European Association of Veterinary Laboratory Diagnosticians

This Newsletter:

Foreword

Dear Colleague,
We start this issue with the announcement of the next EALVD congress and information on «Topics», «registration» and «opportunities for travel grants»
A short article of *Antonio Martínez-Murcia on African Swine Fever Detection and an exciting presentation of lactococcosis specific agents in the context of SUPERTROUT project.*

Work in progress…
next EALVD will be on 24- 26 October 2022

EAVLD
6TH CONGRESS OF THE EUROPEAN ASSOCIATION OF VETERINARY LABORATORY DIAGNOSTICIANS
SEVILLA
OCTOBER 24,25,26 2022

Topics

- Antimicrobial resistance / susceptibility testing
- Biosecurity in animal health
- Emerging and re-emerging diseases
- Eradication and screening programs
- Exotic diseases in developed countries
- Food and water safety
- Fungal and parasitic diseases in animals
- Laboratory sample management / Laboratory Information Management Systems (LIMS)
- News Diagnostic Testing technologies
- Proficiency Testing - participant and organiser perspective
- Quality Systems - revised ISO 17025 (2017) standard
- Role of the wildlife in epizooties
- Vector borne diseases
- Whole Genome Sequencing
- Miscellaneous
- PhD Students Session

Registration

Fee	Early Registration until 16 August 2022	Regular Registration as of 17 August 2022
EAVLD/AVEDILA Members*	415 EUR	485 EUR
EPIZONE, ESCMID and ECVM Members*	425 EUR	495 EUR
Non-Members	475 EUR	545 EUR
PhD Students**	330 EUR	430 EUR
Degree Student	280 EUR	280 EUR
Accompanying Person	135 EUR	170 EUR

REGISTRATION FEE INCLUDES

- Access to all scientific sessions
- Scientific Programme book
- Abstract book
- Lunches
- Congress bag
- EAVLD Welcome Reception in the Venue (24 October 2022)
- EAVLD Congress Dinner in Robles Aljarafe (25 October 2022)

VAT 21 % included.

*EAVLD/EPIZONE discounted fees are available only with good standing membership at the time of the EAVLD congress.

- EAVLD / AVEDILA member registration fee. Your membership fee (€35) is included in the registration fee, so you are paying for your EAVLD/AVEDILA membership fee for the current year (2022) and the next year. The membership will be valid until the end of the month in which the EAVLD 2024 congress will be held.

- Non member registration fee: You will become a EAVLD/AVEDILA member automatically from the end of the EAVLD 2022 conference on until the end of the month in which the EAVLD 2024 congress will be held.

- To select degree student fee, you must attach the degree certificate

**PhD Students: A certificate from the doctoral commission of the study center must be added

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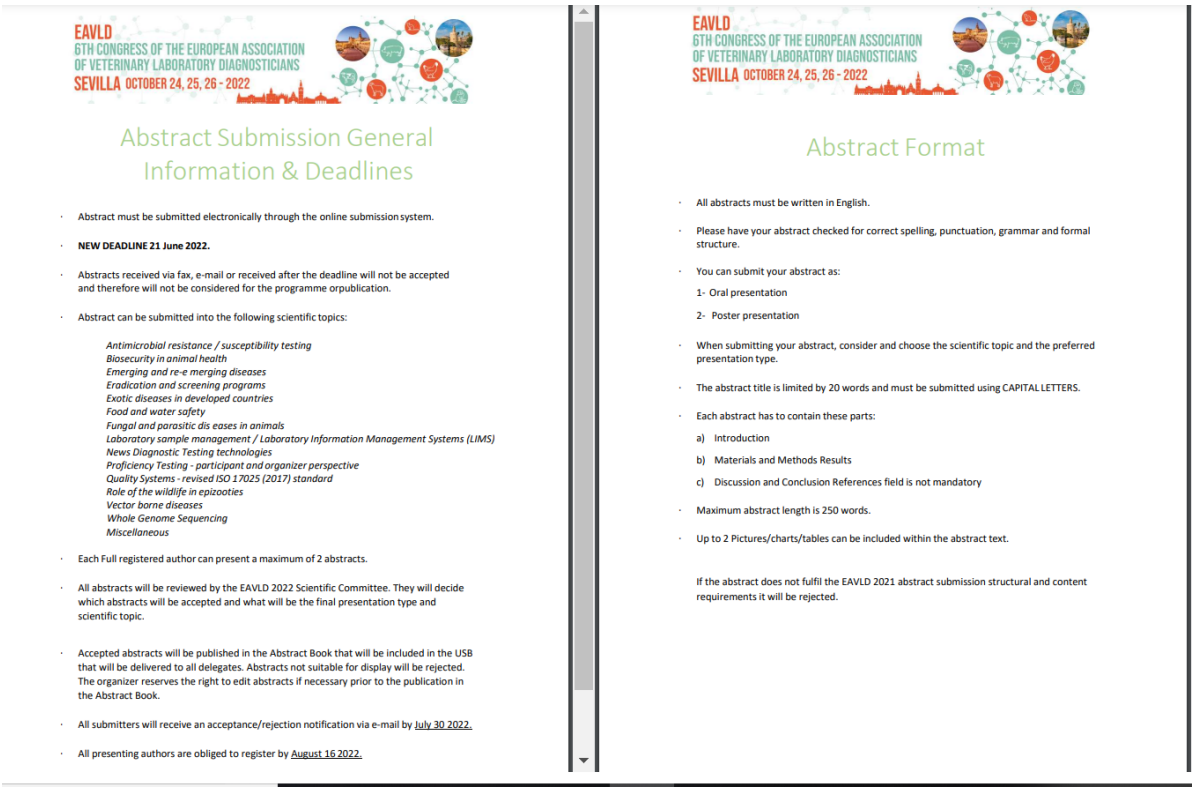
Deadline for abstract submission - 21 june 2022

Abstract instructions are reported on:
https://eavld2022.org/submission-guideline/



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Abstract Submission General Information & Deadlines

- Abstract must be submitted electronically through the online submission system.
- **NEW DEADLINE 21 June 2022.**
- Abstracts received via fax, e-mail or received after the deadline will not be accepted and therefore will not be considered for the programme orpublication.
- Abstract can be submitted into the following scientific topics:

Antimicrobial resistance / susceptibility testing
Biosecurity in animal health
Emerging and re-e merging diseases
Eradication and screening programs
Exotic diseases in developed countries
Food and water safety
Fungal and parasitic dis eases in animals
Laboratory sample management / Laboratory Information Management Systems (LIMS)
News Diagnostic Testing technologies
Proficiency Testing - participant and organizer perspective
Quality Systems - revised ISO 17025 (2017) standard
Role of the wildlife in epizooties
Vector borne diseases
Whole Genome Sequencing
Miscellaneous

- Each Full registered author can present a maximum of 2 abstracts.
- All abstracts will be reviewed by the EAVLD 2022 Scientific Committee. They will decide which abstracts will be accepted and what will be the final presentation type and scientific topic.
- Accepted abstracts will be published in the Abstract Book that will be included in the USB that will be delivered to all delegates. Abstracts not suitable for display will be rejected. The organizer reserves the right to edit abstracts if necessary prior to the publication in the Abstract Book.
- All submitters will receive an acceptance/rejection notification via e-mail by July 30 2022.
- All presenting authors are obliged to register by August 16 2022.

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Abstract Format

- All abstracts must be written in English.
- Please have your abstract checked for correct spelling, punctuation, grammar and formal structure.
- You can submit your abstract as:
  1- Oral presentation
  2- Poster presentation
- When submitting your abstract, consider and choose the scientific topic and the preferred presentation type.
- The abstract title is limited by 20 words and must be submitted using CAPITAL LETTERS.
- Each abstract has to contain these parts:
  a) Introduction
  b) Materials and Methods Results
  c) Discussion and Conclusion References field is not mandatory
- Maximum abstract length is 250 words.
- Up to 2 Pictures/charts/tables can be included within the abstract text.

If the abstract does not fulfil the EAVLD 2021 abstract submission structural and content requirements it will be rejected.

EAVLD_PhD Session

The governing boards of the European Association of Veterinary Laboratory Diagnosticians (EAVLD) and the Spanish Association of Veterinary Laboratory Diagnosticians (AVEDILA) are organizing a **session exclusively dedicated to PhD candidates** working on any of the topics covered by the congress' scientific program for the first time ever
(https://eavld2022.org/topics-2/).
People interested in participating in the 1 st EAVLD PhD Meeting must submit their abstracts through the congress portal (https://eavld2022.org/online-abstract-submission/)
before the 21st of June 2022, selecting 'PhD Session (summary of thesis)' as the presentation type.
Contributions from PhD candidates of all stages will be welcome. For example, students who have recently started their PhD program can present an introduction to their research topic and/or an outline of their project, whereas those close to finish might prefer to focus on presenting the results of a specific chapter of their dissertation.
A total of five abstracts will be selected for oral defense and the rest will be presented in poster format.
For further information about the PhD session and the abstract submission check the congress website (https://eavld2022.org/phd-students-session/) and/or contact Kiki Streng (kiki.streng@wur.nl) or Sergio Álvarez-Pérez (sergioaperez@ucm.es).

Travel Grants Scholarships

•Bursaries to attend the next conference of EAVLD are open to EAVLD members for submission. The EAVLD committee will consider applications covering travel, accommodation and food. Preference will be given to young scientists (under 35) and those giving oral or poster presentations.

•The payment of the two bursaries will be by bank transfer and is up to a total sum of €500. Deadline for the application is June 22, 2022.

•You can find the "APPLICATION FORM" online
https://www.eavld.org/eavld/?q=travelgrantapplication

•FATRO Travel Grant 2022

•For the Congress 2022, the **EAVLD Board would offer a special award**, considering the difficulties related to the SARS CoV-2 pandemic. The award aims to support research projects of young researchers as this period has led to a drastic reduction in the educational action of universities, periodic closures of laboratories, the cancellation of conferences and workshops, the reduction of training opportunities.

•EAVLD will receive the support of the **FATRO Veterinary Pharmaceutical Industry** to sponsor one travel grant of 1000€, to be assigned to a researcher in possession of the requirements specified in the application. The grant will offer the researcher the opportunity to attend the Congress and give impetus to his/her research in the field of veterinary diagnostics, through the presentation of a poster or an oral presentation and the interaction with the scientific community.

•By funding this grant, FATRO strongly supports the contribution of research by young people. The company philosophy can be summarised in its mission statement: "animal health your health". The FATRO will have the opportunity to fulfil its "mission" i.e. to offer a concrete support to the reality of veterinary diagnostics and research safeguarding animal well-being in the perspective of One Health. A ceremony for the assignment of the award will be organised during the Congress.

•The application deadline is June 22, 2022.

•You can find the "APPLICATION FORM" online https://www.eavld.org/eavld/?q=node/87

EAVLD Abstract Prizes

Congress will reward the three best oral communication and the three best abstracts as follows

Oral Communication

First prize to the best Oral Communication: 500€.
Sponsored by INGENASA

Second prize to the best Oral Communication: 200€.
Sponsored by EAVLD

Third prize to the best Oral Communication: 100€.
Sponsored by EAVLD

Poster Communication

First prize to the best Poster Communication: 500€.
Sponsored by HIPRA

Second prize to the best Poster Communication: 200€.
Sponsored by EAVLD

Third prize to the best Poster Communication: 100€.
Sponsored by EAVLD

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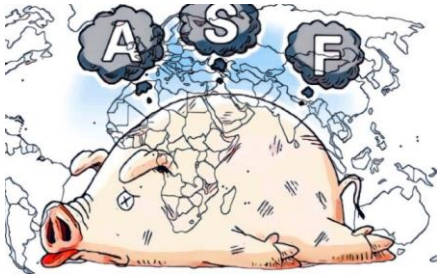

Development and validation of the GPS™ ASFV dtec-qPCR kit for African swine fever virus detection

Antonio Martínez-Murcia [a,b*], Aaron Navarro[b] , Gema Bru[b] , Mayte Jurado[b] , Jose A. Serrano[b]

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[b]Genetic PCR Solutions™, 03300-Orihuela, Alicante, Spain

African swine fever virus (ASFV) is the causative agent of the African swine fever (ASF), a transboundary swine viral disease which affects Suidae family, responsible for massive losses in pig populations and serious economic consequences. African swine fever has generated one of the main crises in the pig industry in recent years, currently present in several regions of the world. This disease not only harms the health and welfare of animals, but also has negative impacts on biodiversity and sources of income for producers. ASFV is a large, enveloped, double-stranded DNA virus, which is the sole member of the genus Asfarvirus within the family Asfaviridae. The virus is highly resistant in the environment, which means it can survive on clothing, shoes, vehicle tires, and other types of equipment. Likewise, it survives in different pork products, such as ham, sausages or bacon. Therefore, if the necessary measures are not applied, numerous behaviors of people can influence the cross-border spread of this disease. For these reasons, the ASF is a notifiable disease and it must be declared to the World Organization for Animal Health (OIE) (Dixon and Chapman, 2008; Gallardo et al., 2019; Wade et al., 2019).

Nowadays, prophylactic vaccine commercially is not available or effective treatments against ASFV. However, it is necessary to develop a rapid and accurate method to detect ASF-affected animals to prevent the virus spreading and to reduce economic losses. In the present study, the GPS™ ASFV dtec-qPCR kit for the ASFV detection was validated following the guidelines of the UNE-EN ISO/IEC 17025:2005. Analytical validation terms include in silico and in vitro specificity (inclusivity/exclusivity), sensitivity (evaluation of the linear regression, validation of the linear model and efficiency) and reliability (repeatability/reproducibility).

Analytical performance (analytical specificity and sensitivity) Analytical Specificity: inclusiveness and exclusiveness Validation terms include in vitro and in silico specificity. The analytical specificity in silico was analyzed comparing target sequences with public data bases as National Center for Biotechnology Information (NCBI). Alignments were performed against all strain sequences of the target species to ensure complete inclusivity. This includes all known genotypes, from I to XXIV described). The primers and probe showed a high level of homology with all sequences available; more than 400 sequences from all the 24 known genotypes suggesting high inclusiveness. Exclusivity was similarly analyzed against virus species close to ASFV. No similar sequences are detected indicating the high exclusiveness of the assay.

In a second phase, the inclusivity in vitro was evaluated with the genetic material of 21 ASFV isolates from the European Reference Laboratory for African Swine Fever: Animal Health Research Center (INIA-CISA). The 21 reference isolates are representative of 7 of the 24 virus genotypes, all yielding positive results using the ASFV dtec-qPCR kit. Also, this study was completed with 8 reference ASFV genomes provided by the Veterinary Hygiene Department, from Warsaw, Poland (PIWet).

Diagnostic performance (diagnostic specificity and sensitivity) The diagnostic specificity and sensitivity were achieved by testing 181 samples of porcine origin, from six different matrix types doped with ASFV reference isolates from CISA-INIA: whole blood, blood serum, kidney, heart, liver and tonsil. Results were compared with two reference detection methods based on real-time PCR. The analysis of the diagnostic data yields a diagnostic specificity of 100 % and a diagnostic sensitivity of 100 %.

The GPS™ ASFV dtec-qPCR kit, available worldwide with full analytical and diagnostic validation, represents a case of efficient transfer of technology. In addition the GPS™ kit has been registered in The Ministry Of Agriculture, Fisheries and Food of Spain – MAPA and has been registered in the corresponding ministry of Bulgaria and is being used currently massively in government labs.

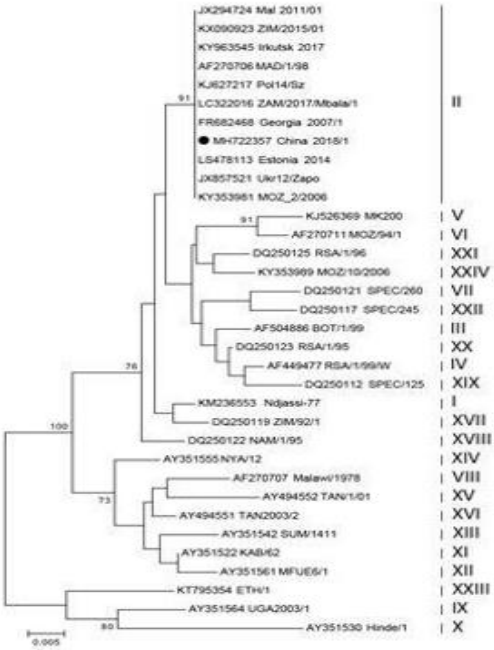

Figure 1. Phylogenetic tree of the ASFV p72 gene by Shengqiang Ge et al. 2018. Phylogenetic tree shows all 24 genotypes of the virus.

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TABLE 1. Summarized results of the validation of GPS™ dtec-qPCR kit for the detection of African swine fever virus following the guidelines of the UNE/EN ISO/IEC 17025:2005

Term of validation	Obtained values		Acceptance criteria	Conclusion
Specificity	**Inclusiveness:** *In silico* studies against all the sequences of the strains of the target species. Tested with 21 reference genomes (CISA-INIA) and 8 reference isolates (Poland) positive for African swine fever virus.		**Inclusividad**: Positive for all strains of African swine fever virus. Positive for all isolates and samples reference.	**Accepted**
	Exclusiveness: *In silico* studies against other virus species close to ASFV, including: CSFV, PRRSV, PCV-I y PCV-II, SuHV-1, FMDV, SVDV, VSIV.		**Exclusividad:** Negative for all strains of closely related virus.	**Accepted**
Standard curve	$Y = -3.369*X + 36.375$ $a = -3.369$ $R^2 = 1.000$		$-4.115 < a < -2.839$ $R^2 > 0.98$	**Accepted**
	$F_{assay} = 3.261$ $F_{fisher} = 5.318$		$F_{assay} < F_{fisher}$	**Accepted**
	Efficiency (e) = 98.1 %		75 % < e < 125 %	**Validated**
Reliability	**Repeatability**	CV (%)	CV < 10 %	**Repeatable**
	Intraensayo	1.14 %		
	Interensayo	1.32 %		
	Reproducibility	1.39 %	CV < 10 %	**Reproducible**
Detection limit (LOD)*	10 copies	n = 15; Posit. = 100 %	Positives ≥ 90 %	**Accepted**
Quantification limit (LOQ)*	10 copies	T *value* = 1.161 $T_{student}$ = 2.145	T value < $T_{student}$	**Accepted**
Diagnostic sensitivity	True Positives: 105 False Negatives: 0 SD = 100 %		DS > 90 %	**Accepted**
Diagnostic specificity	True Negatives: 76 False Positives: 0 ED = 100 %		DE > 90 %	**Accepted**

* Assays were performed for two sets (10 and 5 standard DNA copies) of 15 tests each (n = 15).

European Association of Veterinary Laboratory Diagnosticians

Rapid differentiation between Lactococcus garvieae and Lactococcus petauri based on 16S-23S rRNA intergenic spacer region polymorphisms.

S. Colussi[1], A.I. Vela[2-3], M.M. Blanco[2], C. Kotzamanidis[4], K. Bitchava[4], M. Prearo[1], N. Stoppani[1], I. Altinok[5], R.C. Ozturk[5], L. Fariano[6], D. Volpatti[7], P.L. Acutis[1] and J.F. Fernandez-Garayzabal[2-3]

[1] Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Italy;[2] Department of Animal Health, Veterinary School, Complutense University, Madrid, Spain; [3] VISAVET Health Surveillance Centre, Complutense University, Madrid, Spain; [4] Elgo-Demeter Thessaloniki, Greece; [5] Karadeniz Technical University, Trabzon, Turkey; [6] Azienda Agricola Canali Cavour, Cuneo, Italy; [7] Università degli Studi di Udine, Italy

Lactococcosis is a well-known infectious disease that affects aquaculture farming systems.
It is caused by Lactococcus garvieae, a warm-water pathogen, responsible of significant economic losses in aquaculture worldwide, in particular for rainbow trout.
The loss due to this infection is around 10-60% of the total rainbow trout production; increased mortalities are reported when water temperature exceeds 15 ° C. L. garvieae has been traditionally considered the unique responsible of lactococcosis, but recently Lactococcus petauri has been linked to lactococcosis outbreaks in Europe and North America.
L. petauri shares high similarity in its genome sequence, morphological characteristics and biochemical profile with L. garvieae, which makes the diagnosis extremely difficult.
Currently available molecular diagnostic tools fail to distinguish L. petauri from L. garvieae.
Average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) are commonly used for differentiating species.

In the context of the SUPERTROUT European Project (https://mel.cgiar.org/projects/supertrout), we used the genomes of a collection of L. garvieae isolates from various sources to estimate their degree of DNA-DNA reassociation (dDDN) and ANI, to accurately establish their correct assignment to these species.

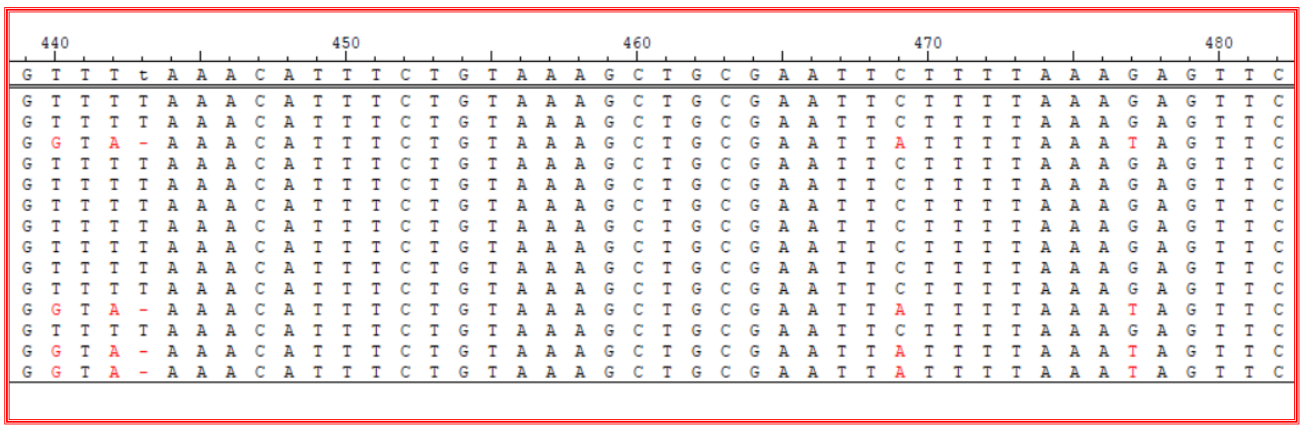
The assembled genome of 21 field Spanish isolates previously identified as L. garvieae and 28 genomes of L. garvieae accessible in Genbank (https://www.ncbi.nlm.nih.gov/genome/browse/#!/prokaryotes/699/) were used for ANI and dDDH calculations compared to the genomes of L. garvieae CCUG 32208T and L. petauri 159469T.
Three representative strains (L. garvieae CCUG 32208T, L. petauri 159469T and the Spanish isolate 8831) were biochemically characterized using the Rapid ID 32 Strep, API 50 CH and API ZYM systems. In addition, the morphological characteristics of the colonies of these three strains, as well as of other field Spanish isolates, were compared after their growth on trypticase soy agar (TSA) supplemented with 5 % sheep blood and M17 agar.

The dDDH and ANI values between 21 (out of the 49 isolates) and the type strain of L. garvieae ranged between 80.7-99.9% and 98.1-100%, respectively, confirming the correct species assignment.
On the other hand, 19 isolates previously identified as L. garvieae exhibited dDDH values between 80.3-90.8% and ANI values between 97.7- 99.0% with the type strain of L. petauri suggesting therefore that they should be reclassified as belonging to this species.
Finally, nine isolates showed dDDH and ANI values lower to the thresholds proposed for species delineation (≥70% for dDDH and 95-96% for ANI) indicating that they do not belong to either species.
Strains correctly identified as L. garvieae, those that should be reclassified as L. petauri and those that are neither species were isolated from different sources including fish, water, mammals and humans. These isolates were identified in many cases by sequencing the 16S rRNA gene or PCRs targeting this gene.
The PCRs assays used in routine laboratory diagnosis to confirm the identification of L. garvieae were developed before L. petauri was described, which together with the high similarity in the 16S rRNA gene sequences of both species (99.93%) could explain the misidentification of many strains of L. petauri as L. garvieae.

Besides, it must be pointed out that colonies of those field isolates that should be reclassified as L. petauri based on dDDH and ANI values, did not exhibit the orange-coloured morphology displayed by the type strain of L. petauri when grown anaerobically for 24 h at 30° C on M17 agar nor did they exhibit the beta-hemolytic activity of L. petauri on blood agar.

These results indicate that many of the isolates identified as L. garvieae from cases of trout lactococcosis are, in fact, L. petauri, and therefore, both species should be considered as the etiological agents of fish lactococcosis.
Furthermore, in order to get a better knowledge of the roles of both infections in the epizootiology of lactococcosis, new molecular diagnostic methods that allow quick and accurate discrimination between the two species are urgently needed.
At this purpose, a study on ITS region among 16S-23S rRNA genes was carried out using as reference strain for L. petauri, the Greek strain LG_SAV_20, isolated in 2007 from rainbow trout and the genome of which was already reported to GenBank, and Italian, Spanish, Turkish, and Greek strains characterized from a phenotypic and genetic point of view in the context of SUPERTROUT.
Its sequencing analysis, through a multiple alignment, showed single nucleotide polymorphisms (SNPs) characteristic of L. petauri. Considering HM241916 as reference sequence for L. garvieae, L. petauri strains exhibited a single base insertion (A) at position 219, a single base deletion at position 443, as well as 5 SNPs at positions 358 (T), 440 (G), 442 (A), 469 (A), and 477 (T) (Fig. 1).
These genetic differences in ITS region could be used as diagnostic markers to easily discriminate between these two very similar species and avoiding misidentification.

Figure1: Alignement of the 16S-23S rRNA ITS sequences that include the reference sequences for L. garvieae HM241916 and L. petauri LG_SAV_20 and field isolates from Italy, Greece, Spain, and Turkey,



European Association of Veterinary Laboratory Diagnosticians

A YOUNG RESEARCHER Across the World

Alonzo Alfaro-Núñez, MSc, PhD

Associate Researcher at the Section for Evolutionary Genomics, GLOBE Institute,

University of Copenhagen -Denmark

In short, I came to Denmark directly from Costa Rica with an undergrad I was very privilege to growth up in Costa Rica, and since a very young age I saw the giant leatherback turtle coming to nest during our summer vacations. This first interaction defined what I wanted to do, to become a researcher marine biologist.

After completing my undergrad education in biology 15 years ago, I applied for an undergrad scholarship in Denmark, as it was well known that leatherback turtles (Dermochelys coriacea) could migrate from the Caribbean warm waters to the cold North Sea in one single season. Thereafter, I got offered to complete my master degree at the University of Copenhagen and for my master thesis project I went back to the Caribbean to determine multiple paternity in green turtles (Chelonia mydas) at Tortuguero.

Then, I wrote my own PhD proposal to study the co-evolution between marine turtles with a herpesvirus infection (ChHV5), which it is believe to cause the fibropapillomatosis diseases in the chelonids. This project allowed me to travel and get collaborations from many different places such as Portugal, Scotland, Greece, Oman, Kuwait, USA, Mexico, Puerto Rico and Thailand. It was an honor and I still maintain most of those collaborations. My first postdoc has also a big part dealing with the pathogenicity of this disease and in particular with the virus.

Along the way, I have also worked with other organisms, in particular with birds solving their evolutionary processes. In 2016 I got an offer for a Lecturer/Researcher position in the Galapagos archipelago and Ecuador, thus, I moved there nearly 4 years. I returned back to Denmark (now my new home) just before the pandemic. I was supposed to start a funded project to develop methods for the environmental detection of viruses, but as many other researchers, I was also forced to work on COVID-19 and the detection on minks and the phylogenetic characterization of SARS-CoV-2. The previous experience obtained with ChHV5 and COVID-19, opened me the doors to work now on the sequencing of the microbiome to determine their association with cancer genes. I am also actively working as an external consultant on the Blue Carbon initiative in a project in Magdalena Bay in Baja California to implement and use eDNA tools to quantify biodiversity. I was invited and went there for two weeks last December 2021 to collect environmental water and sediment samples for our research, a fantastic beautiful place.

Now, little by little I am trying to find my way back to keep working with marine turtles and academia, exclusively I hope. But for now I keep enjoying the ride of scientific research. It has been a long journey, from a boy looking at the sea wondering where those giant dinosaurs came from, to now. But regardless where the path will continue leading me, I know the recipe is to work hard and to produce good data.

I wouldn't dare to call my self an expert in nothing, I know very little of everything. But I am extremely lucky that through my career I have deeply interacted with many fields of knowledge such as virology, environmental sciences, bioinformatics, microbiology, environmental chemistry and genetics, with real experts in those fields. Thus, I may have learned a bit. My humble advice, be always open to collaborate with others despite the differences on background.

One wise man once told me, the secret is to look at the small details, and so, I focus on the small details in a big world.

Be kind to others, all the best,

Alonzo Alfaro-Núñez, MSc, PhD
Associate Researcher and Molecular Biologist at the Clinical Biochemistry Department, Næstved Hospital; and at Section for Evolutionary Genomics, GLOBE Institute, University of Copenhagen
and
External Consultant and Advisory at MarVivo Blue Carbon Project
Magdalena Bay, Baja California

Mind the date!

Bari (Italy) 15-17th September 2022
4th IECVM Conference

The 4th ICECVM is a three-day event dedicated to Veterinary Microbiology, with sessions covering bacteriology, virology, mycology, antibiotic resistance, etc., under the One-Health umbrella.

A satellite event consisting of a training school on veterinary clinical bacteriology, organized by the European Network for Optimization of Veterinary Antimicrobial treatment (ENOVAT) with be held on 14th-15th September 2022.

In parallel will be held the 4th ICECVM scientific conference. There will be sponsored exhibition and workshop events, providing a unique collaborative environment

Link https://icecvmconf.org/

EAVLD Members, we need you!

Would you to contribute to our mission?

Do you have news for the EAVLD newsletter?
Do you are a young researcher working across Europe? Tell us your story!
Please send to Marzia Pezzolato, marzia.Pezzolato@izsto.it before october, 31 2022.

Do you have ideas or proposals to share with us?
Contact marialaura.corrente@uniba.it

Newsletter by Marzia Pezzolato