PROCINORTE

2018 Annual Work Plan Report – Animal Health

Member	Specific Objective	Planned Activities	Expected Results	Date	Venue	Delivery Instrument	Results	Funding Mechanism	Participants
ANIMAL HEALTH	Enhanced cooperation among Mexico, US and Canada	Workshop and TF Meeting: Genomics Tools for Animal Health Research. Subjects covered at 2018 Workshop: * Genomic tools for biodefense * Genomic tools for parasites * Animal genomes and disease resistance * Bacterial genomics * Microbiome discovery with genomics	Scientists are updated on issues related to priority animal diseases.	Oct. 2018	Mexico	workshop	Completed	In kind support from ARS/USDA and CFIA	C. Gay J. Lopez E. Loza
	To have a current summary of emerging animal diseases with potential impact on trade between the northern countries.	To review and update document prepared in 2006 by the AHTF.	Revised summary document with up-to-date information on current and emerging animal diseases of trilateral interest to trade.	June 2018	Mexico, Canada, USA	Virtual meeting	In progress	In kind support from ARS/USDA, INIFAP and CFIA anticipated	C. Gay J. Lopez E. Loza
	To conduct research projects of trilateral interest.	The following collaborative PROCINORTE Research Projects are on-going: • North American Assessment of Bluetongue and *Culicoides* Vectors • Performance of New Reagents and Methods for the Diagnosis and Control of Equine Piroplasmosis - This is a sub-project of the in umbrella project: "Development of Strategies to Control Tick-Borne Babesial Pathogens of Livestock • Collaborative Research on Vesicular Stomatitis Diagnosis	Work plan implemented according to research objectives.	December 2018	Canada, USA, Mexico	Research Activities, Visit	Ebola and Nipah virus project completed. Manuscript published FY 2018	**USDA-ARS** BB - $50K 1Y **CFIA** BT - $39.5K 2Y BB - $12K 1Y **INIFAP** BB - $20K 1 Y	C. Gay J. Lopez E. Loza

	Heartwater Gap Analysis Workshop	Organize workshop with the support of CaribVet in Guadeloupe.	Outcome will u=include a gap analysis report that will include research priorities to address gaps in knowledge and veterinary medical countermeasures	October 2018	Guadeloupe	Workshop	In progress	In kind support from USDA, Mexico, and CaribVet	C. Gay
	Hands on training of Mexican scientists in U.S and Canada	Laboratory training at CFIA and USDA at the Animal Diseases Management Unit, Pullman, Washington).	Customized training for identified scientists in priority topics	Nov, Dec 2018	Canada, USA	Canada, USA	Competencies improved in selected areas	In kind support from ARS/USDA, INIFAP and CFIA	C. Gay J. Lopez E. Loza
	ANIMAL HEALTH SUBTOTAL $15,000.00								

PROCINORTE

Animal Health Task Force Report

Submitted October 15, 2018, to the PROCINORTE Board of Directors

Animal Health Task Force
Dr. Cyril G. Gay, Chair, USDA-ARS, United States
Dr. Elizabeth Loza-Rubio, CENID-Microbiology, INIFAP, Mexico
Dr. José López, CFIA, Canada

Contents

Executive Summary

This year the members of Animal Health Task Force of PROCINORTE met in Mexico City to discuss the timely subject of genomics and its tools as they are used for the advancement of animal health in North America. Canadian, Mexican and U.S scientists, representing the Canadian Food Inspection Agency (CFIA), the United States Department of Agriculture's Agricultural Research Service (USDA-ARS) and the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) got together in a spirit of collaboration to share their experiences in this scientific area, which is gaining momentum and has resulted in outstanding advances in the understanding of biological processes and their strategic manipulation and control for the benefit of animal health, public health and the environment. This year, in addition to the North American participants, the Caribbean Animal Health Network (CaribVet), was invited in order to share experiences of mutual interest in this area and to discuss possible collaborative initiatives to advance animal health in the region. The workshop brought together a total of 32 scientists: 19 from Mexico, eight from the United States, four from Canada, and one scientist from Cuba, representing CaribVet. Additionally, research collaborations have been initiated with one project completed, and scientific exchanges and training was provided for three INIFAP scientists in laboratories in the United States and Canada.

Overview of PROCINORTE and the Animal Health Task Force

PROCINORTE is an important mechanism to facilitate the institutional and technical integration of the Unites States, Canada, and Mexico under the umbrella of the Inter-American Institute for Cooperation on Agriculture's (IICA) Northern Regional Center. PROCINORTE is a cooperative program in research and technology with four task forces (Animal Health, Plant Health, Tropical and Sub-Tropical Fruits & Genetic Resources) that determine common research priorities. Setting work priorities is a major task, because they are strategically important to guarantee the allocation and effective use of national and international resource availability, and they also are important in developing effective collaboration efforts to take advantages of the opportunities of multinational research, especially in those research fields where there is a need to optimize research resources, avoid duplication of efforts, and optimize the use of facilities, and equipment and to facilitate a broad environmental validation of results. PROCINORTE objectives are to:

1. Promote dialogue to identify priority research issues common to the three countries and to influence the regional, hemispheric and global agendas.
2. Facilitate the exchange of experiences, information and training through the building of linkages among public and private country institutions of the Northern region (PROCINORTE) and between the major research and technology transfer actors in the region, hemisphere and the world.
3. Facilitate the collaboration among the countries to solve problems of mutual interest.

Potential mutual benefits from collaborations include:

a) Perform strategic research for agricultural development.
b) Development of technologies for agribusiness benefit.

c) Strengthen technological exchange.
d) Development and use of methodologies for the establishment of standard norms for common use in commodities trade.
e) Provide solutions to common problems and challenges to help the countries to cover their present population needs more efficiently.
f) Develop scientific solutions to agricultural problems, to increase profitability for farmers preserving their land and natural resources.

The PROCINORTE Animal Health Task Force (PAHTF) is comprised of leading government scientists from the three member countries. The PAHTF meets face-to-face at least once a year by holding scientific workshops on animal diseases deemed to be priorities for North America. The primary goal of these workshop is to determine the disease situation and available countermeasures to control and respond to animal diseases that impact agriculture in the three member countries. The ultimate goal of the workshops is to provide scientists the opportunity to get to know each other and establish research collaborations that will advance research to enhance animal health in the three member countries.

The PAHTF identified the following criteria for prioritizing areas of research:

1. Diseases that impact the movement of animals between Canada, U.S, and Mexico.
2. Diseases that have significant economic and/or public health impact.
3. Diseases that are national priorities for either Canada, U.S, or Mexico.
4. Diseases that are endemic in one or two of the three countries where the disease free-country will benefit from the expertise of the endemic country, including the ability to work directly with a foreign animal pathogen, expert scientists, and pathogen-dedicated facilities.
5. Areas of expertise in one country may complement expertise in another country enhancing the formation of multi-disciplinary research teams.
6. Opportunities to enhance the impact of limited financial resources.
7. Opportunities to develop control measures such as diagnostics and vaccines that can be applied within the three countries, resulting in uniform diagnostic tools and control measures.
8. Opportunities to bank and share samples for diagnostic validation and future research.

The expected results from the activities of the PAHTF include:

1. Sharing of research activities and potential changes in high consequence animal disease control program policies, procedures and techniques.
2. Coordination of research activities to promote collaborations between the US, Canada and México.
3. Development of an understanding of measures used in the US, Canada and México for the control and eradication of high consequence animal diseases and harmonization (where appropriate) of policies and procedures.

Research Collaborations

As a result of the workshop, initial discussion is taking place to potentially establish a North American Consortium on Genomics and Barcoding for Vectors of Animal Disease, potentially including the Caribbean Animal Health Network (CaribVet) as well.
Workshop participants from Canada, Mexico, the US and the Caribbean are currently discussing possible collaborative projects to be submitted for support by our respective Agencies and Departments.
In addition to the above collaborations which are starting to be discussed, the following collaborative PROCINORTE Research Projects are on-going:

- North American Assessment of Bluetongue and *Culicoides* Vectors
- Performance of New Reagents and Methods for the Diagnosis and Control of Equine Piroplasmosis - This is a sub-project of the in umbrella project: "Development of Strategies to Control Tick-Borne Babesial Pathogens of Livestock
- Collaborative Research on Vesicular Stomatitis Diagnosis

The following PROCINORTE project has been completed and the final report was submitted:

- Use of IFN-expressing vectors in control of Nipah and Ebola virus diseases in swine

Training

As a result of the workshops organized by the PROCINORTE Animal Health Task Force, the following scientific exchanges and training was achieved Fiscal Year 2018 (also see Appendix III).

Mayra Cobaxin, Instituto Nacional de Investigaciones Forestales, Agricolas y Percuarias, INIFAP, Mexico:
Training to acquire the tools for growing *Anaplasma marginale* in tick cell lines at the Animal Diseases Research Unit, Agricultural Research Service (ARS), USDA, Pullman, Washington.

Edith Roja-Anaya, Department of Biotechnology and Animal Health, INIFPA, Mexico:
Training to initiate collaborative metagenomics research in small ruminants, National Animal Disease Center (NADC), Agricultural Research Service (ARS), USDA, Ames, Iowa.

Elizabeth Loza Rubio, Department of Biotechnology and Animal Health, INIFPA, Mexico:
Training to sort trapped midges, CFIA Lethbridge Laboratory, Alberta, Canada.

Outreach

Students
The workshops organized by the PROCINORTE Animal Health Task Force included graduate students from Mexico and provided opportunities for official presentations and interactions with leading scientists in their field from Canada, Mexico and the United States of America. Opportunities for training and exchange are also considered when the budget provided by the PROCINORTE Executive Committee allows

Caribbean Animal Health Network
The Caribbean islands consist of 34 countries and territories. Animal production is an important industry that is critical to the economies of the island nations and a key contributors to food security. Importantly, the Caribbean are geographically part of North America with the Caribbean islands bordering the United States and Mexico. As such, the emergence of new infectious diseases in the Caribbean with its tropical climate poses a significant threat to North America and the PROCINORTE Animal Health Task Force has reached out to the Caribbean Animal Health Network – CaribVet https://www.caribvet.net/

CaribVet provides strategic opportunities for research collaborations that are likely to help protect animal agriculture in North America against the threat of emerging pests and infectious diseases that are present in the Caribbean. A good example is the Tropical Bont Tick, which is also the tick vector for Heartwater, an important disease of ruminants in Africa and some of the Caribbean islands. Strategic opportunities for collaborations with CaribVet include:

- Epidemiological surveillance, control and eradication of diseases
- Identification of pathogen
- Prevention and emergency preparedness plans for priority diseases

Accomplishments in Fiscal Year 2018, include the participation of CaribVet at the annual PROCINORTE Animal Health Task Force workshop. Participation from CaribVet at the workshop provided a forum for scientific exchanges and areas of common interest research project that are relevant to North America. Future plans for Fiscal Year 2019 include the participation of CaribVet in the annual PROCINORTE Animal Health Task Force Workshop in Mexico City and participation of a PROCINORTE representative in a gap analysis workshop on Heartwater organized in Guadeloupe by USDA and CaribVet.

Appendix I

Agenda

September 3, 2018
Registration, Reception, Key Note Presentations (18:00 – 20:00)

1. **Welcome and review of the workshop program and objectives**

   Jose Lopez, CFIA
2. **CaribVet: Overview of Research Interests**

   Carmen L. Perera González, Centro Nacional de Sanidad Agropecuaria (CENSA), Cuba
3. **Overview of genomics research in animal health**

   Stephen White, USDA-ARS

September 4, 2018
Session 1: Introductions, Purpose, Expected Outcomes (09:00 – 09:30)

1. **Welcome & Introductions**

   Fernando de la Torre Sánchez, Director General, INIFAP, Mexico
   Elizabeth Loza-Rubio, INIFAP – TF Member, Mexico
   Jose Lopez, CFIA – TF Member, Canada
   Cyril Gay, USDA-ARS – TF Leader, USA
   Break (09:30 – 10:00)

Session 2: Genomic tools for biodefense (10:00 – 12:30)

Chair: Cyril Gay, USDA-ARS

2. Identification and Whole Genome Sequencing of Known, Unknown and Unexpected Viruses at the Canadian Food Inspection Agency's National Centre for Foreign Animal Disease (10:00 – 10:30)

   Oliver Lung, National Centre for Foreign Animal Disease Canadian Food Inspection Agency
3. Genomic tools: a Canadian perspective on invasive species, vector species, viral identifications, and food fraud (10:30 – 11:00)

   Rob Young, University of Guelph's Biodiversity Institute of Ontario
4. New approaches to diagnostics of avian respiratory disease (11:00 – 11:30)

   Claudio Afonso, USDA-ARS
5. Virome of backyard swine in Mexico reveals new viruses (11:30 – 12:00)

   Rodrigo Jesús Barrón Rodríguez, UNAM/Elizabeth Loza-Rubio, INIFAP

6. Spatiotemporal distribution of endemic classical swine fever virus in Cuba and molecular characterization of a low virulence strain (12:00 – 12:30)

   Carmen L. Perera González, Centro Nacional de Sanidad Agropecuaria (CENSA), Cuba

Lunch (12:30 – 13:30)

Session 3: Genomic tools for hemoparasites and ectoparasites (13:30 – 15:30)

Chair: Jose Lopez, CFIA

7. Transcriptional analysis of Babesia stage specific parasites to control bovine babesiosis (13:30 – 14:00)

   Massaru Ueti, USDA-ARS

8. Analysis of *Haemonchus* spp trancriptome as a model for anthelmintic resistance drug and immune tolerance studies (14:00 – 14:30)

   María Eugenia Lopez Arellano, INIFAP

9. Genomics insights of *Enterococcus casseliflavus* PAVET15 isolated from the cattle tick *Rhipicephalus microplus* (14:30 – 15:00)

   Bernardo Sachman Ruiz, INIFAP

10. Culicoides barcoding to metabarcoding of an important disease vector species (15:00 – 15:30)

    Rob Young, University of Guelph's Biodiversity Institute of Ontario

Break (15.30 – 16:00)

Session 4: Animal genomes and disease susceptibility (16:00 – 17:30)

Chair: Elizabeth Loza-Rubio, INIFAP

11. Manual annotation of the porcine genome for cross translational human-animal health research (16:00 – 16:30)

    Harry Dawson, USDA-ARS

12. Beyond genome-wide association studies for respiratory diseases in cattle (16:30 – 17:00)

    Eduardo Casas, USDA-ARS

13. Identification of loci associated with susceptibility to Mycobacterium avium subspecies paratuberculosis (17:00 – 17:30)

    Stephen White, USDA-ARS

September 5, 2018

14. Identification of genetic markers associated with goat scrapie resistance (9:00 – 9:30)

    Stephen White, USDA-ARS

Session 5: Bacterial genomics (9:30 – 11:00)

Chair: Jose Lopez, CFIA

15. Whole Genome Sequencing for tracing bovine tuberculosis transmission in Canada: A retrospective study (9:30 – 10:00)

    Olga Andrievskaia, Ottawa Animal Health Laboratory, Canadian Food Inspection Agency

16. Molecular epidemiology of cattle tuberculosis in Mexico through whole-genome sequencing and spoligotyping (10:00 – 10:30)

    Claudia Angélica Perea-Razo/Feliciano Milián Suazo Universidad Autónoma de Querétaro
17. Genome analysis of hemoplasmas in México (10:30 – 11:00)

    Rosa Estela Quiroz Castañeda, INIFAP
    Break (11:00 – 11:30)

Session 6: Microbiome discovery with genomic tools (11:30 – 15:00)

    Chair: Cyril Gay, USDA-ARS
18. Metagenomic study of bacterial microbiome in *Tilapia nilotica* (11:30 – 12:00)

    Rocio Parra Laca-UNAM/Elizabeth Loza-Rubio, INIFAP
19. Gut microbiome in small ruminants (12:00 – 12.30)

    Edith Rojas Anaya, INIFAP
    Lunch (12:30 – 14:00)
20. The Microbiome and Microbial approaches for targeting antibiotic-resistant bacteria in the livestock sector (14:00 – 14:30)

    Edgar Dantan González, Universidad Autónoma del Estado de Morelos
21. Microbiome in migratory wild Ducks (14:30 – 15:00)

    Gary García Espinosa, Universidad Nacional Autónoma de México
    Break (15:00 – 15:30)

Session 7: Research Planning and Next Steps (15:30 – 17:30)

22. Panel Discussion with Presenters (15:30 – 17:00)

    Research needs
    Research priorities
    Potential collaborations

23. Summary/Next Steps (17:00 – 17:30)

    Cyril Gay, USDA-ARS – TF Leader
    Jose Lopez, CFIA – TF Member
    Elizabeth Loza-Rubio, INIFAP – TF Member

APPENDIX II

Abstracts

Identification and Whole Genome Sequencing of Known, Unknown and Unexpected Viruses at the Canadian Food Inspection Agency's National Centre for Foreign Animal Disease

Dr. Oliver Lung, National Centre for Foreign Animal Disease Canadian Food Inspection Agency

The Canadian Food Inspection Agency National Centre for Foreign Animal Disease (CFIA NCFAD) conducts diagnosis, surveillance and research on high consequence veterinary and zoonotic viral diseases in its containment level (CL) 2, 3 and 4 laboratories in Winnipeg, Manitoba. This presentation will include an overview of Canada's only CL3 high throughput sequencing facility and ongoing activities at the new Genomics Unit which includes establishing of methods for sequencing of a broad range of known viruses, as well as unknown or unexpected pathogens in support of diagnostic, surveillance and research activities.

Genomic tools: a Canadian perspective on invasive species, vector species, viral identifications, and food fraud

Dr. Rob Young, University of Guelph's Biodiversity Institute of Ontario

The use of genomics tools has become more prominent in applied research, and many examples are now being reported by mainstream media. My talk will explore cases where genomic tools have been applied both globally and within Canada. These cases will include examples within the scope of animal health and beyond and will include: vector and disease screening, animal vector species biosurveillance, biosurveillance for detection and early warning of invasive species, flora DNA barcode database establishment, viral infection diagnostics using next-generation sequencing, natural health product research, animal processing and packaged food content analysis, and food fraud. I will discuss the importance of these efforts for Canadian producers and consumers and how Canadian agencies and industry are moving toward increased use of genomics tools.

New Diagnostic Approaches for Avian Respiratory Diseases

Exotic and Emerging Avian Viral Diseases Research Unit, Southeast Poultry Research Laboratory, US National Poultry Research Center, ARS, USDA, 934 College Station Road, Athens, GA 30605, USA

Dr. Claudio Afonso, United States Department of Agriculture –Agricultural Research Service

Avian Influenza, Newcastle disease, and Infectious Bronchitis are among the most serious avian respiratory diseases of poultry. These diseases are caused by small RNA viruses and are often associated with bacterial infections. Molecular diagnostics have contributed enormously to the detection and characterization of respiratory infections; however rapid diagnostic tests based on real time PCR have limitations, as they are agent specific, fail to detect mutants or specific strains, and do not provide specific genetic or epidemiological information. Advances in high-throughput sequencing allow for deep sequencing of large amplicons (AmpSeq) or randomly amplified nucleic acids, and the sequencing data in turn provide 1) confirmation of the PCR results, 2) the potential to genetically categorize the result, 3) the potential to identify multiple lineages of a virus in a single

sample tested with a single set of primers, and 4) detection of mixed infections. We used total RNA extracted from infected allantoic fluids, clinical samples or fixed tissues to identify avian infectious agents. Random sequencing of total RNA allowed detection of mixed infections including co-infections of Newcastle disease virus with bacteria, avian Influenza virus and infectious bronchitis virus. More recently, long read sequencing, based on the MinION Oxford Nanopore device provides highly sensitive, specific, and cost effective detection of multiple agents. In summary, respiratory diseases outbreaks can be better understood with new technologies for rapid genome characterization.

Virome of rural backyard swine from Morelos, Mexico

MS. Rodrigo Jesús Barrón Rodríguez, Universidad Nacional Autonoma de Mexico, Dr. Elizabeth Loza-Rubio, Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias (INIFAP)

Domestic swine is an important species for food production and economy, and it has been introduced by the human in a wide variety of environments, keeping close contact with humans and other animals, setting risk interfaces for mutation, adaptation and presence of diseases, even zoonosis. Little is known about the viral communities of backyard swine, one of the most important productive systems in developing geographical regions. The aim of this work was to determine the virome from nasal and rectal swabs of 23 rural backyards porcine in Morelos state, Mexico. The obtained virome showed a different viral diversity than those reported before, with an abundant presence of bacteriophages associated to healthy status of the sampled porcine and new viral variants not reported previously in the country (circular ssDNA viruses, sapovirus, bocaparvivirus and mamastrovirus). All of these viruses must be monitored to better understand their ecological function and prevent infectious and/or zoonotic risks on anthropized environments.

Spatiotemporal distribution of endemic classical swine fever virus in Cuba and molecular characterization of a low virulence strain

In collaboration of Dr.Liani Coronado Báez, Dr. Osvaldo Fonseca Rodríguez, Dr. Laymara Amarán, Dr. María Irian Percedo Abreu, Dr. María Teresa Frías-Lepoureau, Dr. Llilianne Ganges Espinosa and Dr. Carmen L. Perera González
Centro Nacional de Sanidad Agropecuaria (CENSA), Cuba, Laboratorio Nacional de Diagnóstico Veterinario, Ministry of Agriculture, Cuba, Centre de Recerca en Sanitat Animal (CReSA), España
In Cuba, Classical Swine Fever (CSF) has become an endemic disease since 1993 with several outbreaks each year, despite vaccination and control programs that are implemented. Its presentation forms are diverse. From chronic and acute form to subclinical, depending on the virulence of the strain, and the immunological condition and age of the pigs. Characterizing the disease, as well as assessing the field situation would enable to implement measures of effective control to eradicate it. For this study, information provided by Laboratorio Nacional de Diagnóstico Veterinario (National Vet Diagnostic Lab) about 846 CSF confirmed focus at national level between 2010 and 2016 and clinical signs reported were used. The probabilistic space-temporal permutation model (SaTScan 9.4) proved that high and low rates grouping prevail in the West and East, respectively. Some works were made to assess the positive selection pressure on E2 partial gene of classical swine fever viruses from infected animals with non-acute haemorrhagic form of the disease to get insights into the mechanisms governing virulence and the driving forces of classical swine fever virus evolution in swine population under regular vaccination programs. It was found that G761R mutation was caused by positive selective pressure that seemed to be an

important factor in the virulence of the virus inducing variation on the clinical manifestations of the disease in the field. Nevertheless, a pathogenicity study of isolated with the same mutation (Pinar de Rio, Holguín y Santiago de Cuba) showed different clinic performances. This study shows that Pinar del Rio strain, is clearly low virulent during the endemic phase. Next, we analysed the complete nucleotide sequence of the Pinar del Rio virus isolated. More importantly, a novel unique poly-uridine tract was found in the 3'UTR of the Pinar del Rio virus, which was not found in the parental Margarita virus. Some studies on the late finding are carried out at present. These data provide novel insights into viral molecular features associated with adaptation of CSFV for persistence in the field.

Transcriptional analysis of Babesia stage specific parasites to control Bovine Babesiosis

Dr. Massaro Ueti, United States Department of Agriculture –Agricultural Research Service

Tick cattle fever caused by *Babesia bovis* and *B. bigemina*, poses a significant challenge to the livestock industry worldwide. More than half population of cattle that live in tropical and sub-tropical areas are in constant risk of Babesia infection. Both the bovine host and tick vector of these pathogens facilitate endemic disease stability as bovines become persistently infected and serve as reservoirs for transmission by ticks. Parasites are acquired when tick vectors ingest *Babesia* blood stages during feeding. Within the midgut lumen of female ticks, gametogenesis induced and zygote formation occurs. The zygote infected ticks gut epithelial cells and culminate in production of kinetes which are released into hemolymph to invade other tick tissues including ovary epithelial cells. Parasites are transmitted transovarially to the next tick generation. Within larva stages, parasites invade salivary glands where *Babesia* transforms into sporozoites, the infectious stage to bovine hosts. Replication of *Babesia* in the erythrocytes is the cause of hemolytic anemia, hemoglobinemia, hemoglobinuria and icterus, in some case, death. Survived animals become chronically infected and represent reservoirs for tick transmission. The scarcity of circulating *Babesia* in the tick vector is a challenge to obtain sufficient quantities for molecular analysis. A method of *B. bovis* tick specific stages enrichment was developed to facilitate the evaluation of *B. bovis* specific stage transcripts. RNA sequencing was performed to compare transcriptomes from *B. bovis* during its development in bovine erythrocytes and kinetes that traffic through tick hemocoel. The analysis of transcriptome resulted in the discovery of *B. bovis* up-regulated genes during infection of mammalian or tick hosts. The up regulated genes during infection of cattle or tick vector can be used as potential targets for the development of a subunit vaccine to control disease or transmission of *B. bovis*.

Analysis of *Haemonchus* spp. Transcriptome as a model for Anthelmintic Resistance Drug and Immunotolerance Studies

López-Arellano ME, Ph.D. National Centre of Disciplinary Researcher in Veterinary Parasitology of the National Institute for Forestry, Agriculture and Livestock Research

The wide diversity in the biological processes in the nature of parasites is a reflex of their genome diversity. The ruminant parasitic nematode Haemonchus spp. in particular has evolved mechanisms to invade the host tissue and at the same time to evade the host immune response. These mechanisms are challenges for exploring new alternatives in the development of control measures. Under grazing tropical and template regions, *Haemonchus* spp. is the most pathogenic parasite worldwide. Also, these nematode species have shown multiple anthelmintic-drug resistance in small

ruminants and young cattle, which threaten both the animal health and productivity. Mexico is a large country with wide climate diversity, where milk and meat production is carried out in extend tropical and template areas from southern and center of Mexico.
Because of the high prevalence of gastrointestinal nematode (GIN) and the increasing of anthelmintic problems in Mexico different strategies of control are being explores. The study of immune mechanisms related to high responder hosts against GIN have been carried out in our Institution in collaboration with other Academic and Researchers groups. Our interdisciplinary work has focused in the inflammation process and in the study of specific immune molecules against GIN. Through these studies some important cytokines and SNP were identified. In addition, our group is involved in the identification of some secreted products with potential activity to modulate de host-response. Anthelmintic resistance (AR) is a complex process and it is actually difficult to reduce. In order to understand some anthelmintic resistance processes some important tools i.e., molecular techniques have been developed. These techniques have improve the AR diagnosis against drugs such as Benzimidazoles and Ivermectins (macrocyclic lactones) and have contributed to establish other values tools i.e., the sensitive resistance-detection test and also in the inhibition of genes involved in AR processes. The control of parasitic infections caused by the GIN complex is not an easy task; nevertheless the mechanisms of parasites to tolerate the toxicity of anthelmintic drugs and to evade the host immune defense in the nematode species Haemonchus spp. and more recently the genus *Cooperia* spp. are been explored using genomic tools.

Genomics insights of Enterococcus casseliflavus PAVET15 isolated from the cattle tick Rhipicephalus microplus.

Dr. Bernardo Sachman Ruiz, Instituto Nacional de Investigaciones Fostestales, Agricolas y Pecuarias (INIFAP)

Enterococcus casseliflavus PAVET15 was obtained from an engorged female tick that showed symptoms of bacterial infection, such as the presence of an exudate at the genital orifice. The sequence genome obtaining 3,722,480 bp under the accession no. MUBE00000000, comprises 3,594 coding proteins, 58 rRNAs, 50 tRNAs, and 41.93% GC content. We found 93.58% identity with the closest genome *E. casseliflavus* EC20 and high synteny. Interestingly, 641 genes of PAVET15 are not present in EC20, of which were found 28 phages, 6 unknown, 16 putative, 436 hypothetical, 14 transposases and 145 with a known function genes. On the other hand, PAVET15 does not present 236 genes that EC20 has. The genome comparison of *Enteroccocus casseliflavus* determines the lifestyle and adaptive process in the infection of the cattle tick, and its possible biocontrol.

Culicoides barcoding to metabarcoding of an important disease vector species

Dr. Rob Young, University of Guelph's Biodiversity Institute of Ontario

Infectious diseases are a major global concern for their impacts on human health and the health of agricultural animals. The spread of many high impact viruses on both animals and humans is facilitated through arthropod species. One arthropod genus of concern in its role as a disease vector is Culicoides (Latreille, 1809). Using the Bluetongue disease as an example, my talk will focus on the complexity of the relationships between vector, virus, disease, and infected animals concerning

the application of molecular tools. This will include discussion of several different molecular approaches including DNA barcoding of individual specimens, metabarcoding environmental DNA samples, and a targeted assay approach using eDNA as the source of the template DNA. Finally, I highlight the need for a coordinated global effort to research Culicoides and Culicoides transmitted diseases using standardized methodologies, informed response protocols, and standardized, accessible data storage.

Manual annotation of the porcine genome for cross-translational human-animal health research

Harry D. Dawson, Ph.D., United States Department of Agriculture –Agricultural Research Service, Diet, Genomics, Immunology Laboratory, Beltsville Human Nutrition Research Center

The use of swine as biomedical research models for humans has increased dramatically in the last decade. In addition, several recent reports indicate that porcine physiology may benefit by introduction of genes that are missing from pigs. The utility of such models is dependent on a robust and accurate bioinformatics infrastructure. Significant errors exist in machine-assembled genomes. For example, only around 65% of protein coding genes are properly assembled and annotated in the current builds of porcine genome. In addition, many pseudogenes are inaccurately reported annotated as protein coding genes. To address these issues, we have manually assembled and compared the presence and structure of over 10,000 porcine genes, related to immunity and metabolism, in pigs, mice and humans. We found that for immune related genes, the overall frequency of gross protein domain structural preservation between human and pig is nearly 2X that of mouse to human and pig to mouse. Pigs have far less unique immune response genes than mouse. Immune-related gene family expansion in pigs relative to humans has occurred at less than half the rate of mice. Familial gene expansion of pig pattern recognition receptors (PRRs) superfamilies relative to humans has occurred at a reduced rate compared to mice. Last, we observed contraction in components of porcine inflammasome components and PRRs involved in the immune response to viruses and bacteria. Several of these genes are reported as present in the porcine genome. Replacing these pseudogenes with orthologs from bovine or other species may enhance disease resistance as well as improve the utility of pigs for cross-translational human-animal health research.

Beyond genome-wide association studies for respiratory diseases in cattle.

Dr. Eduardo Casas, United States Department of Agriculture –Agricultural Research Service, National Animal Disease Center, Ames, IA, 50010, USA

Loss of profitability is a consequence of diseases caused by respiratory diseases in cattle (known as shipping fever). Studies in genetics of the immune system (Immunogenetics) initially focused on the major histocompatibility complex (MHC), which harbors genes that play key roles in immune response. However, it is now known that additional genes residing outside the genomic region of MHC also play a role in immune response. Identifying the genes throughout the genome, their products, and understanding their function and interaction with other molecules will assist in the development of intervention strategies to improve of resilience, tolerance or, resistance to pathogens associated with respiratory diseases. Since the advent of high throughput sequencing technology, it is possible to identify differences in the genome (DNA), transcriptome (RNA), and proteome

(proteins), which could potentially be associated with the ability of the livestock to cope with a pathogen. Genome-wide association studies have been used to identify putative genes, and variants within or near the genes, that could potentially be associated with the immune response of livestock. Transcriptomic studies have been done to compare gene expression at the messenger RNA level between challenged and control animals. Similarly, expression of small non-coding RNAs (microRNAs, etc.), have been studied to understand their roles in expression of genes in animals infected with a pathogen. The proteome has also been studied to understand the expression differences between infected and non-infected animals when livestock is infected with a specific pathogen. An understanding of how an animal responds to a pathogen is needed for the development of methods to identify exposure to the pathogen, and potentially strategies to modify or improve the animals' immune response.

Identification of loci associated with susceptibility to Mycobacterium avium subspecies paratuberculosis

Dr. Stephen White, United States Department of Agriculture –Agricultural Research Service
Johne's disease is caused by Mycobacterium avium subspecies paratuberculosis (MAP) in many ruminant species. Subclinical infections result in decreased milk production, and clinical cases can involve diarrhea, progressively worsening emaciation, and death. Annual losses to the U.S. dairy industry alone have been estimated in excess of $1.5 billion. An existing vaccine does not change the probability of infection and antibiotic treatment is unlikely to cure animals of MAP, so alternative measures are a critical research need. Naturally occurring genomic variation could be used in selective breeding to improve susceptibility to MAP at the individual animal level, and this approach could have disproportionate benefits at the herd level by eliminating "superspreaders" before they become infected and transmit MAP. The challenge is to identify genetic loci with large effects on MAP susceptibility, and multiple approaches have been used to this end. Genome-wide association studies (GWAS) have been performed using phenotypes of MAP tissue infection as well as MAP tolerance measures, and multiple genomic loci have been identified as associated with each. These analyses have been extended by fine-mapping to refine the physical location of the underlying genomic variants, genetic pathway analyses like GSEA-SNP to robustly identify additional genomic regions of interest, and comprehensive analyses and comparisons of data across populations. While multiple genomic regions have been identified, there are still opportunities to identify causal variants with robust predictive value across populations.

Identification of genetic markers associated with goat scrapie resistance

Dr. Stephen White, United States Department of Agriculture –Agricultural Research Service
Scrapie is the transmissible spongiform encephalopathy of sheep and goats. While the ARR allele of the prion gene in sheep confers strong resistance to classical scrapie, for many years there has not been widely recognized resistance in goats. In some countries, goat scrapie has become a bigger problem than sheep scrapie. Early epidemiological evidence highlighted goats with either S146 or K222 amino acid substitutions in the prion protein as absent in scrapie cases even though present in herds where herd mates developed disease. To test the resistance conferred by these alleles, we conducted an oral scrapie challenge at birth, during the window for maximal protein absorption through the gut. All controls homozygous for the most common goat haplotype showed clinical scrapie by an average age of 2 years. In contrast, no S146 and K222 heterozygotes have become scrapie positive by clinical signs, live animal tests, or post-mortem scrapie confirmatory tests despite long post-inoculation times approximating or exceeding the commercial lifespans of many

goats. These data highlight the strong resistance to classical scrapie afforded by S146 and K222 in goats. The European Food Safety Authority has recently released a comprehensive review and statement supporting the use of S146 and K222 in breeding goats with strong scrapie resistance to enhance scrapie eradication. They also ranked other alleles by weight of existing research evidence for scrapie resistance, and research opportunities exist for clarifying the scrapie resistance provided by some other common alleles that could benefit many goat producer operations. Expanding such research could enhance scrapie eradication and accelerate the opening of import/export markets in many parts of the world.

Whole Genome Sequencing for tracing bovine tuberculosis transmission in Canada: A retrospective study

Dr. Olga Andrievskaia, National Reference Laboratory for Bovine Tuberculosis
Canadian Food Inspection Agency, Ottawa Laboratory (Fallowfield)

Bovine tuberculosis (bTb) is an infectious chronic disease caused by *Mycobacterium bovis* and presents a risk to the public health and livestock industry. We applied whole genome sequencing (WGS) to investigate the genomic diversity among 168 *M. bovis* isolates originating in Canada over the last 30 years, and to evaluate WGS utility in advanced molecular epidemiological investigations of bTb. While WGS-based typing was in agreement with the broad cluster definition by spoligotyping and variable number tandem repeat (VNTR) analysis, it further identified new subclusters of *M. bovis* strains within homogeneous spoligo-VNTR genotypes, and revealed potential transmission routes. For example, WGS data demonstrated that *M. bovis* spoligotype SB0673 isolates recovered in a bTb outbreak in Alberta in 2016 were genetically different by >90 SNPs from *M. bovis* isolates of the same spoligo-VNTR type recovered from Canadian cattle in 2007 and 2011, and represented a new strain in Canada.

Overall, WGS significantly improved the discriminatory power of molecular typing, proved to be an exceptional tool in monitoring transmissions of endemic strains and defining new introductions, and became an important part of bTB epidemiological investigations and eradication activities in Canada. Harmonization of sequencing and bioinformatics algorithms with NVSL, USDA allowed exchange of WGS genotyping results between our laboratories and aided bTb transmission studies in the broader North American context.

Molecular epidemiology of cattle tuberculosis in Mexico through whole-genome sequencing and spoligotyping

Dr. Claudia Angélica Perea Razo, Ph.D. Universidad Autónoma de Queretano, Dr. Elba Rodríguez Hernández, Centro Nacional de Investigación Disciplinaria en Fisiología y Mejoramiento Animal, INIFAP, Dr. Sergio Iván Román Ponce, Centro Nacional de Investigación Disciplinaria en Fisiología y Mejoramiento Animal, INIFAP, Dr. Feliciano Milián Suazo, Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, Querétaro, Qro., México, Dr. Suelee Robbe-Austerman and Tod Stuber, National Veterinary Services Laboratories, United States Department of Agriculture, 1015 N University Blvd, Ames, IA 50011, USA, Germinal Jorge Cantó Alarcón, Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, Querétaro, Qro., México

Mycobacterium bovis infection in cattle persists in Mexico, posing a threat to human health. Control of bovine tuberculosis, through the National Program Against Bovine Tuberculosis, has led to the decrease of disease prevalence in most of the country, except for high dairy production regions. Genotyping of M. bovis has been performed mainly by spoligotyping and variable number tandem repeats (VNTR), but higher resolution power can be useful for a finer definition of the spread of the

disease. Whole genome sequencing and spoligotyping was performed for a set of 322 *M. bovis* isolates from different sources in Mexico: Baja California, Coahuila, Estado de Mexico, Guanajuato, Hidalgo, Jalisco, Queretaro and Veracruz, from dairy and beef cattle, as well as humans. Twelve main genetic clades were obtained through WGS and genetic diversity analysis. A clear differentiation of the Baja California isolates was seen as they clustered together exclusively. However, isolates from the central states showed no specific clustering whatsoever. Although WGS proves to have higher resolving power than spoligotyping, and since there was concordance between WGS and spoligotyping results, we consider that the latter is still an efficient and practical method for monitoring bovine tuberculosis in developing countries, where resources for higher technology are scarce.

Genomic analysis of hemoplasmas in Mexico

Dr. Rosa Estela Quiroz Castañeda, Anaplasmosis Unit, CENID-PAVET, Instituto Nacional de Investigaciones Fostestales, Agricolas y Pecuarias (INIFAP)

The hemoplasmas are Gram-negative and cell wall-less epierythrocytic bacteria that may cause anemia, oedema, reproductive problems and mastitis in cattle. Two hemoplasmas have been reported in cattle in Mexico: *Candidatus* Mycoplasma haemobos INIFAP01 and *Mycoplasma wenyonii* INIFAP02. A comparative genomics approach revealed the presence of two clades that groups hemoplasmas genomes while the annotation of the genomes showed the presence of virulence operons involved in the protein synthesis (SSU and LSU ribosomal proteins) and proteins such as cardiolipin synthase. This fact could contribute to targeting bacterial cardiolipin domains in the membranes of mycoplasmas as an antimicrobial strategy.

Tilapia *Oreochromis niloticus* bacteriome in semi-humid warm climate aquaculture systems

Ms. Parra-Laca Rocío, CENID-Microbiología, Instituto Nacional de Investigaciones Fostestales, Agricolas y Pecuarias (INIFAP); FMVZ- Universidad Nacional Autonoma de Mexico, Dr. Elizabeth Loza-Rubio, Instituto Nacional de Investigaciones Fostestales, Agricolas y Pecuarias (INIFAP)

There are few studies on the bacterial microbiota typical of tilapia culture, most of the studies focus on bacteria with interest in safety that could affect the final consumer. Therefore, knowing the bacterioma of tilapia is important to determine the composition of the bacterial population in healthy cultured organisms and lay the foundations that allow us in cases of mortality or atypical disease events to compare the bacterial population and power determine with greater certainty the origin of the health problem. With the use of modern tools of massive sequencing the analysis of the microbiome of tilapia cultivated in farms with intensive and semi intensive systems was carried out with two types of coating of the pond: cement and geomembrane. This is an innovative study in the area which provides the basis for decision making in animal health in freshwater aquaculture.

Gut microbiome in small ruminants

Rojas-Anaya E1*, Loza-Rubio E1, Barrón Rodríguez RJ1, Parra-Laca R1, Romero Espinoza JAI2, Vázquez Pérez JA2, Gutiérrez-Hernández JL1, Díaz-Aparicio E1, Cortes-Cruz MA3
Centro Nacional de Investigaciones Disciplinarias en Microbiología Animal, INIFAP; 2Centro Nacional de Recursos Genéticos, INIFAP.

The aim of this work was to describe the microbiome in the digestive tract of goats and sheep from different regions of the Mexican Republic using massive sequencing, to identify microbial agents of interest in production. Oral and rectal swabs were taken from apparently healthy adult goats and sheep from the States of Mexico, Sinaloa, Oaxaca, Tlaxcala, Puebla and Chiapas. The DNA was obtained with a commercial kit and sequenced on the platform Miseq Nextera XT 2x150. The results obtained from the sequencing were filtered by quality, obtaining the fastq files that were used to perform the bioinformatic analyzes necessary to obtain sequences and subsequent bacterial taxonomic characterization by gender and in some cases species. From the processing of the samples, more than 7 million filtered readings were obtained by quality and more than 25,000 assembled sequences were obtained. As a result of the comparison of the sequences in Blast the presence of the following bacterial genera was demonstrated: *Escherichia, Pseudomonas, Salmonella, Campylobacter, Serratia, Klebsiella, Pseudomonas, Vibrio, Bordetella, Enterobius, Aeromonas* and *Pasteurella*. Being the most frequent *Escherichia, Pseudomonas* and *Salmonella*. All these genera presented more than 98% homology with sequences reported in the gene bank, after analyzing the sequences obtained using blastn. In most cases, especially Escherichia, Pseudomonas and Salmonella, assembled cotings of more than 1,000 bp were obtained. Additionally, the presence of bacterial sequences was corroborated by the detection of phage sequences of the same bacteria. Of all these bacterial genera there are reports of their presence in ruminants from different regions of the world, but not necessarily in Mexico. In addition, some other findings were found with fewer sequences, or shorter sequences (less than 1000 nt) and therefore these require further analysis. In this study, the presence of several bacterial genera with high abundance and with sufficiently representative sequences that allow concluding that they are infecting herd populations in regions that are important in the production of sheep and goats.

Microbiome in migratory wild waterfowl

Dr. Gary García-Espinosa, Departamento de Medicina y Zootecnia de Aves, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México

Wild waterfowl are considered reservoirs of the *Influenza A virus*, but other viral species have been notify or described in wild ducks during an outbreak or an epidemiological surveillance like *Anatid alphaherpesvirus, Avian avulavirus 1* and the *Avian coronavirus* for example. Most of these viruses are excreted on cloacal through feces, and feces can be a source for water contamination. Feces have a microbiome that includes mostly bacteria, fungi, and viruses; but bacteria are more studied due its implication on intestinal health in humans and farming animals, while viruses for infection diseases transmission. Regarding wild migratory ducks, viruses will be a primary interest for wild life, animal and wetland health. A first description of viroma in ducks' cloacae shows viral families that belong mainly to prokaryotic followed by eukaryotic in free-living farm ducks in India, where eukaryotic viruses include insects, algae, protozoan, mammals and plants. At this time, we are describing the first viroma of feces on wild migratory ducks during the winter season in a fragment marsh in the central high plateau of Mexico where most of the identified viral families belonged to fish, mammals, bacteria, plants and invertebrates. Viromic in wild migratory waterfowl will open new avenues of knowledge in wild life health beyond the avian influenza in North America region from the genomic point of view, which could give us an idea of possible threats to animal health (Project PAPIIT IN218716).

APPENDIX III

Training

PROCINORTE Secretariat
Inter-American Institute for Cooperation on Agriculture
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PCN/CA-060

Ottawa, November 17th, 2017

Dr. Mayra Cobaxin
Researcher "B"
Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias (INIFAP)
Mexico

RE: invitation letter – Training in the Department of Veterinary, Microbiology and Pathology, Agricultural Reserch Service, Washington State University, Pullman, WA, United States

Dear Dr. Cobaxin

As you know, the Cooperative Program of PROCINORTE is committed to supporting collaboration in agricultural research and technologies areas of trilateral relevance.

On behalf of PROCINORTE, we are inviting you to participate in a training at the Department of Veterinary, Microbiology and Pathology, Agricultural Reserch Service, Washington State University, Pullman, WA, United States from January 29 to March 16, 2018. This training is designed to acquire the tools for growing A marginale in tick cell lines for the production of live material for further studies.

PROCINORTE will provide the round trip air travel as well as support for in-kind lodging and meals up to USD $ 1,000.00. Once your attendance is approved, the PROCINORTE Secretariat will be in direct contact with you regarding the final arrangements before the training takes place.

Kindly confirm your attendance by contacting Gloria Ramirez at gloria.ramirez@iica.int or (613) 230-1044.

We are looking forward to the results of this training which will advance the objectives of the Animal Health Task Force.

Kindest Regards,

Audia Barnett, Ph.D.
Executive Secretary of PROCINORTE/
IICA Representative in Canada
C.C. Dr. Cyril Gay, Leader of the Animal Health Task Force

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PCN/CA-059

Ottawa, November 17th, 2017

Dr. Edith Rojas Anaya
Responsable del Departamento de Biotecnología en Salud Animal
CENID-Microbiología
Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias (INIFAP)
Mexico

RE: invitation letter – Training in the National Animal Disease Center ARS, Ames, Iowa, United States

Dear Dr. Rojas

As you know, the Cooperative Program of PROCINORTE is committed to supporting collaboration in agricultural research and technologies areas of trilateral relevance.

On behalf of PROCINORTE, we are inviting you to participate in 5 days training at National Animal Disease Center ARS, from November 27 to December 01, 2017. This training is designed to anable you to acquire skills for manipulating data derived from NGS of small ruminants, in order to obtain the viroma of these species.

PROCINORTE will provide the round trip air travel as well as support for in-kind lodging and meals up to USD $ 1,260.00. Once your attendance is approved, the PROCINORTE Secretariat will be in direct contact with you regarding the final arrangements before the training takes place.

Kindly confirm your attendance by contacting Gloria Ramirez at gloria.ramirez@iica.int or (613) 230-1044.

We are looking forward to the results of this training which will advance the objectives of the Animal Health Task Force.

Kindest Regards,

Audia Barnett, Ph.D.
Executive Secretary of PROCINORTE/
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C.C. Dr. Cyril Gay, Leader of the Animal Health Task Force

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Ottawa, October 20th, 2017 PCN/CA-036

Elizabeth Loza Rubio
Research Scientist
Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias
Mexico

RE: invitation letter – Training in the CFIA Lethbridge Lab to sort trapped midges, Alberta, Canada

Dear Dr. Loza

As you know, the Cooperative Program of PROCINORTE is committed to supporting collaboration in agricultural research and technologies areas of trilateral relevance. The work of Task Forces such as the Animal Health Task Force is therefore important in furthering this goal.

PROCINORTE is therefore pleased to invite you to participate in 3 days practical training at Canadian Food Inspection Agency, Lethbridge Laboratory - Bovine and Equine Indigenous Viral Diseases, from November 14 to November 16, 2017. This training is designed to help Mexico in the trapping, identification and sorting of midges in the Culicoides/bluetongue project before they are sent to Winnipeg.

PROCINORTE will provide the round trip air travel as well as in-kind lodging and meals. Once your attendance is approved, the PROCINORTE Secretariat will be in direct contact with you regarding the final arrangements before the training takes place.

Kindly confirm your attendance by contacting Gloria Ramirez at gloria.ramirez@iica.int or (613) 230-1044.

We are looking forward to the results of this workshop which will advance the objectives of the Animal Health Task Force.

Kindest Regards,

Audia Barnett, Ph.D.
Executive Secretary of PROCINORTE/
IICA Representative in Canada

C.C. Dr Cyril Gay, Chair of the Animal Health Task Force of PROCINORTE