

Project 7

Name/title of the PhD course	Veterinary science
Name of the PhD coordinator	Prof. Dr. Aniello Anastasio
Name/Title of the PhD project	<i>The Wild Boar One Health “Chain” Approach: The Gut Microbiome Prediction for Antibiotic-Resistance and Meat Safety</i>
Department of reference	The Department of Veterinary Medicine of Napoli (DVMAP https://www.mvpa-unina.org/default.php)
Working conditions, research team, infrastructures, equipment	One of the 26 Departments of the University of Naples “Federico II”. Objectives of the DVMAP take into account European Directive No. 2005/36/EU, the current national legislation (Ministerial Decrees No. 509/1999, 270/2004 and Law 240/2010), and the Standard Operating procedures of the European association of establishments for veterinary education (EAEVE SOPs). To integrate the fundamental cycle the Department, offer further courses such as 7 Specialization Degree and a Research Doctorate Course. The department gathers more than 100 professors and researchers in Veterinary Medicine, Animal productions, biology, and other scientific and technical disciplines and 50 Ph.D. students. Research facilities involved in the activity, located within the DVMAP, comprise the classical infrastructure of a microbiology research group as well as molecular platforms, including labs for working with biosafety group 2 bacteria. For taxonomic <i>characterization</i> of pure cultures, a wide range of research tools are available, including the matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Currently, the team is working on different projects in the field of microbial diversity by using MALDI TOF MS and Shotgun sequencing for all metagenomic DNA examination, taxonomy and ecology, food microbiology, infectious diseases, and antimicrobial resistance.
Scientific context	Antimicrobial resistance (AMR) is a growing factor that greatly impacts the world economy, human and animal health. According to the World Health Organization (WHO), antimicrobial resistance (AMR) is one of the biggest threats to global health, food security and development of the 21 st century. Globally, antibiotic-resistant bacteria (ARB) already cause more than 70,000 deaths each year, and it has been predicted that soon, this problem will involve millions of people throughout the world. The over-reliant use of antibiotics for humans and animals has contributed considerably to the dissemination of antibiotics into environments, yet the mechanisms by which antibiotic dissemination influences the assembly of the microbial community continue to remain unclear. In this context, wildlife that, unlike livestock, is not treated with antibiotics can be used as an indicator of the spread of ARB into the environment. Knowledge concerning ARB circulating in wildlife is currently limited, although it could provide important insights into AMR emergence and persistence. Wildlife may spread ARB in the environment via their feces. The spread of resistant strains in the natural environment constitutes a potential hazard for both humans and domestic animals. Wild boars (<i>Sus scrofa</i>) are the most widely distributed large mammals in the world and they occupy a wide variety of habitats including urban areas with easy access to anthropogenic food resources included garbage. They are characterized by the highest reproductive rate among the ungulates and thus, in recent years the wild boar population has dramatically increased in size. Economic interest for these animals is related to the damage of crops and husbandry and the possibility of transmitting the disease to livestock and humans. The microbial population present in the skin and the digestive tract can vary greatly between the different animals because, in contrast to livestock, wild boar roam free and their diet is uncontrolled. Even though wild boars are unlikely of being treated with antibiotics, they are considered a reservoir of pathogenic ARB because of the overlap between habitats. Moreover, the presence of ARB in wild boar can also cause public health concerns since its meat is consumed all over the world. Human exposure to AMR from wild boar meat may occur: (i) directly, through the ingestion of wild boar meat contaminated by pathogens; (ii) indirectly, through the dissemination of resistance genes from commensal bacteria. Different studies have focused their attention on the occurrence of AMR in food-borne pathogens, but little is still known about the AMR profiles of the commensal bacteria. The consumption of the meat of these animals, although still low if compared with domesticated animals, is increasing in Europe. Usually, the meat is eaten cooked, though some wild boar meat products are not heat-treated but only dry-cured, cold smoked, and dried. However, a precise knowledge of bacteria present during the shelf-life in wild boar meat after the application of different preservation techniques is still missing. The microbial population can vary greatly between meat samples belonging to different wild boars because it also depends on the hunting hygiene (e.g. hunting method, shooting, disemboweling) and the spread of microorganisms during the slaughtering process and in particular during the evisceration process. The evolution of AMR genes in wild animals and the close connection with natural, agricultural, animal, and human ecosystems demonstrate that the use of a One Health integrated approach is crucial for understanding and managing AMR. Wild boar represents a perfect sentinel model species in AMR dynamics to unveil the emergence, spread, and persistence of AMR in the “One Health” interface. Thus, it is becoming increasingly important to study the connection between the gut microbiome and the microbial composition of meat products to understand the ecology of food-borne ARB. In this context, rapid identification and characterization of these drug-resistant bacteria have an important role in diagnostic and antimicrobial stewardship. Recently, the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) has been used for the screening of biomarkers to predict antimicrobial resistance.
Project Research plan	In the first phase of the project, a quantitative data collection and analysis will be performed. To evaluate the human impact on the environment, the study area will be divided into different categories considering the distance to the anthropogenic activities. Based on the information collected and analysed, a representative number of hunting areas with different characteristics will be selected for the activities of the project. During different hunting seasons and in each one

	<p>of the selected areas, a representative number of digesta samples will be collected from the wild boars at the end of each hunting session. Moreover, the wild boar carcass surface, along with the fresh meat will be sampled for microbiological analysis. Samples will be collected from a carcass half after evisceration and trimming. Moreover, environmental samples, such as knives, cooling rooms, tables will be collected, as well. All samples will be taken and transported at 4 °C to the laboratory for analysis. Samples will be analyzed by the Ph.D. student through the conventional microbiological method and MALDI TOF MS at the Dept. of VMAP and through the shotgun sequencing at LM-UGent. A cultural method will be adopted to determine the total viable count and the microbiota composition of the digesta and wild boar meat samples. Different media incubated at different temperatures and conditions will be used for the enumeration and isolation of the bacterial population present in the samples. After incubation, isolates will be all picked from the agar plates and analyzed through MALDI- TOF MS. A representative number of isolates (pathogenic and non-pathogenic) will be then selected for the evaluation of antimicrobial resistance. Antibiotic phenotypic resistance of isolates will be evaluated by using both the EUCAST disk diffusion test and MALDI TOF MS. Moreover, for the selected isolates, the phenotypic results will be compared with the genotypic profile. DNA will be extracted from colonies and will be analyzed through Real-Time PCR to reveal the carriage or co-carriage of specific genes. To characterize the microbial community in the samples a culture-independent method will be also used. DNA will be extracted directly from the samples using a commercial DNA extraction kit following the manufacturer’s recommendations and will be analyzed through shotgun sequencing. Data will be then analysed through bioinformatics tools. Moreover, fresh meat samples will be collected, and they will be undergone to different preservation techniques at the farm “La Fattoria del Campiglione”. The microbiological analysis will be carried out on different batches at zero time and during the conservation period to evaluate the evolution of the microbial population after the application of different preservation techniques. Characterization of samples microbiota and isolation/identification of the microbial population (species and strains) will be performed again through culture-independent and dependent methods.</p>
<p>Research and Training Innovative aspects</p>	<p>The presence of AMR bacteria in wildlife is an indicator that resistant microorganisms of human and livestock origin are widespread in the environment. Diet and external environment shape the gut microbiome by modulating the abundance of specific species and their functions as well as the AMR transmission and persistence, harbouring resistance factors for all antibiotics. Since to date exist only speculations on possible sources and sinks of AMR, the isolation of ARB and antibiotic resistance genes (ARGs) from georeferenced wild boar could suggest the role and the impact of wildlife AMR on human, livestock, and domestic animal resistance. Wild boar is one of the perfect sentinel species for AMR surveillance and being highly consumed in Europe as game meat, it could represent a risk for public health in terms of infectious disease and AMR transmission. By using georeferenced sampling areas, we could also evaluate the anthropogenic hand in the spreading of AMR. The impacts of ingress of ARG into microbiomes and the wider issue of AMR spread into the environment will be evaluated. We will perform long-term monitoring of wild boars to collect data about the spread of AMR also with the aim to inform public policies on this topic since wild animals are under-regulated. Moreover, little is still known about the dynamics of the microbes during the production of wild boar meat products using common preservation techniques, such as drying, fermentation, vacuum packing, modified atmosphere packaging. These techniques could be used to extend the shelf life of the wild boar meat and therefore improve the safe conservation and commercialization.</p>
<p>Inter-Multidisciplinary aspects</p>	<p>The Ph.D student will work in a multidisciplinary group of three partners from different countries working in academic and business contexts (UNINA, LM-UGent, and Fattoria del Campiglione). The different members of the multidisciplinary group balance each other with their research and practical expertise, and the synergy among them helped to shape the different activities at the premise of the project. Moreover, the integration of different complementary backgrounds and expertise will foster a holistic approach during project implementation and management that will be crucial for the Ph.D student to achieve each single project objective. Taking into account their specific expertise, the Ph.D student will manage different tasks at each partner organization. In this way, the Ph.D student will be able to take advantage of each partner-specific skill to obtain results as defined in this project proposal, guaranteeing high-quality standards and time efficiency. The Departments of Veterinary Medicine and Animal Production of UNINA with a highly qualified professional environment in which researchers from different fields and disciplines will interact and cooperate with the Lab. of microbiology of UGent. and the Fattoria del Campiglione (see below)</p>
<p>Secondment opportunities</p>	<p>LM-UGent - https://www.ugent.be/we/biochemicro/en/research/microbiology The Lab of microbiology, LM-UGent, founded in 1959, is today a multidisciplinary team of more than 60 biotechnologists, biologists, biochemists, engineers, mathematicians, (bio)-informaticians, veterinarians, and technicians studying all aspects of bacteriology. LM-Ugent will contribute to the project by hosting one Ph.D. student for secondment <u>of six months</u>. Prof. Dr Kurt Houf will act as <i>co-supervisor</i>. Fattoria del Campiglione, a farm in the “Campania region” - Southern Italy. The farm is including within its premises a cutting plant to handle the meat of domestic ungulates and wild animals (i.e. wild boars’ meat), in compliance with the requirements laid down in Chapter V (Hygiene during cutting and boning) of Regulation (EC) 853/2004. La Fattoria del Campiglione will contribute to the project by hosting the Ph.D. for at <u>least three months</u>. During this period they will give the Ph.D. student the possibility to follow their production flows. Mr. Michele Sgamato will act as <i>co-supervisor</i>.</p>
<p>Main Supervisor: Prof Nicoletta Murru (https://www.docenti.unina.it/nicoletta.murru)</p>	
<p>Brief CV</p>	<p>Associate Professor of Inspection of Food of Animal Origin’ (SSD VET/04) at the Department of Veterinary Medicine and Animal Productions, University of Naples Federico II. His expertise is focused on classical and molecular microbiology and the main research activity concerns the detection of microorganisms in the food chain, the evaluation of the antimicrobial resistance of different pathogens, and the characterization of the microbial contamination of different ecosystems through</p>

	MALDI-TOF MS and 16S amplicon sequencing analysis. Supervisor of 5 Ph.D. students and supervisor together with Prof. Kurt Houf (Gent University) of a Joint-PhD student on the <i>Assessment of the microbial contamination on pork and wild boar meat by a culture-dependent and independent approach</i> (2015-2018).
Publications	<p>The total number of her publications is 40 and, among them the 5 most significant publications in the microbiome field are:</p> <ul style="list-style-type: none"> - Peruzzy, M. F., Murru, N.*, Smaldone, G., Proroga, Y. T. R., Cristiano, D., Fioretti, A., & Anastasio, A. (2021). Hygiene evaluation and microbiological hazards of hunted wild boar carcasses. <i>Food Control</i>, 108782. - Peruzzy, M. F., Houf, K., Joossens, M., Yu, Z., Proroga, Y. T. R., & Murru, N. (2021). Evaluation of microbial contamination of different pork carcass areas through culture-dependent and independent methods in small-scale slaughterhouses. <i>International Journal of Food Microbiology</i>, 336, 108902. - Peruzzy, M. F., Capuano, F., Proroga, Y. T. R., Cristiano, D., Carullo, M. R., & Murru, N. (2020). Antimicrobial susceptibility testing for salmonella serovars isolated from food samples: Five-year monitoring (2015–2019). <i>Antibiotics</i>, 9(7), 365. - M.F. Peruzzy, N. Murru, Z. Yu, P.-J. Kerkhof, B. Neola, M. Joossens, Y.T.R. Proroga, K. Houf. (2019) Assessment of microbial communities on freshly killed wild boar meat by MALDI-TOF MS and 16S rRNA amplicon sequencing. <i>International Journal of Food Microbiology</i> 301 (2019) 51–60 - Peruzzy, Maria Francesca, Murru, Nicoletta, Yu, Zhongjia, Cnockaert, Margo, Joossens, Marie, & Houf, Kurt (2018). Determination of the microbiological contamination in minced pork by culture-dependent and 16S amplicon sequencing analysis. <i>International Journal of Food Microbiology</i> 290:27-35
Projects participation	<p>The total number of funded projects participation with microbiome research is 9. Below is a brief list of 5 national more recent funded projects:</p> <p>Ricerca Corrente IZSME 07/20 RC (24 mesi) finanziata dal Ministero della Salute "Insetti e rettili alla tavola del consumatore: quali rischi ?" IZS ME 03/21 RC "Settore Sicurezza Alimentare Istituto Zooprofilattico Sperimentale del Mezzogiorno"</p> <p>Ricerca Corrente IZSME 07/20 RC (24 mesi) finanziata dal Ministero della Salute "Valutazione dell'impatto del consumo delle carni di cinghiale sulla salute umana-SALSUS" RC "Settore Sicurezza Alimentare Istituto Zooprofilattico Sperimentale del Mezzogiorno"</p> <p>Responsabile scientifico del progetto bando GAL Partenio Consorzio - S.S.L. "Terra del Partenio" PSR Campania 2014/2020. MISURA 19 – Sviluppo locale di tipo Partecipativo– LEADER. Sottomisura 19.2. Tipologia di intervento 19.2.1 "Strategie di Sviluppo Locale" Misura 16 "Cooperazione" FORFRESH "Sviluppo di un formaggio fresco probiotico in imballaggio ecosostenibile"</p> <p>Ricerca Corrente IZS ME 02/16 RC (24 mesi) finanziata dal Ministero della Salute "La salmonella negli alimenti: sviluppo di una strategia analitica innovativa per la sua rilevazione in meno di 24 ore" Settore Sicurezza Alimentare Istituto Zooprofilattico Sperimentale del Mezzogiorno</p> <p>Ricerca Corrente IZS ME 11/15 RC (24 mesi) finanziata dal Ministero della Salute "Valutazione dei rischi microbiologici dei prodotti a filiera corta a base di latte dell'Alta Irpinia e miglioramento delle metodologie per la preservazione delle produzioni" Settore Sicurezza Alimentare Istituto Zooprofilattico Sperimentale del Mezzogiorno</p>