapeutic doses. Through a concerted One Health approach, this study revealed that the cattle and their surrounding environments do not represent reservoirs for MRSA. Nonetheless, it would still be interesting to study the prevalence and transmission of methicillin-susceptible S. aureus carrying resistance determinants for other antimicrobial drugs and to extend the study to other bacterial species that also represent major AMR threats.

## ACKNOWLEDGEMENTS

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# Highly pathogenic *Aeromonas hydrophila* in swine

**Duarte EL[1,2], Queiroga MC[1,2], Saavedra MJ [3,4]**

[1]Departamento de Medicina Veterinária, Escola de Ciências e Tecnologia, Universidade de Évora, Évora, Portugal
[2]ICAAM-Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Évora, Portugal
[3] Departamento de Ciências Veterinárias, Lab. Microbiologia Médica, Escola de Ciências Agrárias e Veterinárias
[4]Centro de Ciência Animal e Veterinária (CECAV), Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal

## OBJECTIVE

Autochthonous Iberian pig breeds have been growingly popular due to the increasing demand for locally and extensively produced animals. Due to their different production system, microbiological hazards significantly diverge from industrially reared animals. Within the frame of a broader study to characterize specific pathogens associated with Alentejano pig breed, *Aeromonas hydrophila* was isolated in pure culture from collected organs of septicemic piglets from two farms. These farms had no epidemiological link between them to our knowledge. As A. *hydrophila* is seldom the cause of septicemia in mammals, antimicrobial resistance profile and virulence factors were investigated for these two strains.

## MATERIALS/METHODS

*Aeromonas hydrophila* were phylogenetic characterized using *gyr*B gene sequencing. Antimicrobial resistance profile and the production of extracellular lipases and proteases was evaluated. The presence of several genetic determinants of resistance and virulence were determined by PCR: aminoglycoside resistance associated genes (acetyltransferases-AAC-, phosphotransferases-APH- and nucletildiltranferases-ANT), genes encoding lipases and aerolysin-related toxins and type III secretion system.

## RESULTS

Identification was confirmed by *gyr*B sequencing. A. *hydrophila* isolate from farm 1 was sensitive to gentamicin, oxytetracycline, neomycin, enrofloxacin, colistin sulfate, trimethoprim, ceftiofur and amoxicillin/ clavulanic acid. A. *hydrophila* from farm 2 was resistant to all antibiotics except enrofloxacin. This isolate harboured APH(6)-I and ANT(6)-I genes, but no AAC genes. Genes for all virulence factors tested were present in both isolates. Moreover, all strains displayed lipolytic and proteolytic activity under the conditions tested.

## CONCLUSION

Although described in immunocompromised humans or as a secondary pathogen, *Aeromonas hydrophila* has been unfrequently reported as a cause of septicemia in mammals. The occurrence of several virulence determinants in these emergent pathogens, their multiple resistance profile, along with their ubiquitous nature in terrestrial and aquatic environments, is prone to rise a significant concern to animal health and veterinary microbiologists in the near future.

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# *Clostridioides difficile* in canine puppies: relevance and risk factors for infection

**Mirjam Duijvestijn[1], Miriam Koene[2], Ed Kuijper[3], Lapo Mughini-Gras[1,4], Jaap Wagenaar[1,2]**

[1]Utrecht University, Faculty of Veterinary Medicine, Department of Infectious Diseases and Immunology, Yalelaan 1, 3584 CL Utrecht, The Netherlands.
[2]Wageningen Bioveterinary Research (WBVR), Lelystad, the Netherlands.
[3]Department of Medical Microbiology, Centre for Infectious Diseases, Leiden University Medical Centre, Leiden, the Netherlands
[4]National Institute for Public Health and the Environment (RIVM), Centre for Infectious Disease Control (CIb), PO Box 1 - 3720 BA Bilthoven, The Netherlands.

## OBJECTIVE(S)

To determine prevalence, clinical relevance and risk factors for *Clostridioides difficile* (CD) in faecal samples of canine puppies (<1 year).

## MATERIALS AND METHODS

49 Dutch veterinary practices submitted faecal samples from puppies ≤1 year, with (n=104) and without (n=47) acute diarrhoea (≤10 days) for bacteriological culture, together with epidemiological data on age, clinical signs, living conditions and antibiotic use (2009-2011). The samples were heat shock treated, and after enrichment for 7 days plated on two CD selective media (CDSM and Brazier). Gram-staining and a gluD-PCR were used as confirmation tests. Additionally, CD ribotyping (PCR 16s rRNA, 23s rRNA) and PCR tests targeting toxin genes (TcdA, TcdB CdtA and CdtB) were performed. Multivariate logistic regression was used to identify determinants of CD infection.

## RESULTS

Twenty four samples were positive for CD; 17% (8/47) in clinically healthy control puppies and 15% (16/104) in puppies with acute diarrhoea (p=0.799)[1]. Ribotype 010 was most common (14x), followed by ribotype 039 (4x). Ribotype 09, 012, 031 and 045 were found once. Two strains were undifferentiated. Ribotype 012 was TcdA and TcdB positive, ribotype 045 was TcdA,TcdB, CdtA and CdtB positive. Younger mean age (88 days vs.122 days (p=0,005) spring /autumn season (p=0,036/ p= 0,046), and antibiotic use at sampling (p=0,000) were associated with increased CD detection. Combinations with metronidazole were most frequently mentioned antibiotics.

## CONCLUSION

In this study detection of CD was not associated with diarrhoea in puppies. CD was significantly more often found in younger animals, in spring and autumn, or when antibiotics were used at sampling, as is also reported in other animals and humans. Toxigenic ribotypes were found in only two samples, one of which was ribotype 045, a type that can also be found in humans. A possible zoonotic potential needs further research.

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# First analysis of both viral and chemical contaminants in oyster *Crassostrea gigas* from experimental lanterns in Campania region

**Filomena Fiorito[1], Maria G. Amoroso[2], Denise Di Concilio[2], Sara Lambiase[2], Antonio L. Langellotti[3], Anna Martello[3], Mauro Esposito[2], Giovanna Fusco[2]**

[1]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy.
[2]Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici (Naples), Italy.
[3]Centro di Ateneo per l'Innovazione e lo Sviluppo dell'Industria Alimentare, Università degli Studi di Napoli Federico II, Portici (Naples), Italy.

## OBJECTIVES

In coastal areas anthropogenic activities as shipping, marine facilities, industrial and domestic effluents result in release of several pollutants that may be bioaccumulated in marine bivalves and threat human health. Oyster, could concentrate enteric viruses, as noroviruses (NoVGI/GII), astrovirus (AsV) and rotavirus (RV) and their consumption is recognized as cause of foodborne disease in human [1]. Furthermore, oyster might accumulate non-dioxin-like polychlorinated biphenyls (NDL-PCBs), known to provoke toxic effects, in both vertebrates and invertebrates, including endocrine disruption, carcinogenesis and immunosuppression [2]. This study was aimed to monitor viral and chemical pollution in oyster *Crassostrea gigas* from experimental stations.

## MATERIALS AND METHODS

Oysters, collected from November 2016 to September 2018, were analysed through validated methods, as quantitative RT-PCR to detect viruses and high-resolution gas chromatography/high-resolution mass spectrometry to assess NDL-PCBs levels.

## RESULTS

A simultaneous contamination, both viral and chemical, was found in 62.5% of samples. NoVGII was the most frequently detected virus (43.8%), followed by NoVGI (37.5%), RV (25%) and AsV (18,7%). The levels of ∑6 NDL-PCBs (ICES) ranged from 0.80 to 7.12 ng g-1 wet weight, below the European maximum limits. Overall, virus-positive samples showed levels of chemicals higher than negative ones. A similar relationship between environmental pollutants and foodborne viruses was previously described in mussels [3].

## CONCLUSIONS

Herein, our results suggest that oysters, a product with high nutritional and economic values, represent suitable bioindicators for viruses and chemicals in marine environment which should be constantly monitored for human safety food.

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# Full-length genome characterization of canine parvovirus strains circulating in Nigeria

**Annalisa Guercio[1], Kenneth Ikejiofor Ogbu[2,3], Francesco Mira[1], Giuseppa Purpari[1], Chika Nwosuh[4], Guido Ruggero Loria[1], Giorgia Schirò[1], Gabriele Chiaramonte[1], Metthew Terzungwe Tion[5], Santina Di Bella[1], Gianluca Ventriglia[6], Nicola Decaro[6], Boniface Maduka Anene[3]**

[1]Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Via Gino Marinuzzi 3, 90129 Palermo, Italy.
[2]Department of Animal Health, Federal College of Animal Health and Production Technology, National Veterinary Research Institute Vom, Plateau State, Nigeria.
[3]Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.
[4]Viral Reseach Division, National Veterinary Research Institute Vom, Plateau State, Nigeria.
[5]Department of Veterinary Medicine, College of Veterinary Medicine, Federal University of Agriculture, Makurdi, Benue State, Nigeria.
[6]Department of Veterinary Medicine, University of Bari, Strada provinciale per Casamassima Km 3, 70010 Valenzano, Bari, Italy.

Canine parvovirus type 2 (CPV-2) emerged suddenly in the late 1970s as pathogen of dogs and, soon after its emergence, the original CPV-2 was replaced by three antigenic variants, CPV-2a/-2b/-2c, which to date have gained a worldwide distribution [1]. Previous molecular analyses conducted in Nigeria were based on partial VP2 gene sequences [2-4]. The aim of this study was to provide a full-length genome analysis of CPV strains collected in Nigeria, Africa. Rectal swab samples (n=320) were collected in 2018 and tested by means of an immunochromatographic assay. Among the positive samples (n=144), 59 were selected for further analyses using different molecular assays. The results revealed a high prevalence of CPV-2c (91.5%) compared to the CPV-2a variant (8.5%). The VP2 gene sequence showed a divergence from the strains analysed in 2010 in Nigeria. A closer connection with CPV strains of Asian origin was observed. The non-structural genes analysis evidenced amino acid changes never previously reported. The molecular analysis evidenced a geographical pattern of distribution of the analysed strains, suggesting a potential common evolutionary origin with CPV of Asian origin. This first CPV molecular characterization including all the encoding gene sequences conducted in the African continent contributes to further define the CPV spreading variants worldwide.

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# Bdellovibrio and like organisms (BALOs): eclectic predators

**J. Hattab[1], F. Mosca[1], D. Ottaviani[2], G. Angelico[2], C.E. Di Francesco[1], A. Pallavicini[3], V. Iebba[4], P.G. Tiscar[1]**

[1]Università degli Studi di Teramo, Facoltà di Medicina Veterinaria – Teramo (TE), Italy
[2]Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche – Ancona (AN), Italy
[3]University of Trieste, Department of Life Sciences Laboratory of Genetics – Trieste (TS), Italy
[4]Institut National de la Santé et de la Recherche Medicale (INSERM) U1015 and Equipe Labellisée-Ligue National contre le Cancer, Istituto Pasteur Cenci Bolognetti Foundation, Public Health and Infectious Diseases Department

*Bdellovibrio* and like organisms (BALOs) are small Gram-negative ubiquitous bacteria, obligate predator of other Gram-negative bacteria. BALOs enter the periplasmic space of the host and replicate until daughter cells lyse the host, starting a new cycle.

To date, the potential applications of BALOs have been experimentally investigated in microbiological food control, environmental safety and as alternative therapeutic approach towards infectious diseases.

The aim of the present study was to test the effectiveness of two *Halobacteriovorax* strains isolated from the Adriatic Sea (Italy) against V. *parahaemolyticus* and *Salmonella enterica*. Bacteriolytic activity of the predators was measured by reduction in prey cell viability by using standardized colony count method. As regards V. *parahaemolyticus*, *in vitro* test was performed setting up seven predator/prey ratios, resulting in a maximum decrease of the prey of 2.4 log at a 1:100 ratio. The *in vivo* Vibrio *parahaemolyticus* test was conducted as a depuration trial on *Mytilus galloprovincialis* to evaluate the effectiveness of *Halobacteriovorax* on experimentally contaminated mussels. In this case, the maximum decrease was 2.2 log at a 1:100 predator/prey ratio. Regarding S. *enterica*, four strains (S. Napoli, S. derby, S. typhimurium and its monophasic variant 1, 4 [5], 12:i) were tested *in vitro*, obtaining a statistically relevant decrease of the bacterial load in S. Napoli (1.7 log). and S. derby (2.0 log) after 24 hours, both at 1:1 ratio.

Results were different depending on the prey, suggesting that the kind of prey influences the predatory activity of BALOs.

This study confirms the efficacy of BALOs' halophilic strains in reducing bacterial load of Gram-negative. There are many application fields for halophilic strains of BALOs still to do research on, for this reason is important to know exactly the action some strains have on each prey.

# The Antimicrobial Stewardship and Pets-project (ASAP): Antimicrobial use in 44 Dutch companion animal clinics prior to and after implementation of an Antimicrobial Stewardship Programme

**Nonke E.M. Hopman[1], Lützen Portengen[2], Marlies E.J.L. Hulscher[3], Dick J.J. Heederik[2], T.J.M. Verheij[4], Jaap A. Wagenaar[1,5], Jan M. Prins[6], Tjerk Bosje[7], Louska Schipper[2], Ingeborg M. van Geijlswijk[2,8], Els M. Broens[1]**

[1]Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584 CL Utrecht, the Netherlands
[2]Institute for Risk Assessment Sciences, Utrecht University, Yalelaan 2, 3584 CM Utrecht, the Netherlands
[3]Scientific Center for Quality of Healthcare (IQ healthcare), Radboud Institute for Health Sciences, Radboud university medical center, Geert Grooteplein 21, 6525 EZ Nijmegen, the Netherlands
[4]Julius Center for Health Sciences and Primary care, University Medical Center, Universiteitsweg 100, 3584 CG Utrecht, the Netherlands
[5]Wageningen Bioveterinary Research, Houtribweg 39, 8221 RA Lelystad, the Netherlands
[6]Amsterdam UMC, University of Amsterdam, Department of Internal Medicine, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands
[7]Medical Center for Animals, Isolatorweg 45, 1014 AS Amsterdam, the Netherlands
[8]Pharmacy Department, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 106, 3584 CM Utrecht, the Netherlands

## OBJECTIVES

To curb increasing antimicrobial resistance (AMR) rates, responsible antimicrobial use (AMU) is needed. Antimicrobial Stewardship Programmes (ASPs) are implemented worldwide in human healthcare to improve the appropriateness of AMU. No ASPs were implemented in companion animal clinics yet. Therefore, the ASAP-project was started to develop, implement and evaluate the effect of an ASP in Dutch companion animal clinics.

## METHODS

Baseline AMU data were collected (2012-2015) from 44 Dutch companion animal clinics. Then, a multifaceted ASP, containing educational training, benchmarking of AMU data, an information leaflet for pet owners on AMU and individual feedback per clinic, was implemented in these clinics. Number of Defined Daily Doses for Animals (DDDAs) per clinic was used to quantify systemic AMU, and calculated from prescription data, for total, 1st, 2nd and 3rd choice AMU (according to Dutch policy on veterinary AMU). Statistical modelling was used to evaluate the effect of the ASP on AMU across participating clinics.

## RESULTS

Before start of the ASP, mean total AMU was decreasing from 1.82 DDDA/year to 1.56 DDDA/year and a shift towards 1st choice AMs was present. A statistically significant stepwise decrease in total (GMR 0.85, 95% CI 0.78-0.93), 1st (GMR 0.85, 95% CI 0.76-0.95) and 2nd choice (GMR 0.74, 95% CI 0.66-0.83) AMU could be attributed to participation in the ASP. Change in (linear) time trend was statistically significant for total AMU as well. Participation in the ASP did not affect 3rd choice AMU that was already low before start of the ASP.

## CONCLUSIONS

Participation in an ASP can contribute to a further reduction and optimisation of AMU in Dutch companion animal clinics, on top of ongoing time trends. Participants reported to be more aware of AMU after participation and the project was positively evaluated.

## ACKNOWLEDGEMENTS

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# ESBL-production and Ciprofloxacin resistance in *Enterobacteriaceae* in high AMU Pig and Broiler Farms in Belgium & the Netherlands

**Franca Jonquiere[1], Moniek Ringenier[2], Sien De Koster[6], Nele Caekebeke[2], Tijs Tobias[1], Hans Vernooij[1], Francisca Velkers[1], Nathalie Sleeckx[4], Angelique van den Hoogen[1], Merel Postma[2], Manon Houben[3], Marjolein Kluytmans[5,6], Jan Kluytmans[5,6], Arjan Stegeman[1], Herman Goossens[6], Jeroen Dewulf[2], i-4-1-health Study Group**

[1]Utrecht University, The Netherlands
[2]Ghent University, Belgium
[3]GD Animal Health, Deventer, The Netherlands
[4]Experimental Poultry Centre, Belgium
[5]Amphia Hospital, Breda, The Netherlands
[6]UMC Utrecht, The Netherlands

## OBJECTIVE

The i-4-1-health project aims to study associations between antimicrobial resistance (AMR) in *Enterobacteriaceae* and antimicrobial use (AMU) on high AMU pig and broiler farms in the cross-border region of The Netherlands (NL) and Flanders (BE).

## MATERIALS AND METHODS

On 29 Broiler and 31 multiplier pig farms in NL and BE with high AMU (based on quality assurance system data of the previous year) 30 fecal samples (Fecal Swab, Copan Italy) were collected. Non-selective broth enrichment (TSB, Copan Italy) and selective agar plates (ChromID ESBL, bioMérieux; McC cipro, in-house) were used to grow extended-spectrum beta-lactamase (ESBL-E)-producing and Ciprofloxacin-resistant (Cipro-R) Enterobacteriaceae, followed by antimicrobial susceptibility testing and phenotypic ESBL confirmation. Associations of AMR with AMU, country and species were analysed using logistic regression models, with random intercept for farm and unit.

## RESULTS

ESBL-E-coli were detected on 2/16 NL pig and 10/14 broiler farms, whereas ESBL-E was found on 13/15 BE pig and 15/15 broiler farms. The Odds Ratio (OR) to find ESBL-E in NL compared to BE broiler samples, was 0.007 (95%CI; 0.001-0.048) and for pig samples 0.004 (95%CI;0.000-0.042). Cipro-R-E were detected on 2/16 NL pig and 14/14 broiler farms and 14/15 BE pig and 15/15 broiler farms. The OR to find Cipro-R in NL broiler samples compared to BE was 0.61 (95%CI; 0.38-0.97) and for pig samples 0.25 (95%CI; 0.17-0.37). Proportion of co-resistance to ciprofloxacin was found in 0.33 of BE and 0.13 of NL ESBL positive E. coli isolates from broilers, compared to only 0.17 of BE pig isolates and none for NL. In 2/15 BE and in 10/14 NL broiler farms Flumequine was used, whereas Enrofloxacin was only used in 4/14 NL broiler farms.

## CONCLUSION

ESBL-E and Cipro-R-E results vary between farms and are associated with livestock species and country. Associations with previous AMU are not straightforward.

# In vitro evaluation of the effect of different topical formulations on (methicillin resistant) *Staphylococcus pseudintermedius* isolated from canine skin

**M.M. Kannekens-Jager[1], N. Elfrink[2], N. Hoes[3], C.M.M. van Damme[2], E.M. Broens[1]**

[1]Veterinary Microbiological Diagnostic Centre (VMDC), Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Netherlands.
[2]Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Netherlands.
[3]Small Animal Dermatology Clinic "Dierendermatoloog Vroom", Udenhout, Netherlands.

## OBJECTIVE

Local therapeutic options for superficial pyoderma in dogs are preferred to systemic treatments with antimicrobials. Few studies have shown the in vitro effect of sodium hypochlorite [1] and chlorhexidine [2] but the effect of exposure time has not been evaluated yet. The aim of this study was to evaluate the effect of different topical formulations as well as the exposure time on (methicillin resistant) *Staphylococcus pseudintermedius*.

## MATERIALS AND METHODS

Four *Staphylococcus pseudintermedius* isolates were used during the experiment, 2 Methicillin Resistant (MRSP) and 2 Methicillin Susceptible (MSSP) isolates. Three formulations were tested in different concentrations; a shampoo with sodium hypochlorite (0.05-1%), a shampoo with chlorhexidine (0.2-2%) and sodium hypochlorite (household bleach) (0.005-1%). A standardized concentration of MRSP or MSSP ($10^6$ CFU/ml) was mixed 1:1 with the formulation in different concentrations. Then 100ul of each mixture was plated on blood agar after 0, 3, 5 and 10 minutes exposure at room temperature. Colonies were counted after O/N incubation at 37°C. All experiments were performed in duplicate.

## RESULTS

All isolates were inhibited by sodium hypochlorite and the shampoo with chlorhexidine 2% instantly (0 minutes). For the shampoo with sodium hypochlorite (0.05-1%) and with chlorhexidine (0.2%) all isolates were inhibited, however longer exposure time (3-10 minutes) was needed.

## CONCLUSION

This study shows that sodium hypochlorite and a shampoo with chlorhexidine 2% reduce the number of MRSP and MSSP instantly. Exposure time for shampoos with sodium hypochlorite and lower concentrations of chlorhexidine appeared to be longer. Further studies are warranted to validate the results of this limited study in vivo.

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## ACKNOWLEDGEMENTS

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# Growth abilities of *Taylorella equigenitalis*: survival in low temperatures *in vitro*

**Karpíšková Renáta[1], Gelbíčová Tereza[1], Koláčková Ivana[1]**

[1]Veterinary Research Institute, Department of Bacteriology, Brno, Czech Republic

## OBJECTIVE

*Taylorella equigenitalis* is the bacterium responsible for sexually transmitted contagious equine metritis (CEM) that may occur in both natural and artificial breeding. *T. equigenitalis* is described as a fastidious bacterium with minimum ability to survive outside the urogenital tract. However, long-term persistence in horse breeding farms suggests that survival of this etiological agent is also possible in the outdoor environment. The study was aimed at comparison of *T. equigenitalis* growth *in vitro* in liquid medium to test the ability to survive at different temperatures.

## MATERIALS AND METHODS

The study was conducted with *T. equigenitalis* ST56 isolated from stallion in the Czech Republic in 2017. The inoculation density of bacteria was log 3 CFU /ml. The growth of the strain was tested in bioreactors RTS-1C (Biosan), at different temperatures (38**°C,** 25**°C,** 15**°C and** 8**°C) under microaerophilic atmosphere in Bolton broth (Oxoid) without blood.**

## RESULTS

The ability of *T. equigenitalis* to grow or survive in Bolton broth (without supplements) was detected at various temperatures. Increase of *T. equigenitalis* cells by more than log 1 CFU/ml at 38**° C was observed the second day of cultivation.** The tested strain was able to survive in Bolton broth also at 25**° C and 15° C for five days while maintaining of bacte**ria number (log 3 CFU/ml). At 8**° C the number of** *T. equigenitalis* decreased by one logarithm after 5 days. At the high initial density of *T. equigenitalis* (log 8 CFU /ml) survival under aerobic condition was detected.

## CONCLUSION

The ability of *T. equigenitalis* to survive at low temperature supports the hypothesis about its possible presence in outdoor environment of horse breeding farms if appropriate conditions are available.

## ACKNOWLEDGEMENTS

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# CRISPR/Cas9 vectors reduce bacterial loads in a *Brucella melitensis* ovine macrophage infection model

**Garyfalia Karponi[1], Spyridon K. Kritas[1], Eleni Papanikolaou[2], Evanthia Petridou[1]**

[1]Department of Microbiology and Infectious Diseases, School of Veterinary Medicine, Aristotle University of Thessaloniki, Greece
[2]Laboratory of Biology, School of Medicine, National and Kapodistrian University of Athens, Greece

## OBJECTIVE

Brucellosis, caused by the bacterium *Brucella melitensis*, is considered one of the most severe zoonotic diseases worldwide [1]. For almost four decades in southern Europe and elsewhere [2], eradication of the disease has been based on ambiguously effective programs [3, 4], rendering massive sanitation of livestock urgent and indispensable. Viral vectors could possibly constitute an alternative option towards a permanent cure for brucellosis, by aiding in the deletion or inactivation of genes associated with the replication of *Brucella* in the host cytoplasm.

## MATERIALS AND METHODS

We infected ovine macrophages with *B.melitensis*, to simulate the host cell/microorganism interaction *in vitro*, and transduced the infected cells with CRISPR/Cas9 lentiviral vectors that target *Brucella*'s RNA polymerase or virulence-associated gene VirB10 at a multiplicity of infection of 60. Mock-transduced cells infected with *Brucella* as well as infected cells transduced with a conventional vector expressing the green fluorescence protein (GFP) served as controls at all times.

## RESULTS

We demonstrate a decrease in the bacterial loads per cell, at two time points (Day 1 and 4 post transduction), especially when infected cells are transduced with the RNA polymerase vector. Additionally, the number of internalized brucellae per cell remained unaffected when macrophages were exposed to the GFP vector, thus underlining the bactericidal effect of our CRISPR/Cas9 systems.

## CONCLUSION

Pending *in vivo* verification of our findings, overall, these results may prove critical not only for the treatment of human and animal brucellosis, but for other infectious diseases in general.

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# Resistances to Cephalosporins and Fluoroquinolones in Veterinary medicine

**Heike Kaspar[1], Anne-Kathrin Karaalp[1], Britta Ballhausen[1], Maria Kluge[1], Juergen Wallmann[1], Ulrike Steinacker[1]**

[1]Federal Office of Consumer Protection and Food Safety (BVL), Berlin, Germany

## OBJECTIVES

Cephalosporins and Fluoroquinolones are classified from WHO as “highest priority critically important antimicrobials” and from OIE as “critically important antimicrobials”. Nevertheless, they are important substances to treat bacterial infections in human and veterinary medicine. Since 2001, an annual representative German-wide monitoring study (GERM-Vet) on bacterial isolates from diseased animals generates resistance data amongst others against a set of five different cephalosporins and three different fluoroquinolones.

## METHODS

The bacterial isolates were investigated by using the broth microdilution method according to CLSI document VET01 5th ed. The MIC values were assessed with their corresponding clinical veterinary breakpoints (CLSI VET08). If no breakpoints were available, $MIC_{90}$ values were used for classification.

## RESULTS

- Mastitis: *S. aureus* isolates showed low resistance rates against cephalosporins (0-1.5%) and fluoroquinolones ($MIC_{90}$ 0.25 mg/L), *E. coli*: $MIC_{90}$ values were increasing over a period of 2 years (Cefquinom from $MIC_{90}$ 0.12 to 0.5 mg/L, Ceftiofur under 2% in 2005 to 8% in 2016).
- Calves: $MIC_{90}$ values for cephalosporins of the 3rd and 4th generation and for fluoroquinolones are high for bacterial strains isolated from calves (for all tested cephalosporins >32 mg/L, fluoroquinolones >16mg/L).
- Pigs: $MIC_{90}$ values for cephalosporins of the 3rd and 4th generation and fluoroquinolones were much lower for bacterial strains isolated from pigs than for those isolated from calves (for all tested cephalosporins 0.12 – 0.5 mg/L, fluoroquinolones 1 mg/L).
- Poultry: Cephalosporins are not approved for veterinary use in poultry. Nevertheless, we see high $MIC_{90}$ values for broilers although the ESBL rates for *E. coli* are still at 3.6%. Resistance rates for isolates from turkeys and broilers for fluoroquinolones are low (about 7%).

## CONCLUSIONS

Depending on the affiliation to animal and bacterial species we see large differences in resistance data and a very different impact on resistance situation in veterinary medicine.

# *Brucella* species detected in tissues of aborted fetuses and seropositive slaughtered ruminants in Greece

**Aristomenis Katsiolis[1], Nektarios Giadinis[2], Eleni Papanikolaou[3], Garyfalia Karponi[1], Spyridon Kritas[1], Athanasia Stournara[4], Evanthia Petridou[1]**

[1]Department of Microbiology & Infectious Diseases, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece
[2]Farm Animal Clinic, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece
[3]Laboratory of Biology, School of Medicine, National and Kapodistrian University of Athens, Athens 11527, Greece
[4]National Reference Laboratory for Brucellosis, Veterinary Laboratory of Larissa, Ministry of Rural Development and Food, Greece

## INTRODUCTION

Brucellosis is a worldwide infectious disease. Humans, ruminants and other species of productive animals as well as many wild mammals can be affected [1]. The disease in most European countries has been eradicated [2]. In Greece, eradication programs are applied. The aim of the study was to investigate the *Brucella* species presence in tissues of seropositive slaughtered ruminants or aborted fetuses in Greece.

## MATERIALS AND METHODS

Ninety-two (92) tissue samples originated from 48 bovines and 31 sheep and goats, were subjected for further investigation. From all samples DNA was extracted. For the initial screening real time PCR was performed. All positive samples were further subjected to a multiplex PCR assay for the identification of *B. melitensis*, vaccine strain Rev1, *B. abortus* and strain RB51 [3].

## RESULTS

The results have shown that of bovine samples 23 fetuses and 16 lymph nodes were PCR positive for *B. abortus* while only one was positive to the vaccine strain RB51, while of the ovine/caprine samples only one was positive to the vaccine strain REV-1.

## DISCUSSION

The PCR analysis indicated that the majority of the bovines were positive to *B. abortus* infection while the majority of the sheep and goats were positive to *B. melitensis* infection. Only in two cases vaccine strains RB51 and REV-1 were detected. The samples were originated from fetuses of a dairy cattle and a dairy sheep, respectively. In both cases the females that aborted were accidentally vaccinated at the age of 12 months when they were already pregnant.

## CONCLUSIONS

Despite of the current reservations that part of the bovine population is *B. melitensis* affected due to the high prevalence of the microorganism in sheep and goats' populations and mainly due to the use of common pastures, the present study indicates that such concern is not documented while further investigation is needed.

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# Occurrence of *Escherichia coli* carrying predilection factors for APEC in different stages of poultry industry

**Koláčková Ivana[1], Kučerová Dana[1], Vacková Zdenka[1], Karpíšková Renáta[1]**

[1]Veterinary Research Institute, Department of Bacteriology, Brno, Czech Republic

## OBJECTIVE

Avian pathogenic *Escherichia coli* (APEC) are the etiological agent of serious avian disease associated with variety of clinical signs from localized infections to systemic septicaemia. It is an endemic disease, which is responsible for high economic losses in farms globally. Current diagnostic is based on anamnestic, clinical and pathological findings and bacteriological examination. Recent studies have been focused on selection of virulence encoding genes as predilection factors. Johnson et al. (2008) proposed a set of genes *iut*A, *hly*F, *iss*, *iro*N, *omp*T for verifying APEC strains. The aim of this study was to assess the occurrence of APEC predilection factors in *E. coli* isolated from different levels of the poultry industry.

## MATERIALS AND METHODS

Poultry with clinical signs of colibacillosis (72), healthy animals at farms (8) and slaughterhouse level (66), swabs from the environment of farms (19) and slaughterhouse (21) were examined using plating on MacConkey agar (37 °C/24 h) and suspect *E. coli* colonies were confirmed by MALDI TOF/MS. Screening for selected genes was performed by PCR.

## RESULTS

APEC predilection factors were confirmed in 50% of isolates from poultry with suspect colibacillosis but most often were selected genes found in samples of bedding from broiler breeding houses (58%), even if bred animals did not show any clinical signs of this illness. Positive findings were obtained also from the environment of slaughterhouse (33%) and in healthy animals before slaughtering (28%).

## CONCLUSION

The results show that *E. coli* carrying genes of APEC predilection factors can be found in many stages of the poultry industry and could be part of commensal microflora. The overall health status of the animals will therefore be an essential factor influencing the onset and course of the disease.

## ACKNOWLEDGEMENTS

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# Comparison of the bacterial composition of the faecal microbiota between European and Asian wild boars (*Sus scrofa*) by metagenomic methods

**Balázs Libisch[1], Tibor Keresztény[1], Zoltán Kerényi[2], Róbert Kocsis[2], Péter P. Papp[1] and Ferenc Olasz[1]**

[1]Laboratory of Microbiology, Agricultural Biotechnology Institute, National Agricultural Research and Innovation Centre (NARIC), Gödöllő, Hungary
[2]Hungarian Dairy Research Institute Ltd., Mosonmagyaróvár, Hungary

## OBJECTIVES

The aim of this study was to comparatively analyse the bacterial composition of the faecal microbiota between European and Asian wild boars (*Sus scrofa*) by metagenomic methods to assess the potential effects of the various environmental or genetic factors.

## MATERIALS AND METHODS

Faecal samples were collected from five hunted wild boars in Hungary. Purified DNA was sent to a service provider for amplicon (V3-V4 regions of the 16S rRNA gene) sequencing on Illumina MiSeq. For one of these samples shotgun sequencing was also performed. rRNA gene amplicon reads of the manure of seven South Korean wild boars were accessed from the MG-RAST database. Bioinformatic analyses were performed using EBI Metagenomics and SILVAngs [1].

## RESULTS

Taxonomical profiling at the phylum level revealed significant differences ($p<0.05$) between the European and Asian wild boars, where the phyla Proteobacteria, Verrucomicrobia and Fibrobacteres were more abundant in the European animals, while mean abundances for Firmicutes and Tenericutes were higher in the Asian boars. The overall ratios of the three main phyla (Bacteroidetes, Firmicutes and Proteobacteria) for the European boars were more similar to that of domestic pigs (determined by a meta-analysis) [2]. Further bioinformatic analyses identified various components of the diet of the animals and also indicated that parasitic nematodes of the Trichocephalida were present in some of the Asian boars.

## CONCLUSION

Our observations on the overall ratios of the three main phyla (Bacteroidetes, Firmicutes and Proteobacteria) between the European and Asian wild boars and domestic pigs may indicate a more pronounced anthropogenic impact on the gut microbiota of the sampled Hungarian animals.

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## ACKNOWLEDGEMENTS

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# Detection of Mycoplasma DNA in 122 cases of abortion, stillbirth and neonatal mortality in dogs and cats

**Chierchia Filomena[1], Rampacci Elisa[1], Stefanetti Valentina[1], Sylla Lakamy[1], Marenzoni Maria Luisa[1]**

[1]Department of Veterinary Medicine, University of Perugia

## OBJECTIVE

Abortion and neonatal mortality are relatively common in dogs and cats and they are linked to several causative factors [1]. *Mycoplasma* spp. have been involved in canine and feline infertility, although existing studies are limited and somewhat contradictory [2, 3, 4]. Intrauterine inoculation of *Mycoplasma* spp. had previously been associated to endometritis, abortion, and neonatal mortality in cat [3]. Conversely, several *Mycoplasma* spp. have been isolated in vaginal swabs of healthy bitch [2]. The purpose of the present study was to retrospectively detect *Mycoplasma* DNA in a caseload of canine and feline abortion, stillbirth and neonatal mortality.

## MATERIALS AND METHODS

Specimens from 122 cases (114 dogs and 8 cats) of abortion, stillbirth and neonatal mortality were investigated to detect *Mycoplasma* DNA by PCR.

## RESULTS

Eight out of 122 cases (6.6%, all dogs) tested positive for Mycoplasma DNA. From five of them (62.5%), other microorganisms were identified, particularly Canine herpesvirus-1 (2/8, 25%) and *Escherichia coli* and/or *Staphylococcus pseudintermedius* (3/8, 37.5%), notoriously responsible for infertility in the bitch. In two different litters, only one puppy of each was positive to Mycoplasma DNA. Moreover, Mycoplasma DNA was identified from vaginal swab and foetal membranes collected during caesarean section of a bitch whelping *Mycoplasma*-negative puppies, further supporting that *Mycoplasma* spp. is part of the normal microflora of the female genital tract. No positive case was observed in feline samples, even if a very limited number of cases were collected.

## CONCLUSION

Mycoplasma DNA was seldom detected from cases of abortion, stillbirth and neonatal mortality in dogs. The detection of Mycoplasma DNA in association with other main pathogens and its detection from the female genital tract in the absence of transmission to puppies support the hypothesis that Mycoplasma is an autochthonous genital microflora or can play a secondary role in the canine infertility.

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# Ovine herpesvirus 2 infection in sheep flocks of Umbria

**Raffaele Oriana[1], Del Rossi Emilia[1], Castelli Lorenzo[2], Stefano Pignani[2], Mandara Maria Teresa[1], Marenzoni Maria Luisa[1]**

[1]Department of Veterinary Medicine, University of Perugia
[2]Regional Breeder's Association of Umbria

## OBJECTIVE

Ovine herpesvirus-2 (OvHV-2) is a gammaherpesvirus that causes an asymptomatic infection in sheep and a severe systemic disease in other ruminants and pigs, called malignant catarrhal fever (FCM) [1,2]. The control of the infection in sheep is the key to avoid the FCM [3,4,5]. Moreover, a role of this virus in the pathology of the sheep can not be excluded [1]. Aim of the present study was to investigate the presence of OvHV-2 in sheep flocks of Umbria and understand the role of some characteristics of the farms for the infection.

## MATERIALS AND METHODS

Nasal swabs of 39 sheep and 11 goats coming from 9 different farms were investigated for the presence of the OvHV-2 DNA by nested PCR.

## RESULTS

Twenty-nine out of 39 (74.4%) sheep and 3 out of 11 (27.3%) goats were positive. The role of the species, the age of the animals, the productive attitude, the breed mixed with cattle and/or goats, and the effect of the single farm were evaluated as risk factors for the infection by univariable and multivariable statistical analysis. All these factors resulted significant by univariable analysis, whereas only the effect of the farm remained significant in the multivariable analysis.

## CONCLUSION

Probably the farm contains other variables that are able to explain variation in the prevalence of OvHV-2. Further factors of the farm will have to be investigated to understand the epidemiology of the infection.

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# PCR conducted on pooled milk samples collected from the older animal of dairy farms constitutes a sensitive early diagnostic indicator of bovine tuberculosis

**Antonia Mataragka[1*], Virginia Fytani[1*], Kyriaki Sotirakoglou[2], John Ikonomopoulos[1]**

[1]Department of Anatomy and Physiology of Farm Animals, Faculty of Animal Science and Aquaculture, Agricultural University of Athens, 75 Iera Odos, Athens, 11855, Greece
[2]Department of Plant Breeding and Biometry, Faculty of Crop Science, Agricultural University of Athens, 75 Iera Odos, Athens, 11855, Greece
*Equal contribution

## OBJECTIVES

The aim of this investigation was to assess whether the polymerase chain reaction (PCR) can be used as a diagnostic indicator of mycobacterial infection in bovine dairy farms that are routinely monitored with the tuberculin skin test (TST).

## MATERIALS AND METHODS

Samples of milk (n=536, 1 sample/animal, sample volume: 20ml) were collected from all animals older than 8 years of age in the four dairy farms (Farms A-D) located in a part of the Attika Prefecture of Athens that is considered enzootic for bovine tuberculosis. Information regarding year of establishment, number and age of the animals, and TST farm record was collected on site. The milk samples (n=78) were pooled and processed for DNA isolation and PCR for the detection of DNA belonging to slow growing members of the genus *Mycobacterium*, using two assays targeting *16S-rRNA* and *65-kDa heat shock protein*. Detection of PCR-inhibitors was conducted with a PCR assay targeting *β-actin*. The specificity of the PCR analysis was assessed through sequence analysis of randomly selected amplification products. DNA isolation and PCR testing were conducted in compliance with ISO17025 accreditation requirements.

## RESULTS

The overall percentage of positivity was 47.4%, and sequence analysis confirmed that all amplification products corresponded to slow growing mycobacteria at 100% alignment query cover and percentage of identity, using BLAST. Farms B and D that were TST-positive at the time of investigation, reacted strongly positive by PCR. Farm C that was TST-negative tested also negatively with PCR, whereas Farm A that was negative with TST since 2012 but had a long prior record of high level TST-positivity, tested positively.

## CONCLUSION

In conclusion it can be stated that PCR conducted on pooled samples of milk collected from the older animals of dairy farms can be used as an early and sensitive diagnostic indicator to improve detection of farms, in which TST-routine monitoring should be intensified [1].

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# Hydroxypyridinone-based iron-chelating co-polymer (DIBI) has antibacterial and antimycotic activity against pathogens associated with canine skin diseases.

**Francesca Paola Nocera[1], Maria Del Carmen Parquet[2], Anna De Filippis[1], Filomena Fiorito[1], Bruce E. Holbein[2], Luisa De Martino[1].**

[1]Department of Veterinary Medicine and Animal Production, Infectious Diseases Unit, University of Naples "Federico II", 80137 Naples (Italy);
[2]Chelation Partners Inc., 1411 Oxford St., Halifax, NS, B3H 3Z1 Canada.

## OBJECTIVES

The increasing of multi-resistance developed in most human and animal community can be attributed to the high use of antibiotics prescribed by medical staff or consumed by patients without a prescription. In any case, the more and more limited therapeutic options, both in human and veterinary medicine, underline the interest and the increase of studies on new alternative therapies. DIBI, a novel water-soluble hydroxypyridinone-containing iron chelating polymer, developed by Chelation Partners Inc. (Canada), provides a potential new antibacterial treatment by denying pathogens of iron as needed for their growth [1]. Herein, we tested DIBI against different strains isolated from dogs suffering from skin disorders as pyoderma or otitis externa.

## MATERIALS AND METHODS

Bacterial isolation and MALDI-TOF-MS identification of pathogens associated with canine skin diseases was performed. The antibiotic resistance profiles were evaluated by disk diffusion method. Then MIC susceptibilities to DIBI were evaluated using the broth microdilution method in 96-well round-bottomed plates against eighteen microbial strains.

## RESULTS

DIBI activity was found to be strongly inhibitory to selected gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus pseudintermedius*,) and two *Malassezia pachydermatis* strains, while resulted to be moderately inhibitory to the selected gram-negative bacteria (*Pseudomonas aeruginosa* and *Proteus mirabilis*). Precisely, gram-positive bacteria and mycetes displayed a DIBI MIC range lower to 4 µg/mL. *Pseudomonas aeruginosa* and *Proteus mirabilis* showed a wider spectrum of sensitivity with values lower to 128 µg/mL.

## CONCLUSIONS

Widespread emergence of multidrug-resistant bacterial pathogens represents an important problem to animal health and an increasing therapeutic challenge in veterinary medicine. Thus, new alternative approaches are necessary, and this study demonstrated that DIBI represents a promising non-antibiotic alternative therapy against different microbial agents involving in cutaneous infectious diseases of companion animals.

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# First case of *Serratia liquefaciens* isolated from urinary tract infection in sows

**Vasileios G. Papatsiros[1], Labrini V. Athanasiou[1], Victoria Marina Spanou[1], Georgios Papakonstantinou[1], Michalis Letsios[2], Charikleia Styliani Villioti[1], Nikolaos Tsekouras[1], George Maragkakis[1], Georgios Christodoulopoulos[1]**

[1]Clinic of Medicine, Faculty of Veterinary Medicine, School of Health Sciences, University of Thessaly, Karditsa, 43100, Greece
[2]Swine Veterinarian Mitsopoulos Farm S.A, Kalentzi Corinth, 20001, Greece

## OBJECTIVE

To present the first report of *Serratia liquefaciens*is in a commercial pig farm. This pathogen is one of the most important causes of hospital-acquired human infections, it has been rarely reported in animals mainly in companion animals and in dairy herds, although it is found in animal products such as dairy milk and pork meat.

## MATERIAL, METHODS & RESULTS

An incident of sudden deaths in the breeding stock was reported from a farrow-to-finish commercial pig farm in Greece. The 8.4% of sows during lactation and gestation period presented anorexia, fever, hematuria, return-to-oestrus, and sudden deaths (mortality rate: 2.3%). Blood and urine samples were collected from 4 diseased sows. Furthermore, swabs from urine bladders were collected from 2 dead sows and 4 culled sows at the slaughterhouse. Blood testing revealed mild leukocytosis and absence of azotemia. Urinalysis revealed hematuria, proteinuria, bilirubinuria and active urine sediment with bacteria, mainly bacilli, numerous epithelial and leukocytes, calcium oxalate and bilirubin crystals, and granular casts. The bacterial culture revealed the presence of S. *liquefaciens*. The antibiotic susceptibility testing showed high resistance to the most common antibiotics, with the highest sensitivity of the isolate towards enrofloxacin. After the administration of a single dose of 0.075mg/Kgr enrofloxacin intramuscularly, the mortality rate decreased to less than 0.5% along with a remarkable reduction in the severity of clinical signs.

## CONCLUSION

Based on our findings, S. *liquefaciens* induced severe clinical signs and deaths in sows, mainly due to urinary infection. Inadequate water sanitation might have been responsible for increased exposure to S. *liquefaciens*.

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# Correlation of *TMEM154* gene polymorphisms with susceptibility against small ruminant lentiviral infections in sheep of Chios breed

**Pappas F.[1,2], Boukouvala E.[1], Papadopoulos A.[2], Ekateriniadou L.[1], Gelasakis A.[3], Bouzalas I.[1]**

[1]HAO-DEMETER, Veterinary Research Institute, Campus of Thermi, Thessaloniki, Greece
[2]Laboratory of Animal Physiology, Department of Zoology, School of Biology, Faculty of Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece
[3]Department of Animal Science and Aquaculture, Agricultural University of Athens, Athens, Greece

## OBJECTIVE

Small ruminant lentiviruses (SRLVs) are retroviruses that infect sheep and goats, causing chronic incurable disease with long incubation period and diverse clinical manifestations including pneumonia, encephalomyelitis, arthritis and mastitis. Genome-wide association studies conducted in the U.S.A. revealed molecular markers related to reduced susceptibility of sheep of different breeds to these viruses, with the most significant one being a single nucleotide polymorphism (SNP) at the 35th codon (E to K) of the transmembrane protein TMEM154 [1]. Since then, these markers have been evaluated in more breeds worldwide. In this study, we evaluated the association of the E35K polymorphism with the serological status against SRLVs in Chios breed dairy sheep.

## MATERIALS AND METHODS

Sheep (N=280) from 3 different herds, older than 2 years of age, were tested for their serological status and genotyped for the locus of interest using an indirect ELISA and a Real-Time PCR method (Taqman), respectively. Chi-squared test was used to assess allele frequency differences between seropositive and seronegative animals.

## RESULTS

The total seroprevalence in the studied farms appears to be 32.1% while the relative frequencies of the genotypes were 88.04% for EE, 11.96% for EK, and 0% for KK. Interestingly, the serological status was significantly correlated (P=0.02861) with allelic variants revealing that animals carrying the K allele (the putative less susceptible allele) had lower relative risk of infection.

## CONCLUSION

Our results indicated that K allele seems to be a potential marker for improving resistance against SRLVs in Chios breed through marker-assisted selective breeding. However, factors such as frequencies of allelic variants, seroprevalence and infectious viral strain are involved in the epidemiology of the disease and thus more extensive studies are required in order to evaluate the protective role of K35 allele.

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# Exploratory Analysis of Zoonotic Agents and Biosecurity Level in sheep and goat flocks in Decisions Making

**Evanthia Petridou[1], Nektarios Giadinis[2], Odysseas Moschidis[3], Spiridon Kritas[1].**

[1]Laboratory of Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
[2]Clinic of Farm Animals, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
[3]Department of Business Administration, University of Macedonia, Greece

## OBJECTIVE

Sheep and goat farming is a growing sector of the economy for Greece. However, livestock management always carries risks associated with the emergence of zoonotic agents in farming. The aim of the study was to investigate the relationship between the occurrence of zoonotic agents in sheep and goat rearing and the biosecurity level of rearing in administrative decisions making.

## MATERIALS AND METHODS

For the needs of the survey, 46 sheep, goat or mixed sheep herds were selected originated from different Regional Units of the country. A questionnaire of 89 questions was constructed. All questions were closed-ended with a single answer. The first four questions concerned the location of the flocks. There were demographic-type questions concerning the manager of the rearing, and there were separate questions about the collection of administrative data on the rearing and data for determining the size and type of rearing. Moreover, informations were collected on the level of rearing biosecurity, the vaccine program, information on the legislation in force, and how to make administrative decisions while additonal informations on how to manage rearing, the most serious health problems faced by rearing, the protocol used in breast hygiene as well as nutrition information were collected. Data were analyzed using the Correspondance Analysis method and the CAH. For this purpose, the M.A.D software program was used.

## RESULTS AND CONCLUSION

The analysis showed a correlation between absence in management and decision-making of individuals seeking cooperation with scientists while at the same time do not wish to be informed about the legislation in force by the relevant competent authorities, they have no interest in attending training seminars nor adopting good practices while lack of biosecurity in the above flocks was observed. In conclusion, the State should educate human resources for the benefit of the nation's primary production.

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ECDC: European Centre for Disease Prevention and Control. An agency of the European Union: https://ecdc.europa.eu/en/home

EFSA : European Food Safety Authority: https://www.efsa.europa.eu

EUROSTAT: http://ec.europa.eu/eurostat

FAO: Food and Agriculture Organization of the United Nations: http://www.fao.org/home/en/

OIE: Office International des Epizooties: World Organization for Animal Health: http://www.oie.int

WHO: World Health Organization: http://www.who.int/en/

# Antibiotic resistance in *Staphylococcus pseudintermedius* causing skin and soft tissue infections in pets

**Catarina Morais[1], Sofia Santos Costa[1], Constança Pomba[2], Isabel Couto[1]**

[1]Global Health and Tropical Medicine, GHTM, Unit of Medical Microbiology, IHMT/UNL, Lisbon, Portugal
[2]Laboratory of Antimicrobial and Biocide Resistance, CIISA, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal

## OBJECTIVES

The emergence of antimicrobial resistance (AMR) in staphylococci of animal origin is a public health concern. In this work we characterized *S. pseudintermedius* causing skin and soft tissue infections (SSTIs) in pets to document the AMR burden in this group of companion animals.

## MATERIALS AND METHODS

A collection of 163 *S. pseudintermedius* isolates associated with SSTIs in dogs and cats was collected between 2014 and 2018 at two laboratories in Lisbon, Portugal. Identification was confirmed by amplification of the *spsJ* gene and the antibiotic susceptibility profile determined by Kirby-Bauer, interpreted according to the VET08 CLSI recommendations (2018) or alternative guidelines when necessary. All isolates were screened for the *blaZ* and *mecA* genes, whereas other resistance determinants, such as *tet* and *erm genes*, were tested only for those showing a resistance phenotype.

## RESULTS

Among the 163 *S. pseudintermedius* isolates studied, 135 (85.3 %) were resistant to penicillin and 133 carried the *blaZ* gene, whereas 55 (33.7 %) were methicillin resistant (*mecA*$^+$, MRSP) and 76 (46.6 %) presented a multidrug resistance (MDR) phenotype, which included resistance to penicillin, macrolides, lincosamides and tetracyclines. We also detected resistance to aminoglycosides (36.8%), trimethoprim-sulfamethoxazole (29.4%), fluoroquinolones (25.2%), chloramphenicol (11.7%) and fusidic acid (4.9%). No resistance was observed for linezolid, novobiocin or quinupristin-dalfopristin. Resistance was associated with different combinations of determinants, which up to now include *tetM*, *tetK*, *tetL*, *ermC* and *ermB*.

## CONCLUSIONS

This undergoing study revealed an increasing trend of antibiotic-resistant *S. pseudintermedius* associated with SSTIs in pets [1], particularly an elevated frequency of MDR. The close contact of these animals with humans may be a possible source of transmission of antibiotic-resistant staphylococci. These results also highlight relevant therapeutic limitations for the treatment of SSTIs in pets which already include critical important antimicrobials.

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## ACKNOWLEDGEMENTS

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# Detection of a High-Risk human ST410 *Escherichia coli* clone producing OXA-181 from a dog in Portugal

**Constança Pomba[1], Michael Brilhante[2], Juliana Menezes[1], Alexandra Collaud[2], Vincent Perreten[2]**

[1]CIISA - Centre of Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Portugal, Av. da Universidade Técnica, 1300-477 Lisbon, Portugal
[2]University of Bern, Institute of Veterinary Bacteriology, Bern, Switzerland

## OBJECTIVE

This study aimed to describe a case report from a dog colonized by a carbapenemase-producing (CP) *Escherichia coli* from Portugal.

## MATERIALS AND METHODS

A 7-year-old female dog was attended at a reference University Veterinary Teaching Hospital (UVTH). Prior to admission, the dog had several courses of antimicrobial treatment. At the UVTH, the dog was diagnosed with a methicillin-resistant *Staphylococcus pseudintermedius* skin infection and treated with minocycline BID for 21 days. To evaluate the colonization of the infected dog and of the human household members by CP, fecal samples were collected. Samples were plated onto MacConkey agar plates supplemented with an antibiotic discs containing meropenem (10µg), temocillin (30µg) and CAT-ID™ (mastdiscs™ ID for screening of CPE). Whole genome sequence of *E. coli* strain PT109 was obtained using both Illumina MiSeq platform and Oxford Nanopore MinION.

## RESULT

Fecal samples from the dog at UVTH admission were positive for an *E. coli* ST410 producing an OXA-181 carbapenemase but not for the two humans living in the same household. One month after the antimicrobial treatment the dog was still colonized, but not after two months. The $bla_{\text{OXA-181}}$ was located together with a *qnrS1* gene on a 51,479-bp IncX3 plasmid pPT109-OXA-181.

## CONCLUSION

To our best knowledge, this is the first report of a dog colonized with the new international high-risk ST410 *E. coli* clone [1] harboring the $bla_{\text{OXA-181}}$ gene in an IncX3 plasmid, similarly to what was described in China in humans [2]. IncX3 plasmids are also a common vehicle for spreading NDM-type carbapenemases in Enterobacteriaceae in China [3].

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## ACKNOWLEDGEMENTS

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# Detection of multidrug-resistant bacteremia in dogs and cats associated with sepsis, Portugal

**Constança Pomba[1,2], Cláudia Santos[2], Catarina Aboim[1], Laura Fernandes[2], Ana Catarina Rodrigues[2]**

[1]CIISA - Centre of Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Portugal, Av. da Universidade Tecnica, 1300-477 Lisbon, Portugal
[2]GeneVet, Veterinary Molecular Diagnostic Laboratory, Lisbon, Portugal

## OBJECTIVE

The aim of this study was the early detection of bacteraemia, namely multidrug-resistant bacteria (MDR) in the dog and cat associated with sepsis.

## MATERIAL AND METHODS

Between January 2016 and July 2019, a total of 81 samples from dogs (*n*=64) and cats (*n*=17) were submitted for blood culture from several veterinary hospitals and clinics to the Genevet Laboratory. The samples were collected according to the proper asepsis rules (trichotomy and disinfection of the venipuncture site and bottle extremity) and were received in paediatric aerobic blood culture bottles. The detection of bacteraemia was performed as depicted in Figure 1. After bacteria isolation, susceptibility testing was performed by the disc diffusion method and/or minimum inhibitory concentrations. CLSI VET08 clinical breakpoints were applied.

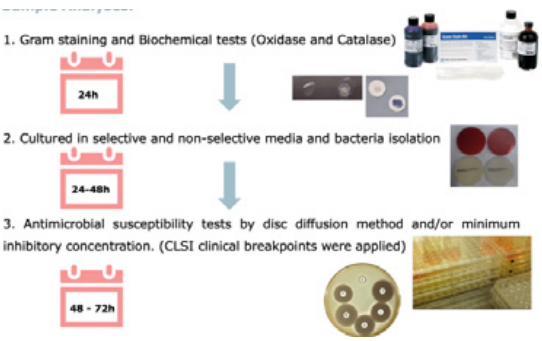
## RESULTS

From the 81 blood cultures received, 43% (35/81) were positive. Diverse bacterial agents were isolated, mainly *Staphyloccoccus* spp. (13/41, 32%), *Enterobacter* spp. (6/41, 15%), *Pseudomonas* spp. (6/41, 15%), *Klebsiella* spp. (5/41, 12%), *Serratia marcescens* (5/41, 12%), among others. The most frequent resistance was to amoxicillin/clavulanic acid (27/40, 68%), tetracycline (23/40, 58%), and trimethoprim/sulfamethoxazole (20/40, 50%). It is important to emphasize the high-number (26/41, 63%) of MDR isolates (resistant to more than 3 different antimicrobial classes). It should also be noted the isolation of two methicillin-resistant *Staphyloccoccus aureus* (MRSA) and two methicillin-resistant *Staphyloccoccus pseudintermedius* (MRSP) and extended-spectrum beta-lactamases producer Enterobacteriaceae (ESBL) resistant to third generation cephalosporins (8/19, 42%).

## CONCLUSION

In this study almost half of the blood cultures was positive. The detection of MDR, MRSA, MRSP and ESBL-producer Enterobacteriaceae is a cause for great concern, with inherent therapeutic limitations. This study illustrates that blood cultures are a key tool for the early diagnosis of sepsis as well as oriented antimicrobial therapy.

Figure 1. Flowchart analysis of blood culture



# Detection of KPC-3-producing Enterobacteriaceae isolates in an animal zoo, Portugal

**Saavedra, M.J.[1], Menezes, J.[2], Martins, J.[3],** *Poirel, L.*[4]**, Duarte A.[5], Pomba, C.[2]**

[1]Department of Veterinary Sciences, School of Agrarian and Veterinary Sciences and CITAB-Centre for the Research and Technology Agro-Environmental and Biological Sciences, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal
[2]CIISA - Centre of Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Portugal
[3]Animal Science Department, School of Agrarian and Veterinary Sciences and CECAV- Animal and Veterinary Research Centre, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal
[4]Medical and Molecular Microbiology Unit, Faculty of Science and Medicine, University of Fribourg
[5]Department of Microbiology and Immunology, Faculty of Pharmacy, University of Lisbon, Portugal

## OBJECTIVE

The spread of carbapenemase-producing Enterobacteriaceae (CPE) is a great problem of healthcare worldwide. *Klebsiella pneumoniae* carbapenemase (KPC)-producing bacteria are a group of emerging highly drug-resistant with significant morbidity and mortality. KPC is mainly identified in *K. pneumoniae* but has also been identified in other Gram-negative species. It is mainly a nosocomial problem, but several studies showed that this carbapenemase might also been found in the environment. The aim of this study was investigate the presence of CPE in captive black-and-white ruffed healthy lemurs (*Varecia variegata*).

## MATERIALS AND METHODS

Rectal swabs were collected from 9 lemurs from a zoo in the Oporto region, Portugal. Positive carbapenem-resistant isolates were plated on MacConkey agar plates supplemented with meropenem (1 mg/L). Isolates were identified by 16S rRNA sequencing. Antimicrobial susceptibility testing was performed by microdilution methods. Carbapenemase resistance genes were characterized by PCR.

## RESULTS

A single *E. coli* and two *K. pneumoniae* isolates were recovered from two heathy lemurs, respectively. Those isolates were resistant to ampicillin, amoxicillin/clavulanic acid, cefotaxime, ceftazidime / clavulanic acid, ertapenem, imipenem, and piperacillin/tazobactam. They were also resistant to sulfamethoxazole /trimethoprim. One *K. pneumoniae* isolate was additionally resistant to ciprofloxacin, levofloxacin, norfloxacin, tetracycline and colistin (MIC at 4µg/mL). PCR and sequencing identified the KPC-3 carbapenemase encoding gene in all three isolates.

## CONCLUSIONS

This study reports for the first time the occurrence of KPC-3-producing bacteria in captivity animals in Portugal. Those animals may represent an underestimated reservoir of carbapenemase genes. Epidemiological surveys are further needed to understand the process of acquisition of such threatening resistance determinants in captive animals.

## ACKNOWLEDGEMENTS

With financial support of CITAB and FCT through Project UID/AGR/04033/2019, CIISA and FCT through Project UID/CVT/00276/2019 and PET-Risk Consortium Project JPIAMR/0002/2016.

# Prevalence and Molecular Characterization of Class 1 Integrons in Gram Negative Bacteria Recovered from Domestic, Wild and Food-Producing Animals

**Domingues S.S.[1,2] Dias C.[3,4], da Silva G.J.[1,2], Saavedra M.J.[3,4,5]**

[1]Microbiology Lab., Faculty of Pharmacy, University of Coimbra Portugal
[2]Center of Pharmaceutical Sciences, Faculty of Pharmacy, University of Coimbra, Portugal
[3]Medical Microbiology Lab., Dep. of Veterinary Sciences, School of Agrarian and Veterinary Sciences, University of Trás-os-Montes and Alto Douro 5001-801 Vila Real, Portugal
[4]Centre for the Research and Technology of Agro-Environmental and Biological Sciences-CITAB
[5] Centre for Animal and Veterinary Science-CECAV, University of Trás-os-Montes and Alto Douro 5001-801 Vila Real, Portugal

## OBJECTIVE

Integrons can be considered one of the major genetic carriers of and vectors for dissemination of antibiotic resistance determinants in bacteria. Their distribution pattern suggests dissemination through horizontal gene transfer. Class 1 integrons have been strongly associated with the ongoing spread of antimicrobial resistance genes and we have screened the presence of class 1 integrons in Gram-negative bacteria collected from domestic, wild and food-producing animals.

## MATERIALS AND METHODS

Seventy-four bacterial samples were isolated from animal of different origin: caecum of rabbits; faeces of cats, dogs, deer, eagle, emus, fox, owls, snake, chickens and rabbit; intestine of squirrels, chickens and rabbits; skin of gilthead breams. The bacterial species were grown in selective culture media and identified by API systems (BioMérieux) or by sequence of the 16S rDNA [1] or gyrB [2] genes. Intermediate or resistant strains to co-trimoxazole (SXT) were screened by PCR for class 1 integrase (IntI1) gene presence [3]. Positive isolates for IntI1 were selected for amplification [4]. Gene cassette arrays were identified by genomic sequencing.

## RESULTS

57% of the bacterial isolates studied were intermediate or resitant to SXT. Of these, 67% were positive for Intl1 and 37% for class 1 integrons. The results show that the same integron is present in different bacterial species suggesting the horizontal genetic transfer of the whole structure.

## CONCLUSIONS

The analysed bacterial isolates were detected in animals that inhabit in diverse environments, highlighting that class 1 integrons are well disseminated among domestic, wild and food-producing animal isolates, acting as integron-borne resistance gene reservoirs. These results suggest serious implications for human and animal health, not only because integrons can end up in food products but also because manure is often used as a fertilizer and sewage easily contact with water streams, with consequent contamination of soil and water, fruits and vegetables, and wildlife.

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# Changes in host gene expression following CAstV infection of chickens

**Joanna Sajewicz-Krukowska[1], Karolina Tarasiuk[1], Katarzyna Domańska-Blicharz[1]**

[1]Department of Poultry Diseases, National Veterinary Research Institute, Pulawy, Poland

## OBJECTIVE

Astroviruses are known to cause enteritis not only in humans but also in various animal species [1]. In poultry they have caused enteritis combined with growth depression and higher mortality but their presence was also described in healthy flocks [2-7]. Chicken astrovirus (CAstV) was recently indicated as the factor of the "white chicks" condition associated not only with increased embryo/chick mortality but also with weakness and white plumage of hatched chicks [7-9]. The aim of our study was to detect transcriptome expression changes during infection with CAstV in chicken spleens.

## MATERIALS AND METHODS

RNA-seq was used to determine the expression levels of mRNA transcripts from chicken spleens after infection with CAstV at 4 dpi. Differentially expressed genes (DEGs) were functionally classified and clustered into pathways using GO and KEGG analyses.

## RESULTS

In total, RNA-seq based analysis identified 204 genes in chicken spleens that were differentially expressed (p-value<0.05) between the infected and uninfected groups (152 upregulated, 52 downregulated). The annotated DEGs were classified into biological processes, cellular components, and molecular functions. GO analysis indicated that enriched terms mainly included cell cycle, cellular process, immune system proces, response to stimulus, biological regulation, cellular component organization, signal transduction and developmental process. KEGG analysis revealed enriched NOD-like receptor signaling pathway, influenza A and cell cycle. The key DEGs in these pathways included JUN, BIRC3, GBP1 and STAT1. These genes play an important role in the regulation of viral transcription, signal transduction pathways, apoptotic proces, ubiquitination and regulation of defense response to virus by host.

## CONCLUSION

To our knowledge, this is the first study to use high-throughput sequencing methods to investigate CAstV infection in chicken spleens. The results of the present study will assist in the understanding of the molecular pathogenesis of CAstV infection.

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# *Bacillus* probiotics as an alternative to antibiotics: fighting growth and biofilms of fish bacterial pathogens

**Rafaela A. Santos[1, 2, 3, 4], Aires Oliva-Teles[1, 2], Maria J. Saavedra[1, 3, 4, 5], Paula Enes[1,2], Claudia R. Serra[1]**

[1]CIIMAR - Centro Interdisciplinar de Investigacao Marinha e Ambiental, Terminal de Cruzeiros do Porto de Leixoes, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal.
[2]Departamento de Biologia, Faculdade de Ciencias, Universidade do Porto, Rua do Campo Alegre s/n, Ed. FC4, 4169-007, Porto, Portugal.
[3]CITAB - Centro de Investigacao e Tecnologias Agroambientais e Biologicas, Universidade de Tras-os-Montes e Alto Douro, Quinta de Prados, 5000-801 Vila Real, Portugal.
[4]CECAV – Centro de Ciencia Animal e Veterinaria, Universidade de Tras-os-Montes e Alto Douro, P.O. Box 1013, 5001-801, Vila Real, Portugal.
[5]Departamento de Ciencias Veterinarias, ECAV, Universidade de Tras-os-Montes e Alto Douro, Quinta de Prados, 5000-801, Vila Real, Portugal.

Bacterial diseases outbreaks are a major constraint in aquaculture, an industry responsible for more than 50% of global seafood production. Diseases emergence are associated with antibiotics misuse, posing serious threats to public health. Thus, to improve human, animal, and environmental health(*One Health* approach) it is urgent to find alternatives to antibiotics. One promising strategy is the use of probiotics. *Bacillus* spp. have been recognized as attractive probiotics for aquaculture due to their endospore-forming nature and their production of Natural Antimicrobial Compounds(NACs) antagonistic of bacterial pathogens. Harnessing the fish-gut microbial potential, we aimed to isolate and characterize *Bacillus* spp. from the gut of aquaculture fish capable of producing NACs antagonistic of fish bacterial diseases.

Heat-treated intestinal contents of *Sparus aurata*, *Diplodus sargus*, and *Dicentrarchus labrax* were used to obtain the gut sporeforming community. Isolates were screened for anti-growth and anti-biofilm activities. Significance of inhibition was evaluated by repeated-measures ANOVA or one-way ANOVA. A total of 172 sporeformers representing different colony morphologies and samples were selected. From these, 52% displayed antimicrobial activity against at least one of the pathogens tested, including *Aeromonas salmonicida*, A. *hydrophila*, A. *veronii*, A. *bivalvium*, *Vibrio anguillarum*, V. *parahaemolyticus*, V. *vulnificus*, and V. *harveyi*. By characterizing the localization of the inhibitory molecules, the cell-free supernatants of 3 isolates (identified as B. *subtilis* by 16S rRNA sequencing), significantly ($p<0.05$) inhibited the growth and biofilm formation of the pathogens tested. These strains are being further studied to be used as future probiotics or source of bioactive molecules as tools to prevent aquaculture fish diseases.

## ACKNOWLEDGEMENTS

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# Extended Spectrum β-Lactamase and AmpC-producing *Enterobacteriaceae* (ESBL/AmpC-E) shedding in petting zoos: A zoonotic hazard?

**Anat Shnaiderman-Torban[1], Amir Steinman[1]*, Gal Meidan[1], Yossi Paitan[2,3], Wissam Abu Ahmad[1], Shiri Navon-Venezia[4]***

[1]Koret School of Veterinary Medicine (KSVM), The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel
[2]Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Israel
[3]Clinical Microbiology Lab, Meir Medical Center, Israel
[4]Department of Molecular Biology, Faculty of Natural Science, Ariel University, Ariel, Israel
*equal contribution

## OBJECTIVES

To investigate the prevalence, molecular epidemiology and risk factors for ESBL/AmpC-E shedding in petting zoos animals, since this population is in close contact with children and may transfer zoonosis.

## MATERIALS AND METHODS

A prospective cross-sectional study was performed in eight petting zoos. Shedding was determined in two body sites: (i) gut (feces from 228 animals) and (ii) fur/skin/feathers (60% of animals). Enriched samples were plated and sub-cultured (CHROMagarESBL plates). ESBL/AmpC production was determined according to EUCAST. Bacterial identification, antibiotic susceptibility profiles and sequence types were determined (Vitek-2 and MLST). Genes (CTX-M, SHV, TEM, CMY-2) were identified via PCR and sequencing. Owners' questioners were reviewed for risk factor analysis (SPSS).

## RESULTS

Shedding rate was 12% (n=28/228), with 35 recovered bacteria; 77% from feces and 23% from skin/fur/feathers. Isolated bacteria included *Enterobacter cloacae* (55%), *Escherichia coli* (31%), and *Citrobacter freundii* (14%). ESBL genes included CTX-M-1 group (17%), SHV-2 (9%), CTX-M-9 group, SHV-31 and SHV-12 (4% each), and 20% of the AmpC-E were CMY-2-positive. Eight *E. cloacae* sequence-types were identified: ST750, ST350, ST557, ST170, ST102, ST112, ST182 and ST511. Six *E.coli* STs were identified: ST656 (enterotoxigenic), ST648, ST127 (uropathogenic), ST4981, ST2521 and ST224. Shedding was associated with antibiotic therapy (p=0.038 and p=0.011 for gut and skin/fur/feathers, respectively) and with petting permission policy (p=0.023). In a logistic regression model, antimicrobial therapy was identified as a risk factor (OR=7.34).

## CONCLUSIONS

Our findings demonstrates the diverse and alarming potential reservoir of ESBL/AmpC-E in petting zoos.

## ACKNOWLEDGEMENT

To petting zoo owners, employees, and veterinarians, and to Kira Kondratyeva for graphic assistance.

# Extended-spectrum β-lactamase-producing *Enterobacteriaceae* in hospitalized neonatal foals: Prevalence, risk factors for shedding and association with infection

**Anat Shnaiderman-Torban[1], Yossi Paitan[2,3], Haia Arielly[3], Kira Kondratyeva[4], Sharon Tirosh-Levy[1], Gila Abells Sutton[1], Shiri Navon-Venezia[4*], Amir Steinman[1*]**

[1]Koret School of Veterinary Medicine (KSVM), The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel
[2]Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Israel
[3]Clinical Microbiology Lab, Meir Medical Center, Israel
[4]Department of Molecular Biology, Faculty of Natural Science, Ariel University, Ariel, Israel
*Equal contribution

## OBJECTIVES

To determine prevalence and risk factors for ESBL-E shedding and infection in neonatal foals and mares.

## MATERIALS AND METHODS

Pairs of mares and their foals were sampled on admission and on the third day of hospitalization. Rectal swabs and clinical samples were collected, enriched, plated onto Chromagar ESBL plates and bacteria were verified for ESBL production. Species identification and antibiotic susceptibility profiles were determined (Vitek2). For pathogenic bacteria were genotyped by MLST. ESBL genes were identified by PCR and sequencing (CTX-M, OXA-1, OXA2, OXA10, TEM and SHV). Medical data was analyzed for risk factors (SPSS).

## RESULTS

On admission, 55 pairs were sampled, of which 33 pairs were re-sampled. Shedding rate on admission in foals and mares were 33% (95% CI 21-47%) and 16% (95% CI 8-29%), respectively, and during hospitalization increased significantly to 85% (95% CI 70-94%) and 58% (95% CI 40-73%), respectively. Foals' shedding was associated with umbilical infection on admission (P=0.013) and with ampicillin treatment during hospitalization (P=0.006). Foal shedding was independent of its mare's shedding status. Overall, 127 ESBL-E bacterial isolates were analyzed. The major fecal bacterial species on admission was *E. coli*. During hospitalization, the diversity of ESBL-E species increased in both populations. The main *bla*ESBL gene group was CTX-M-1. A total of 24 clinical samples were collected from 18 foals. Four foals were infected with an ESBL-E strain, including infected umbilici and wounds. Pathogenic strains included *E. coli*, *K. pneumoniae* and *S. enterica*, sharing identical sequence types infecting different foals. Infected foals also shed ESBL-E, on admission and/ or during hospitalization.

## CONCLUSIONS

This study substantiates an alarming prevalence of ESBL-E shedding in hospitalized neonatal foals. These findings should be further investigated in order to reduce infections and resistance rates.

## ACKNOWLEDGEMENTS

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# Prevalence of *S. aureus* in nostrils and buccal mucosa of camels from Gran Canaria Island

**Vanessa Silva[1-4], Margarita González-Martin[5], Juan Alberto Corbera[5], María Teresa Tejedor-Junco[5], Gilberto Igrejas[2-4], Patrícia Poeta[1,4]**

[1]Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[2]Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal
[3]Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[4]Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Lisboa, Caparica, Portugal
[5]Research Institute of Biomedical and Health Sciences, University of Las Palmas de Gran Canaria, Spain.

The aim of this study was to investigate the prevalence of *Staphylococcus aureus* in camels from Gran Canaria island. Samples were collected from nostrils and buccal mucosa of 32 camels. The swabs were inoculated into BHI (broth containing 6.5% NaCl and incubated for 24 h at37°C. The inoculum was seeded onto Mannitol Salt agar and Baird-Parker agar plates. One presumptive S. *aureus* colony was recovered from each plate and confirmed by Gram staining, coagulase, DNase and catalase tests. Sixteen (50%) S. *aureus* were isolated from camels. A higher number of isolates were recovered from oral samples (n=9) than from nasal samples (n=7). None of the animals were positive for both nasal and oral samples. A high rate of S. *aureus* **was found in samples of camels. Therefore, these animals may act as a reservoir of** S. *aureus* which can carry many antimicrobial resistance determinants that could be a risk for humans in contact with camels, in particular, tourists that visit the island and go on camel rides. Further studies, including the antimicrobial resistance, virulence and genetic lineages will be carried out.

## ACKNOWLEDGEMENTS

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# Antimicrobial resistance profile of *Staphylococcus aureus* isolated from Maronesa cattle

**Vanessa Silva[1-4], Susana Correia[1-4], Juan García-Díez[5], Paula Teixeira[6], Kevin Pimenta[1-4], María Teresa Tejedor-Junco[7], Gilberto Igrejas[2-4], Patrícia Poeta[1,4]**

[1]Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[2]Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal
[3]Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[4]Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Lisboa, Caparica, Portugal
[5]Animal and Veterinary Research Centre (CECAV), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[6]Associação de Criadores do Maronês (ACM), Cooperativa Agricola de Vila Real, Vila Real, Portugal
[7]Research Institute of Biomedical and Health Sciences, University of Las Palmas de Gran Canaria, Canary Islands, Spain

The objectives of this study were to isolate *Staphylococcus aureus* from Maronesa cattle and to investigate their antimicrobial resistance phenotypes. Oral and nasal swabs were collected from 195 healthy Maronesa cows. Samples were recovered in 12 different farms located near the hospital centre in Vila Real city in the North of Portugal. Swabs were incubated into Brain Heart Infusion broth tubes containing 6.5% of NaCl and incubated at 37°C for 24h. The inoculum was seeded onto Mannitol Salt agar and Baird Parker agar plates. Presumptive S. aureus colonies were recovered and confirmed by biochemical tests. The susceptibility of the isolates was tested by the Kirby-Bauer disc diffusion method against 14 antimicrobial agents and according to EUCAST (2018) standards with the exception of kanamycin that followed the CLSI guidelines (2017). Twenty-nine (14,9%) S. *aureus* were isolated from Maronesa cows. More than half of S. *aureus* (n=16) isolates showed sensibility to all antibiotics tested. Resistance to penicillin (n=8), tetracycline (n=5), gentamicin (n=3), tobramycin (n=3), kanamycin (n=2), chloramphenicol (n=1) and fusidic acid (n=1) was detected among the isolates. A moderate rate of S. *aureus* **carriage was detected in healthy Maronesa cattle showing low rates of antibiotic resistance which value this important autochthonous breed.**

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# Detection of ST2855-MRSA-III in wild hares (*Lepus granatensis*) from Portugal

**Vanessa Silva[1-4], Vera Manageiro[5,6], Eugénia Ferreira[5,6], Manuela Caniça[5,6], José Eduardo Pereira[1], Gilberto Igrejas[2-4], Patrícia Poeta[1,4]**

[1]Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[2]Department of Genetics and Biotechnology, Functional Genomics and Proteomics' Unit, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal
[3]Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[4]Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Lisboa, Caparica, Portugal
[5]National Reference Laboratory of Antibiotic Resistances and Healthcare Associated Infections (NRL-AMR/HAI), Department of Infectious Diseases, National Institute of Health Dr Ricardo Jorge, Av. Padre Cruz, 1649-016, Lisbon, Portugal
[6]Centre for the Studies of Animal Science, Institute of Agrarian and Agri-Food Sciences and Technologies, Oporto University, Oporto, Portugal

The present study was undertaken to investigate the occurrence of MRSA strains in wild Iberian hares, as well as, its antimicrobial resistance profile, virulence factors and genetic lineages. Eighty-three wild hares (*Lepus granatensis*) were captured in the north of Portugal. Samples were collected from nostrils, buccal mucosa and perianal skin using only one swab per animal. Isolation of MRSA was accomplish using ORSAB medium with 2mg/L of oxacillin. The susceptibility of the isolates was tested by the Kirby-Bauer disc diffusion method against 14 antimicrobial agents. The presence of resistance genes and virulence factors was studied by PCR. Isolates were characterized by MLST, *agr*, SCC*mec* and *spa* typing. From the 83 samples, 3 (3,6%) MRSA strains were isolated. All MRSA strains showed resistance to penicillin, cefoxitin and erythromycin. One MRSA isolate also showed resistance to gentamycin exhibiting a multidrug-resistant profile. All MRSA strains were ascribed to ST2855, t1190 and SCC*mec* type III. MRSA carriage by wild hares may be explained by the uptake of these strains or resistance determinants from the natural environment since these animals are not in direct contact with antibiotics.

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# Pathogens in Animal Effluents and Their Different Capacity of Survival

**Ana Sofia Soares[1], Carla Miranda[1], Carlos Afonso Teixeira[1], Henrique Trindade[1], Ana Claudia Coelho[2]**

[1]Centre for Research and Technology of Agro-Environmental and Biological Sciences (CITAB), Universidade de Tras-os-Montes e Alto Douro, Portugal
[2]Animal and Veterinary Research Centre (CECAV), Universidade de Tras-os-Montes e Alto Douro, Quinta de Prados, Vila Real, Portugal

The intestinal microbiota of animals and humans is constituted by a multiple set of bacteria. Most of these microorganisms are beneficial to intestinal motility. The *Enterococcus* species have long been known as commensals of the gastrointestinal tract and as one of the present bacteria in the environment. For many years, *Enterococcus* species were believed to be harmless to humans and unimportant medically. Although these bacteria do not produce toxins, have virulence factors in the form of aggregation substances, so they can cause disease based in the way they adhere to the host tissues. Based on this virulence factors production and their high antibiotic resistance *Enterococcus* are now considered emerging pathogens. The animal manures including poultry, cattle, and swine are the primary source of water resources contamination by water-borne pathogens, as well as *Escherichia coli* or *Salmonella* sp. Salmonellosis is considered the most widespread zoonosis in the world. As the transmission cycle of *Salmonella* involves practically all vertebrates, their control poses a challenge to public health in both developed and developing countries. As this infection have long periods of latency, it's possible that occurs release of bacterial cells from feces of the infected animals, contaminating environment, food and water.

The aim of the study was to evaluate the survival capacity of *Salmonella* sp. (presence/absence criterion) and Enterococcus sp. (CFU mean calculation) in the liquid fraction of dairy cow slurry stored during 90 days. Different treatments were applied to the liquid fraction of the slurry (previously obtained by mechanical separation): addition of biochar, addition of sulfuric acid and the combination of the addition of biochar and sulfuric acid. Each treatment was repeated in triplicate and the samples for detection of *Salmonella* sp. and *Enterococcus* sp. (by microbiological methods) were collected on the third day after the start of the test and repeated at 30, 60 and 90 days until the end of storage. In the present study the treatments containing acidification seem to favour the survival of the pathogens studied (mean of 5,62 CFU for *Enterococcus* sp and presence of *Salmonella* sp. in the treatment containing acid at 90 days of storage), comparing to the treatments that not have acidification. The type of pathogens and levels of contamination of animal effluents vary with health status, age and diet of the animals as well as with the physical and chemical characteristics of the effluent. The survival time of microorganisms in the animal manures, according to our study, is increased by acidification.

# Endogenous retroviruses in equine epitheliochorial placenta: differential expression pattern of *env* gene

**Valentina Stefanetti[1], Fabrizio Passamonti[1], Katia Cappelli[1], Stefano Capomaccio[1], Luisa Pascucci[1], Martina Crociati[1], Maurizio Monaci[1], Mauro Coletti[1], Maria Luisa Marenzoni[1]**

[1]Department of Veterinary Medicine, University of Perugia, Italy

## OBJECTIVE(S)

Endogenous retroviruses (ERVs) are proviral phases of exogenous retroviruses [1]. Previous studies identified the envelope (env) protein genes of retroviral origin (named *syncytins*) preferentially expressed in the placenta [2]. Until now, all the characterized *syncytins* have been associated with the invasive placentation type [3]. Only recently, a study found a retroviral *env* gene in horses, a species characterized by epitheliochorial placenta which is highly expressed in the placenta when compared to other tissues [4]. The aim of the present study was to evaluate whether there is a further different ERVs *env* expression pattern in various areas of the same placentas.

## MATERIALS AND METHODS

Primers were designed on the *env* region of the candidate full-length ERV. Ten placentas were collected immediately after an eutocic delivery and a sampling of seven specific areas of each placenta (amnion, avillous cervical star, villous corion near the cervical star, avillous chorionic girdle, villous chorionic girdle, gravid horn and non-gravid horn) was carried out. Total RNA was extracted, treated with DNase I and reverse-transcribed into cDNA, before qPCR reaction. **β-actin** and *RPL32* were used for the normalization of qRT-PCR data.

## RESULT(S)

Among the selected full-length ERVs, we found only one candidate showing an ORF longer than 300 aa suitable for downstream investigation. Interestingly, qPCR assay showed that the retroviral *env* gene is regularly higher expressed in the areas with the highest density of villi compared to the avillous areas.

## CONCLUSION

Mare's chorion is covered by microcotyledonary villi everywhere the chorion had contact with the endometrium. The reduced EqERV *env* expression value in areas laking villi could suggest a role in horse placentation that deserves investigation. A putative immunological role of this gene in relation with maternal-fetal tolerance and the relationship with the other exogenous retroviruses should also be evaluated.

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# Sea turtles as sentinel species of the mediterranean sea: Isolation of ESBL producing bacteria

**Adriana Trotta[1], Marialaura Corrente[1], Georgia Diakoudi[1], Sunčica Bosak[2], Stefano Ciccarelli[1], Delia Franchini[1], Mariarosaria Marinaro[3], Domenico Buonavoglia[1]**

[1]Department of Veterinary Medicine, University of Bari, St prov. per Casamassima Km 3, 70010, Valenzano (BA), Italy
[2]Department of Biology, Faculty of Science, University of Zagreb, Rooseveltov trg 6-10000 Zagreb, Croatia
[3]Department of Infectious Diseases, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy

## OBJECTIVE

The significant anthropogenic influence on the marine ecosystems can be observed on many levels and a widespread phenomenon is the continuous spreading of antimicrobial resistance (AMR) determinants among bacteria. Gram-negative bacteria producers of extended-spectrum-beta-lactamases (ESBLs) show the highest resistance rate against β-lactam antibiotics [1] and may also show multidrug resistance (MDR) [2]. The bio-monitoring of AMR is required, especially in the coastal areas [3] and we propose that wild animals, such as sea turtles, may serve as sentinels as they have no history of therapeutic antibiotic exposure.

## MATERIALS AND METHODS

Fifty-two loggerhead sea turtles (*Caretta caretta*) hospitalized at the Sea Turtle Clinic of the University of Bari (2016-2018), were sampled for bacterial identification, antimicrobial susceptibility testing, according with EUCAST guidelines and ESBLs gene analysis. A total of 52 samples were collected [24 external wounds (45%), 16 BAL (32%), 11 biopsies (21%), 1 cloacal swab (2%)]. ESBLs genes were screened by PCRs, sequenced and compared with antimicrobial resistance databases.

## RESULTS

Forty Gram-negative strains were isolated. The antimicrobial susceptibility test revealed that 74% of the isolates were MDR and 100% were resistant to Imipenem, a drug licensed for human use only. Fifty percent of the strains resulted positive for at least one ESBL gene, 15% for two genes and 5% for three genes. The most frequently detected genes were $bla_{\text{Amp-C}}$ (45%), $bla_{\text{CTX-M}}$ (17.5%) and $bla_{\text{TEM}}$ (12.5%), followed by $bla_{\text{SHV}}$ (2.5%). All of them encode for enzymes involved in antibiotic inactivation and were firstly reported in human urine and feces.

## CONCLUSION

We report ESBLs producers and MDR clinical isolates found in sea turtles. ESBLs could be a problem for the therapeutic treatment of these animals. The role of the marine sea turtle as a reservoir of MDR bacteria could be linked to the impact of human activities on marine ecosystem.

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# Antimicrobial resistance of coliform isolates collected from drinking water samples

**Tzimotoudis Nikolaos[1], Petsios Stefanos[1], Mpatra Aikaterini[1], Filiousis George[2]**

[1]Laboratory of Microbiology, Hellenic Army Biological Research Center, Athens, Greece
[2]Laboratory of Microbiology & Infectious Diseases, School of Veterinary Medicine, Aristotle University, Thessaloniki, Greece

## OBJECTIVE

The aim of the present study was to investigate the antimicrobial resistance pattern of coliform strains isolated from drinking water samples.

## MATERIALS AND METHODS

A total of 138 coliform strains were isolated from drinking water samples collected from various regions Greece. The identification of the isolates by biochemical tests resulted in 26 Enterobacter spp, 56 Citrobacter spp, 51 Klebsiella spp and 5 Serratia spp strains. The disc diffusion method on Mueller-Hinton Agar was used for the determination of the antimicrobial susceptibility. The test was performed by using standard discs containing Ciprofloxacin (5μg), Tetracycline (30μg), Gentamicin (10μg), Trimethoprim-Sulfamethoxazol (1,25/23,75μg), Ampicillin (10μg), Amoxicillin - Clavulanic acid (15μg), Cefadroxil (30μg), Ceftazidime (10μg), Cefoxitin (10μg), Cefotaxime (5μg), Meropenem (10μg). The microbial strains were evaluated for resistance to the antimicrobial agents according to the guidelines and criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

## RESULT(S)

All strains were susceptible to Ciprofloxacin, Gentamicin, Trimethoprim-Sulfamethoxazol and to Meropenem. 114 isolates (82.6%) were resistant to at least one antimicrobial. Resistance against Ampicillin, Amoxicillin-Clavulanic and Cefoxitin was detected in 74.1%, 44.9% and 50.8% of the isolates, respectively. 16.4% of the isolates were resistant against the third-generation Cephalosporins Ceftazidime and Cefotaxime. Additionally, it is noticeable that 2 isolates were resistant to 8 antimicrobials (Klebsiella and Citrobacter) and 9.4% and 16.7% of the isolates were resistant to 6 and 4 antimicrobials.

## CONCLUSION

Under certain circumstances the drinking water could be a significant reservoir and a mean of disseminating bacteria which are resistant to various antimicrobial substances.

# Evaluation of the antibiotic sensitivity of bacterial strains isolated from *Caretta caretta* sea turtles in Sicily

**D. Gambino[1], G. Schirò[1], V. Randazzo[1], A. Gentile[1], M. Vitale[1], D. Vicari[1]**

[1]Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Via Gino Marinuzzi 3, Palermo (PA) 90129, Italy.

Due to their ecological and physiological characteristics sea turtles are considered sentinel species useful as bioindicators of the health of the marine and coastal environment. The *Caretta caretta* frequents the coastal areas to feed and reproduce, and remain faithful to a site where returns during the course of their lives. The turtles are therefore exposed to anthropic factors and to the resistant bacteria coming from human wastewaters, which are inevitably transported elsewhere during migration [1-2]. The susceptibility to antibiotics of 23 strains isolated from 14 C. caretta sea turtles (cloacal / buccal or organs) and from eggs from 2 nests was determined. The antibiograms were performed with the Kirby-Bauer method on Muller Hinton agar and 8 antibiotics were tested: amoxicillin + clavulonic acid, cefazolin, ceftrizxone, colistin, streptomycin, enrofloxacin, sulfamethissazole + trimethoprim, tetracyclin. Organisms belonging to the genera *Aeromonas* (13), *Citrobacter* (3), *Enterobacter* (1), *Enterococcus* (2), *E. coli* (1), *Klebsiella* (1), *Pseudomonas* (1) and *Staphylococcus* (1) have been isolated. Many of the tested strains showed resistance to multiple antimicrobials; in particular high frequency of resistance has been found to cefalozolin, streptomycin (60,8%), colistin (52,1%) and amoxicillin + clavulonic acid (39,5%); while only sulfamethissazole + trimethoprim (86,9%) and tetracycline (65,5%) showed high sensitivity. The results obtained, in accordance with what reported by others author, highlight the presence of microbial antibiotic resistance from sea turtles [2]. More research is needed on antibiotic resistant bacteria present in marine organisms animals to determine the degree of diffusion of antibiotic resistance in the marine environment.

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# Surveillance system for zoonotic pathogens and antimicrobial resistance in companion animals in the Netherlands: results 2010-2018

**M.A.M. van Dijk[1], E.M. Broens[1], E.R. Nijsse[1], M.M. Kannekens – Jager[1], A.H.W. Schoormans[1], J.A. Wagenaar[1,2,3]**

[1]Veterinary Microbiological Diagnostic Centre (VMDC), Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Netherlands.
[2]Central Veterinary Institute of Wageningen University and Research Center, Lelystad, Netherlands
[3]WHO-Collaborating Center for Campylobacter/OIE Reference Laboratory for Campylobacteriosis.

## OBJECTIVE

In the Netherlands a surveillance system for zoonotic pathogens and antimicrobial resistance (AMR) in companion animals has been implemented since 2011 (retrospective analysis for 2010) as part of the national integrated human-veterinary risk analysis structure for zoonoses. The aim of the surveillance is to observe trends in prevalence of zoonotic pathogens and AMR.

## METHODS

The surveillance system consists of 3 pillars:

1. Passive surveillance: data of routine tests performed at the Veterinary Microbiological Diagnostic Centre (VMDC).
2. A helpdesk for (veterinary) healthcare professionals concerning zoonoses and AMR.
3. articipation in the Signaling Forum Zoonoses

## RESULTS

Relevant signals include a rise in leptospirosis in dogs in 2014, which coincided with a rise in autochthonous cases in humans [1]. In 2016 the VMDC diagnosed the first two cases of canine brucellosis in the Netherlands. The first case was a *Brucella suis* biovar 1 infection, with the dogs raw meat-based diet as the most likely source of infection [2]. The second case was a *Brucella canis* infection in a dog imported from Eastern Europe. For all signals, communication took place via messaging services for (veterinary) health professionals to create awareness and provide information. The surveillance on AMR indicated a decline in Extended Spectrum Beta-lactamase producing bacteria (4.3% positive isolates in 2010, 2.3% in 2018). The Methicillin Resistant (MR) fraction of isolated *Staphylococcus aureus* (SA) and *Staphylococcus pseudintermedius* (SP) is 5.3%-14.8% and 6.7%-12.4% respectively, with MRSA occurring more than ten times less frequently than MRSP in absolute numbers.

## CONCLUSION

Surveillance of zoonoses and AMR in companion animals provides insight in trends and signals, generating valuable information for (veterinary) health professionals and authorities.

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# Investigation of antimicrobial resistance patterns in commensal *Escherichia coli* isolates from broilers in Greece

**A. Xexaki[1], G. Filioussis[1], E.N. Sossidou[2], E. Petridou[1], D. Timofte[3]**

[1]Aristotle University, Faculty of Veterinary Medicine, Laboratory of Microbiology and Infectious Diseases, 54124 Thessaloniki, Greece
[2]Hellenic Agricultural Organization-DEMETER, Veterinary Research Institute, 57001 Thessaloniki, Greece
[3]Institute of Veterinary Science, University of Liverpool, Liverpool, United Kingdom

## OBJECTIVE

With the advent of the frequent use of antimicrobials in the poultry industry increasing antimicrobial resistance (AMR) among *Escherichia coli* is a major concern. The aim of the current study was to evaluate the fecal carriage and the pattern of antimicrobial-resistant *E. coli* from broilers which were raised in intensive production systems in Greece.

## MATERIALS AND METHODS

A total of 317 fecal samples were collected from 75 broiler chicken flocks in Greece. The samples were enriched overnight in Brain-Heart Infusion broth with ampicillin (10 mg/L) and plated on Tryptone Bile X-glucuronide Agar. The *in vitro* antimicrobial-susceptibility testing was assessed for 14 antimicrobials by disc-diffusion, whilst colistin minimum inhibitory concentration (MIC) was determined by broth microdilution [1,2]. Furthermore, *E. coli* isolates were assessed for extended-spectrum beta-lactamase (ESBL) production. PCR/DNA sequencing was used to characterize beta-lactamase and *mcr*-1 resistance genes [3] Phylogrouping was performed using an established PCR method [4].

## RESULTS

The overall prevalence of ampicillin-resistance in the *E.coli* isolated in the present study was 94.5% (301/317). The highest rate of co-resistance to ampicillin detected was for nalidixic acid (73.4%), followed by tetracycline (70.8%), streptomycin (70.4%) and enrofloxacin (60.1%). Colistin resistance was present in 11 *E. coli* isolates which all carried the *mcr*-1 gene and had a colistin MIC of 4 to 8 mg/L. Ten percent of isolates (n=32) showed resistance to cefpodoxime and 28 of these were ESBL-producers and PCR showed that they carried $bla_{\text{CTX-M}}$ (n=19), $bla_{\text{SHV}}$ (n=7), $bla_{\text{CIT-M}}$ (n=4) and $bla_{\text{OXA}}$ (n=2). Fifty nine percent of isolates were assigned to phylogroup B1(n=178) while 19.6% (n=59), 9.3% (n=28) and 12% (n=36) were assigned to A, B2 and D respectively.

## CONCLUSION

The high incidence and the different patterns of AMR identified in broilers identified in this study, suggests that there is a need to reduce antimicrobial use in the poultry industry in Greece.

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# Microbiological monitoring of laboratory animal facilities

**Argyro Zacharioudaki[1], Maria-Anna Tsoutsou[1], Vasileios Ntafis[2]**

[1]Experimental Educational Research Center ELPEN, Athens, Greece
[2]BSRC "Alexander Fleming", Vari, Greece

## OBJECTIVE

To present considerations and guidelines regarding microbiological monitoring of laboratory animal facilities.

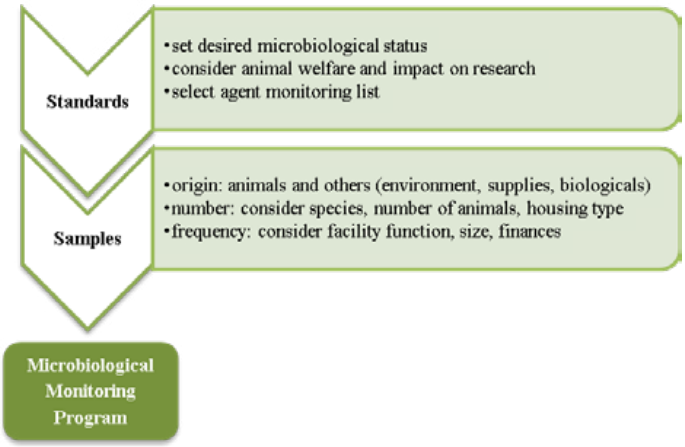
## DISCUSSION

Laboratory animal facilities need to breed and/or maintain animals with a microbiological standard that does not affect animal welfare and research results. A microbiological monitoring program must be tailored to the specific needs of each facility in order to ensure that premises and animals meet the individual standards. Animals (sentinel or primary/resident), environment (rooms, cages, air filters etc.), supplies (water, feeds, bedding etc.), biological material (of either animal or human origin, such as cell lines) or other specimens may be sampled and tested. The Federation of European Laboratory Animal Science Associations (FELASA) issues recommendations for health monitoring of common laboratory animal species[1], which are widely accepted. Suggested monitoring frequency ranges from 1 to 4 times per year. Factors considered in the selection of specimens and agents to be monitored include: facility function(s), biosecurity level, housing style, number of animal rooms and microbiological units, presence of breeding lines, number, species and immune status of animals, zoonotic potential, previous disease incidence, use of biologicals and type of research conducted[2]. These will define the desired microbiological status. The level of monitoring and any positive results should be critically evaluated to balance cost and facility needs.

## CONCLUSION

A proposed decision rubric for the formulation of a microbiological monitoring program of laboratory animal facilities is to prioritize needs regarding standards and samples (Figure 1).

Figure 1. Decision rubric for microbiological monitoring of laboratory animal facilities

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# American foulbrood in honeybee colonies: PCR-based quantification of *Paenibacillus larvae* spores in honey

**Urška Zajc[1], Irena Zdovc[1], Alenka Žugelj[2], Bojan Papić[1], Jana Avberšek[1], Tina Pirš[1], Metka Pislak Ocepek[3], Matjaž Ocepek[1], Darja Kušar[1]**

[1]University of Ljubljana, Veterinary Faculty, National Veterinary Institute, Institute of Microbiology and Parasitology, Gerbičeva 60, SI-1000 Ljubljana, Slovenia
[2]University of Ljubljana, Veterinary Faculty, National Veterinary Institute, Unit Maribor–Ptuj, Šentiljska cesta 109, SI-2000 Maribor, Slovenia
[3]University of Ljubljana, Veterinary Faculty, National Veterinary Institute, Institute of Pathology, Wild Animals, Fish and Bees, Gerbičeva 60, SI-1000 Ljubljana, Slovenia

## OBJECTIVES

American foulbrood (AFB) is a highly contagious and devastating disease of honeybees, caused by *Paenibacillus larvae*. Reliable quantification of *P. larvae* spores in hive-associated materials is necessary for the implementation of improved control measures to reduce the spread of AFB. Plate counting of *P. larvae* spores is proven as highly unreliable. Several PCR-based protocols have been developed, but only real-time PCR (qPCR) or digital PCR (dPCR) can be regarded as reliable for the quantification of *P. larvae* spores. This study aimed at constructing a novel qPCR assay, based on TaqMan technology, for the quantification of *P. larvae* spores in honey. TaqMan assays are more specific, sensitive and reproducible compared to others.

## MATERIALS AND METHODS

TaqMan qPCR protocol with a probe targeting the metalloproteinase gene of *P. larvae* was developed. Total DNA was extracted from AFB-positive honey in 3 biological replicates using a commercial extraction kit and a 5-fold DNA dilution series was prepared. Each sample was subjected to qPCR in 3 technical replicates. To enable absolute quantification, the qPCR assay was also employed for dPCR to determine the number of spores in honey and thus allow the calibration of qPCR independent of plate counting.

## RESULTS

The constructed qPCR was successfully calibrated using dPCR, which was employed for *P. larvae* for the first time. For qPCR, the observed limit of quantification (LOQ) was 13 and limit of detection (LOD) 3 spores of *P. larvae* per gram of honey, which is the lowest theoretically possible LOD in complex samples.

## CONCLUSION

The developed TaqMan probe-based qPCR assay represents an important contribution to the early diagnostics of AFB by quantification of *P. larvae* spores in honey.

## ACKNOWLEDGEMENTS

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# Infection control in Veterinary Hospitals using Acuitas Resistome, a new rapid molecular approach for typing multidrug-resistant Gram-negative bacteria

**Flavia Zendri[1], Catherine McGowan[1], Vanessa Schmidt[1,2], Dale Shelton[3], Dorina Timofte[1,2]**

[1]Institute of Veterinary Science, University of Liverpool, UK
[2]Institute of Infection and Global Health, University of Liverpool, UK
[3]OpGEN, Gaithersburg, Maryland, USA

## BACKGROUND

Hospital-acquired infections associated with multidrug-resistant Gram-negative (MDR-GN) bacteria are an emerging concern in veterinary healthcare settings, especially within intensive care units (ICUs).

## METHODS

To understand the molecular epidemiology of MDR-GN isolates in two veterinary hospitals (Equine and Small Animal Hospitals), we performed a six month pilot study during which faecal and environmental samples were obtained from selected patients admitted to our ICUs, during the first and after 48 hours from admission. In total, 317 MDR-GN were collected and analysed using the Acuitas Resistome Test (OpGen,Gaithersburg, Maryland) [1] a PCR-based microfluidic array assay which screens for 50 antimicrobial resistance gene families, including those encoding production of extended spectrum beta-lactamase (ESBLs), TEM/SHV/OXA or AmpC beta-lactamases and carbapenemases. Combining organism identification and antimicrobial susceptibility data to genotyping results, unique 'Acuitas Profiles' were generated that can be used for typing the isolates and tracking transmission events.

## RESULTS

The most prevalent MDR-GNs isolates circulating in both the Small animal and the Equine Hospital, consisted of *Pseudomonas aeruginosa* and *Enterobacter cloacae* (21.8% each), *Klebsiella pneumoniae* (15%), *Acinetobacter baumannii* (14%) and *Escherichia coli* (12%), all harbouring a combination of genes encoding for beta-lactamases and ESBLs. One important finding was the identification of isolates carrying transmissible resistance to last resort antimicrobials (i.e. carbapenems) within the hospital environments, represented by three *Acinetobacter* spp harbouring $bla_{\text{OXA-23}}$ and one *E. coli* with $bla_{\text{OXA-48}}$.

## CONCLUSION

The results of this project will allow the rapid application of targeted infection control policies in our hospitals in order to prevent the transmission of relevant nosocomial infections.

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## ACKNOWLEDGEMENTS

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# Occurence of ESBL-producing *Escherichia coli* ST131 including the H30Rx and C1-M27 sublineages amongst seagulls from UK

**Flavia Zendri[1], Iuliana E. Maciuca[1], Simon Moon[2], Philip Jones[3], Andy Wattret[1], Richard Jenkins[4], Andy Baxter[5], Dorina Timofte[1,6]**

[1]Institute of Veterinary Science, University of Liverpool, UK
[2]Somerset West and Taunton Council, Taunton, UK
[3]Animal and Plant Health Agency (APHA), Shrewsbury, UK
[4]School of Allied Health Sciences, De Montfort University, Leicester, UK
[5]Birdstrike Management Ltd., York, UK
[6]Institute of Infection and Global Health, University of Liverpool, UK

## OBJECTIVES

Faecal *Escherichia coli* strains were isolated from seagulls (fam. *Laridae*) to determine the prevalence of extended-spectrum cephalosporin resistant (ESC-R) and fluoroquinolone-resistant (FQ-R) *E. coli* among gull species from two cities in the United Kingdom (one coastal and one inland). We also characterized the genetic background as well as carriage of plasmid-mediated resistance genes in extended spectrum beta-lactamase (ESBL)-producing *E. coli* obtained from these birds.

## METHODS

Herring gull (*Larus argentatus*) and lesser black-backed gull (*Larus fuscus*) faecal samples were screened for ESC-R *E. coli* which were then analysed for ESBL production, presence of plasmid-mediated quinolone resistance (PMQR) genes and genes conferring colistin and carbapenem resistance. All seagull *E. coli* isolates were assigned to phylogenetic groups and those belonging to the ST131-O25b international clone were further typed to the C/H30 and C/H30Rx sublineages. Extensive antimicrobial susceptibility testing was performed on the isolates displaying plasmid-mediated ESC and/or FQ resistance.

## RESULTS

Sixty ESC-R *E. coli* isolates were obtained from 38 seagulls, of which 28 (46.7%) were positive for plasmid-mediated CTX-M and/or AmpC **β-lactamase resistance genes. Among these,** $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-14}}$ and $bla_{\text{CMY-2}}$ predominated. Three isolates belonging to the ST131-O25b clone were detected, of which two harboured $bla_{\text{CTX-M-15}}$ (typed to C2/H30Rx) and one harboured $bla_{\text{CTX-M-27}}$ typed to C1/H30-R (recently described as the C1-M27 sublineage). PMQR gene carriage prevalence was 11.7% (n=7) and consisted of *aac*(6')-*Ib-cr* and *qnrB* genes. No carbapenem or colistin resistance genes were detected among these seagull *E. coli* isolates.

## CONCLUSION

Wild seagulls in the UK are colonized, and can spread, major antimicrobial resistant *E. coli* isolates harbouring ESBL and PMQR determinants, including clinically important strains such as the the pandemic clone ST131-O25b and the C1-M27 subclone. To the best of our knowledge this is the first report of ST131-C1-M27 subclone in wildlife in the UK and in seagulls across Europe.