



# Proceedings of the 3<sup>rd</sup> Scientific Meeting of the Faculty of Veterinary Medicine (University of Liege - Belgium)

October 11, 2013

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Edited by C. Bayrou, J.-F.Cabaraux, C. Delguste, T. Jauniaux, A. Sartelet, D.-M. Votion

## Welcome to the 3<sup>rd</sup> scientific meeting of the Faculty of Veterinary Medicine

Each year, the Scientific Staff of the Faculty of Veterinary Medicine stimulates the meeting of new and old members of the Scientific Staff at its Annual General Meeting.

The purpose of this meeting is to promote the social life and scientific research within the Faculty as well as to enhance the interactions within the Faculty regarding the different research topics. This reunion is an opportunity for the members of the Scientific Staff to practice writing an abstract submitted for selection, and preparing a poster and/or an oral presentation as well as their defense. Finally, this event can show to the students the career opportunities in the Faculty (clinics and research).

Submission of abstracts for the 3<sup>rd</sup> Scientific Meeting was dedicated to members of the Scientific Staff of the FVM, and to the new-graduates (as part of an award-winning study) of Liege Faculty. The third edition is a success again, with a total of 95 abstracts submitted, of these, 19 were selected for an oral presentation by a scientific committee composed of PhD members of the Scientific Staff.

Jean-François CABARAUX,

President of the Scientific Staff

# Bienvenue à la 3<sup>éme</sup> édition de la réunion scientifique de Faculté de Médecine vétérinaire

Chaque année, le Personnel Scientifique de la Faculté de Médecine vétérinaire (FMV) stimule la rencontre des nouveaux et anciens membres du PSc lors de son assemblée générale. L'objectif de cette journée est de promouvoir la vie sociale et la recherche scientifique au sein de la Faculté et de favoriser la rencontre et les interactions des membres du personnel académique et scientifique en faisant connaître les différents sujets et programmes de recherche de la FMV.

C'est l'occasion pour les membres du Personnel Scientifique de s'exercer à la rédaction d'un abstract, à la préparation d'un poster et/ou d'une présentation orale, ainsi qu'à leur défense. Enfin, cet événement permet de montrer aux étudiants les possibilités de carrières à la Faculté (cliniques et recherches).

La soumission d'abstract pour la 3<sup>ème</sup> réunion scientifique de la FMV était réservée aux membres du PSc ainsi qu'aux nouveau diplômés (dans le cadre d'un travail de fin d'études primé) de l'Université de Liège. Cette 3<sup>ème</sup> édition est à nouveau un succès avec un total de 95 abstracts soumis. Parmi ces abstracts, 19 ont été sélectionnés pour une présentation orale par un comité scientifique composé exclusivement par des membres du Personnel Scientifique titulaires d'une Thèse de Doctorat.

Jean-François CABARAUX,

Président du Personnel Scientifique

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Diseases

#### **TECHNICAL SUPPORT**

Nathalie Guillaume Marie-Eve Lechanteur Michel Motkin

## **Acknowledgments**

















## **Program**

**08:30 - REGISTRATION** (lecture hall C, building B45)

09:00 - OPENING AND WELCOME SPEECH

Jean-François CABARAUX, President of the Scientist Staff

**09:15 - Oral Session 1** (lecture hall C, building B45)

CHAIRMEN: Prof Stefan DELEUZE and Jérôme PONTHIER, ULg

**09:15 Invited Speaker:** Functional genetics in mice: the Itpkb case: from immune deficiency to Alzheimer disease

Prof Stéphane SCHURMANS, ULg

09:45 Diversity of psychrophilic and psychrotrophic Clostridium sp. in cattle faecal samples collected at a slaughterhouse

Ana Rodrigues, Food Science, ULg

10:00 Impact of latent murid herpesvirus 4 infection on the development of an antipneumovirus vaccine-induced respiratory immunopathology

Michaël Dourcy, Immunology-Vaccinology Laboratory, ULg

10:15 Preliminary etiological data on the epidemiology of sub-maxillary adenitis in Belgian slaughter pigs

Marc Govaerts, Mycobacteria Unit, CODA-CERVA

10:30 Long term follow up in dogs with eosinophilic bronchopneumopathy treated with inhaled glucocorticosteroid therapy: preliminary results

Morgane Canonne, Clinical Sciences, ULg

10:45 Effect of omeprazole on canine cerebrospinal fluid composition **Maud Girod,** Small Animal Clinic, ULg

**11:00 - Coffee Break and Poster Session 1** (Room P, building B45)

11:30 - ORAL SESSION 2: EQUINE RESEARCH (lecture hall C, building B45)

CHAIRMEN: Prof Stefan DELEUZE and Jérôme PONTHIER, ULg

11:30 Haematology and plasma metabolites in horses offered linseed oil over a 4 months period

Stéphanie Patoux, presented by Louis Istasse, Nutrition Unit, ULg

11:45 Exercise modifies the innate immune response in equine bronchial epithelial cells

Linda Frellstedt, Center of Sports Medicine, ULg

12:00 Early indicators for exertional rhabdomyolysis in standardbred racehorses using high-resolution respirometry

Rosa Houben, Equine Clinic, ULg

12:15 Altered mitochondrial oxidative phosphorylation capacity in horses suffering from polysaccharide storage myopathy

Irene Tosi, Center of Sports Medicine, ULg

12:30 - LUNCH AND POSTER SESSION 2 (Room P, building B45)

**14:00 - Oral session 3: BOVINE RESEARCH** (lecture hall C, building B45)

CHAIRMEN: Mutien-Marie GARIGLIANY and Axel MAUROY, ULg

14:00 INVITED SPEAKER

Veterinary radiology team, ULg

14:30 A gammaherpesvirus uses alternative splicing to regulate its tropism and its sensitivity to neutralization

Bénédicte Machiels, Immunology-Vaccinology Laboratory, ULg

14:45 Experimental infection of cattle with bluetongue virus serotypes circulating in Europe

Ludovic Martinelle, Epidemiology, ULg

15:00 Bovine noroviruses in Belgium: from a molecular and evolutionary perspective **Axel Mauroy**, Virology, ULg

15:15 The role of alcelaphine herpesvirus 1 semaphorin 7A-homologue secreted 115 kDa glycoprotein during malignant catarrhal fever

Françoise Myster, Immunology-Vaccinology Laboratory, ULg

15:30 Milk fatty acids as influenced by energy metabolism in early lactating dairy cows

Emilie Knapp, Food Science, ULg

15:45 A splice-acceptor site variant in the bovine PIGH gene causes glycosylphosphatidyl inositol deficiency and lethal arthrogryposis syndrome Arnaud Sartelet, Animal Genomics, ULg

**16:00 - Coffee Break and Poster Session 3** (Room P, building B45)

**16:30 - Oral session 4: FISH RESEARCH** (lecture hall C, building B45)

CHAIRMEN: Mutien-Marie GARIGLIANY and Axel MAUROY, ULa

16:30 Rational design of an attenuated recombinant vaccine against Cyprinid Herpesvirus 3 using BAC cloning mutagenesis and in vivo imaging system

Maxime Boutier, Immunology-Vaccinology Laboratory, ULg

16:45 The mechanism of behavioral fever induced by Cyprinid herpesvirus 3 in common carp (Cyprinus carpio L.)

Krzysztof Rakus, Immunology-Vaccinology Laboratory, ULg

17:00 Sensitivity and permissivity of Cyprinus carpio to cyprinid herpesvirus 3 infection according to host ontogenesis

Maygane Ronsmans, Immunology-Vaccinology Laboratory, ULg

17:15 Laboratory validation of a lateral flow device for the detection of CyHV-3 antigens in gill swabs

Robert Vrancken, Okapi Sciences

#### 17:30 - AWARDS

Catherine DELGUSTE, President of the Scientific Committee

#### 17:45 - CLOSING SESSION

Prof Bernard RENTIER, Rector at the University of Liège
Prof Pascal LEROY, Dean of the Faculty of Veterinary Medicine

**18:00 - Cocktail and Poster Session 4** (Room P, building B45)

**20:00 - DINNER** (paella) and **DANCING PARTY** at the "Office des Cours" room (building B42)

## **Table of Contents**

## **O**RAL PRESENTATIONS

09:45 - Diversity of psychrophilic and psychrotrophic Clostridium sp. in cattle faecal samples collected at a slaughterhouse p.2  Rodrigues A. <sup>1</sup> , Taminiau B. <sup>2</sup> , Daube G. <sup>3</sup>	
10:00 - Impact of latent murid herpesvirus 4 infection on the development of an anti- pneumovirus vaccine-induced respiratory immunopathology p.2  Dourcy M.1*, Machiels B.1, Dewals B.1, Desmecht D.2, Vanderplasschen A.1, Gillet L.1	0
10:15 - Preliminary etiological data on the epidemiology of sub-maxillary adenitis in Belgian slaughter pigs p.2  Govaerts M.1*, Denoël J.2, Cassart D.3, Saegerman C.2	
10:30 - Long term follow up in dogs with eosinophilic bronchopneumopathy treated with inhaled glucocorticosteroid therapy: preliminary results  Canonne A-M. <sup>1</sup> , Billen F. <sup>1</sup> , Clercx C. <sup>1</sup>	1
<b>10:45 - Effect of omeprazole on canine cerebrospinal fluid composition p.2</b> Girod M. <sup>1</sup> , Allerton F. <sup>1</sup> , Gommeren F. <sup>1</sup> , Tutunaru F. <sup>1</sup> , De Marchin J. <sup>2</sup> , Van Soens I. <sup>1</sup> , Peeter D. <sup>1</sup> .	
11:30 - Haematology and plasma metabolites in horses offered linseed oil over a 4 months period p.2  Patoux S. <sup>1</sup> , Fabry C. <sup>1</sup> , Dotreppe O. <sup>1</sup> , Haeghens G. <sup>1</sup> , Hornick J-L. <sup>1</sup> , Istasse L. <sup>1</sup>	2
11:45 - Exercise modifies the innate immune response in equine bronchial epithelial cells	_
<b>p.2</b> Frellstedt L. <sup>1,5</sup> *, Gosset P. <sup>2,5</sup> , Desmet C. <sup>3,5</sup> , Pirottin D. <sup>3,5</sup> , Bureau F. <sup>3,5</sup> , Dupuis-Tricau MC. <sup>4,5</sup> , Waldschmidt I. <sup>4,5</sup> , Lekeux P. <sup>1,5</sup> , Art T. <sup>1,5</sup>	
12:00 - Early indicators for exertional rhabdomyolysis in standardbred racehorses using high-resolution respirometry p.2  Houben R.M.A.C. <sup>1</sup> *, Leleu C. <sup>2</sup> , Fraipont A. <sup>1</sup> , Amory H. <sup>1</sup> , Serteyn D. <sup>1</sup> , Houben R.M. <sup>3</sup> , Votio D.M. <sup>1</sup>	
12:15 - Altered mitochondrial oxidative phosphorylation capacity in horses suffering from polysaccharide storage myopathy  Tosi I. 1*, Art T. 1, Cassart D. 2, Serteyn D. 3, Votion D. 3	4
14:30 - A gammaherpesvirus uses alternative splicing to regulate its tropism and its sensitivity to neutralization  Machiels B. 1*, Stevenson P.G. 2, Vanderplasschen A. 1, Gillet L. 1	4
14:45 - Experimental infection of cattle with bluetongue virus serotypes circulating in Europe  Martinelle L. <sup>1</sup> , Dal Pozzo F. <sup>1</sup> , Thys C. <sup>1</sup> , De Leeuw I. <sup>2</sup> , Van Campe W. <sup>3</sup> , De Clercq K. <sup>2</sup> , Thir E. <sup>4</sup> , Saegerman C. <sup>1</sup> *	
<b>15:00 - Bovine noroviruses in Belgium: from a molecular and evolutionary perspective p.2</b> Mauroy A. <sup>1</sup> , Scipioni A. <sup>2</sup> , Daube G. <sup>3</sup> , Thiry E. <sup>1</sup>	5
15:15 - The role of Alcelaphine herpesvirus 1 Semaphorin 7a-homologue secreted 115 kDa glycoprotein during malignant catarrhal fever p.2  Myster F. <sup>1*</sup> , Palmeira L. <sup>1</sup> , Sorel O. <sup>1</sup> , Vanderplasschen A. <sup>1</sup> , Dewals B. <sup>1</sup>	6
<b>15:30 - Milk fatty acids as influenced by energy metabolism in early lactating dairy cows p.2</b> Knapp E. <sup>1*</sup> , Dotreppe O. <sup>1</sup> , Hornick JL. <sup>1</sup> , Istasse L. <sup>1</sup> , Dufrasne I. <sup>1</sup>	6

15:45 - A splice-acceptor site variant in the bovine PIGH gene causes glycosyl	phosphatidyl
inositol deficiency and lethal arthrogryposis syndrome	p.27
Sartelet A. <sup>1a</sup> *, Li W. <sup>1a</sup> , Pailhoux E. <sup>2</sup> , Tamma N. <sup>1</sup> , Karim L. <sup>1</sup> , Fasquelle C. <sup>1</sup> ,	Druet T.1, Coppi-
eters W. 1, Georges M. 1, Charlier C. 1	

- **16:30 Rational design of an attenuated recombinant vaccine against Cyprinid Herpesvirus 3 using BAC cloning mutagenesis and in vivo imaging system p.27**Boutier M.<sup>1</sup>, Ouyang P.<sup>1</sup>, Fournier G.<sup>1</sup>, Ronsmans M.<sup>1</sup>, Reschner A.<sup>1</sup>, Scohy S.<sup>2</sup>, Vanderplass-
- **16:45 The mechanism of behavioral fever induced by Cyprinid herpesvirus 3 in common carp (***Cyprinus carpio* **L.)**Rakus K. <sup>1\*</sup>, Ronsmans M. <sup>1</sup>, Boutier M. <sup>1</sup>, Ouyang P. <sup>1</sup>, Forlenza M. <sup>2</sup>, Wiegertjes G. <sup>2</sup>, Becco C. <sup>3</sup>, Frédéric Farnir F. <sup>4</sup>, Vanderplasschen A. <sup>1</sup>
- 17:00 Sensitivity and permissivity of *Cyprinus carpio* to Cyprinid herpesvirus 3 infection according to host ontogenesis p.28

  Ronsmans M.<sup>1\*</sup>, Boutier M.<sup>1</sup>, Szpirer C.<sup>2</sup>, Rougeot C.<sup>3</sup>, Vandecan M.<sup>3</sup>, Mélard C.<sup>3</sup>, Vandernlasschen A.<sup>1</sup>
- 17:15 Laboratory validation of a lateral flow device for the detection of CyHV-3 antigens in gill swabs

  Vrancken R. 1\*, Boutier M. 2, Ronsmans M. 2, Reschner A. 2, Goris N. 1, Leclipteux T. 3, Lieffrig F. 4, Collard A. 4, Mélard C. 5, Wera S. 1, Neyts J. 1, Vanderplasschen A. 2

## **P**OSTERS

1.	KNN-MDR Approach for Detecting Gene-Gene Interactions Abo Alchamlat S. 1, Farnir F. 1	p.32
2.	Comparative study of 3 DNA extraction protocols for recovery of genomic microbial and from the Algerian traditional date product "Btana". Abekhti $A^{1,2}$ , Taminiau $B^{1}$ , Kihla $M^{2}$ , Daube $G^{1*}$	ADN p.32
3.	Quality assessment of marketed eggs in Eastern Algeria Ait Kaki A. <sup>1</sup> , Kacem Chaouche N. <sup>1</sup> , Kara Ali M. <sup>1</sup> , Moula N. <sup>2</sup> *	p.33
4.	Mechanical Torsional Properties of Tibiae Following Modified Maquet Technique or Ti Tuberosity Advancement Barthélémy N. <sup>1*</sup> , Brunel L. <sup>1</sup> , Laurent C. <sup>2</sup> , Farnir F. <sup>3</sup> , Balligand M. <sup>1</sup>	bial p.33
5.	Identification of pregnancy-associated glycoproteins and alphafetoprotein in fallow ( <i>Dama dama</i> ) placenta  Beriot M. <sup>1</sup> , Tchimbou A. F. <sup>1</sup> , Barbato O. <sup>2</sup> , Beckers J-F. <sup>1*</sup> , Melo de Sousa N. <sup>1</sup>	deer p.34
6.	Growth performance and meat quality of stress negative Piétrain pigs in the tropics: case of Vietnam  Bo H. X. <sup>1</sup> , Luc D. D. <sup>1,2</sup> *, Moula N. <sup>2</sup> , Clinquart A. <sup>3</sup> , Ton V. D. <sup>4</sup> , Binh D. V. <sup>1</sup> Farnir F. <sup>2</sup> , Leroy P.	p.34
7.	Post-translational modification of the Myxoma virus chemokine-binding protein M-T2 a virally encoded a-2,3-sialyltransferase  Boutard B. <sup>1*</sup> , Vankerckhove S. <sup>1</sup> , Canis K. <sup>2</sup> , Markine-Goriaynoff N. <sup>1</sup> , Sarlet M. <sup>3</sup> , Wattie Leprince P. <sup>5</sup> , Desmecht D. <sup>3</sup> , Haslam S. <sup>2</sup> , McFadden G. <sup>6</sup> , Dell A. <sup>2</sup> , Vanderplasschen A. <sup>1</sup> , Gille	<b>p.35</b> z R. <sup>4</sup> ,
8.	Strandings of the common porpoise ( <i>Phocoena phocoena</i> ) in the southern North Sea modeling of backtrack drift.  Brihaye E. <sup>1</sup> *, Degraer S. <sup>2</sup> , Dulière V. <sup>2</sup> , Haelters J. <sup>2</sup> , Jauniaux T. <sup>1</sup>	and p.35
9.	Thelazia callipaeda ocular infection in two dogs in Belgium Caron Y.1*, Premont J.2, Losson B.1, Grauwels M.2	p.36
10	. Three cases of <i>Parafilaria bovicola</i> infection in Belgium and a few recent epidemiologobservations on this emergent disease.  Caron Y. <sup>1*</sup> , Groignet S. <sup>1</sup> , Saegerman C. <sup>2</sup> , Losson B. <sup>1</sup>	gical p.36
11	. Characterization of host factors involved in resistance/susceptibility to influenza A infection in Mx-negative mouse models  Casanova T. <sup>1</sup> , Desmecht D. <sup>2</sup> , Garigliany M. <sup>1</sup>	p.37
12	. <b>Detection of </b> <i>Mycobacterium celatum</i> <b>in wild boars in Southern Belgium</b> Ceuleers V. <sup>1*</sup> , Vionnet A. <sup>1</sup> , Grégoire F. <sup>1</sup> , Volpe R. <sup>1</sup> , Jouant L. <sup>1</sup> , Paternostre J. <sup>1</sup> , Govaert Linden A. <sup>1</sup>	<b>p.37</b> s M. <sup>2</sup> ,
13	. <b>Prevalence Of PRRS virus in Wallonia (Belgium)</b> Czaplicki G. <sup>1</sup> , Thilmant P. <sup>2</sup> , Hooyberghs J. <sup>3</sup> , Ceulemans K. <sup>4</sup> , Lomba M. <sup>1</sup> , Wavreille J. <sup>5</sup> , Laita	<b>p.38</b> t M. <sup>6</sup>
14	Effects of Q-fever vaccination in Belgian goat farms.  Dal Pozzo F. <sup>1</sup> *, Czaplicki G. <sup>2</sup> , Bertels G. <sup>3</sup> , Saegerman C. <sup>1</sup>	p.38
15	. Risk of introduction of alphaviruses responsible for American equine encephalitides Belgium  De La Grandiere M.A. <sup>1</sup> , Dal Pozzo F. <sup>2</sup> , Francis F. <sup>3</sup> , Caij A.B. <sup>4</sup> , Thiry E. <sup>1</sup>	in p.39

Desmecht D. 1*, Garigliany M-M. 1, Bayrou C. 1, Schirrmeier H. 3, Beer M. 3, Paternostre J. 2, R. 2, Linden A. 2	, Volpe
17. Health screening to identify opportunities to improve preventive medicine in cats ar	
<b>dogs: focus on nutrition status</b> Diez M. <sup>1*</sup> , Picavet P. <sup>2</sup> , Ricci R. <sup>3</sup> , Dequenne M. <sup>1</sup> , Renard M. <sup>4</sup> , Bongartz A. <sup>4</sup> , Farnir F. <sup>5</sup>	p.40
18. Validation of a LC-MS analytical method for the measurement of aldehydes in meat	
oil Douny C. <sup>1</sup> *, Tihon A. <sup>1</sup> , Bayonnet P. <sup>1</sup> , Brose F. <sup>1</sup> , Degand G. <sup>1</sup> and Scippo ML. <sup>1</sup>	p.40
19. Tolerance and anesthetic effects of 1% tetracaine hydrochloride and 0.4% oxybuprocaine hydrochloride on healthy feline cornea.  Dufour V. <sup>1</sup> , Storms G. <sup>1</sup> , Farnir F. <sup>2</sup> , Grauwels M. <sup>1</sup> , Monclin S. <sup>1</sup> *	p.41
20. Atypical presentation of an orbital osteosarcoma in a two year old dog: a case report literature review  Dufour V.¹, Storms G.¹, Heymann H.², Busoni V.¹, Billen B.¹, Monclin S.¹*	t and p.41
21. Ultrasonographic percutaneous anatomy of the atlanto-occipital region and indirect ultrasound-guided cisternal puncture in the dog and the cat  Etienne AL.1*, Audigié F.2, Peeters D.3, AGabriel A.4, Busoni V.1	p.42
22. Ultrasonography of the collateral ligaments of the distal interphalangeal joint in hot technique and reference images  Evrard L. <sup>1</sup> , Bolen G. <sup>1</sup> , Maquet N. <sup>2</sup> , Busoni V. <sup>1</sup>	ses: p.42
<b>23. Circulation of Schmallenberg virus in Belgian red and roe deer populations</b> Garigliany M. <sup>1*</sup> , Volpe R. <sup>1</sup> , Paternostre J. <sup>1</sup> , Desmecht D. <sup>1</sup> , Linden A. <sup>1</sup>	p.43
<b>24.</b> Vitrification of immature equine oocytes: investigation of volume variations during a process  Gatez C. <sup>1</sup> , Ectors F.J. <sup>2</sup> , Farnir F. <sup>3</sup> , Beckers J-F. <sup>1</sup> , Deleuze S. <sup>4</sup>	the p.43
process	p.43 urel p.44
process Gatez C. <sup>1</sup> , Ectors F.J. <sup>2</sup> , Farnir F. <sup>3</sup> , Beckers J-F. <sup>1</sup> , Deleuze S. <sup>4</sup> 25. Etude des caractéristiques des élevages du mouton Koundoum dans son habitat nat au Niger Hamadou I. <sup>1,3</sup> , Marichatou H. <sup>3</sup> , Moula N. <sup>1</sup> , Siddo S. <sup>1</sup> , Moumouni I. <sup>3</sup> , Leroy P. <sup>1</sup> , Antoine	p.43 urel p.44
process Gatez C. <sup>1</sup> , Ectors F.J. <sup>2</sup> , Farnir F. <sup>3</sup> , Beckers J-F. <sup>1</sup> , Deleuze S. <sup>4</sup> 25. Etude des caractéristiques des élevages du mouton Koundoum dans son habitat nat au Niger Hamadou I. <sup>1,3</sup> , Marichatou H. <sup>3</sup> , Moula N. <sup>1</sup> , Siddo S. <sup>1</sup> , Moumouni I. <sup>3</sup> , Leroy P. <sup>1</sup> , Antoine siaux N. <sup>1,2</sup> 26. Perception des causes et les conséquences de la régression de l'effectif du mouton Koundoum du Niger par les éleveurs	p.43 urel p.44 -Maus- p.44
<ul> <li>process     Gatez C.¹, Ectors F.J.², Farnir F.³, Beckers J-F.¹, Deleuze S.⁴</li> <li>25. Etude des caractéristiques des élevages du mouton Koundoum dans son habitat nat au Niger     Hamadou I.¹,³, Marichatou H.³, Moula N.¹, Siddo S.¹, Moumouni I.³, Leroy P.¹, Antoine siaux N.¹,²</li> <li>26. Perception des causes et les conséquences de la régression de l'effectif du mouton Koundoum du Niger par les éleveurs     Hamadou I.¹,³, Marichatou H.³, Moula N.¹, Siddo S.¹,³, Leroy P.¹, Antoine-Maussiaux N.¹,²</li> <li>27. Using conjoint analysis to estimate farmer's preferences for breeding ram traits in t Middle Niger     Hamadou I.¹,³, Marichatou H.³, Moula N.¹, Siddo S.¹, Moumouni I.³, Leroy P.¹, Antoine</li> </ul>	p.43 urel p.44 -Maus- p.44 -he p.45 -Maus-
process Gatez C.¹, Ectors F.J.², Farnir F.³, Beckers J-F.¹, Deleuze S.⁴  25. Etude des caractéristiques des élevages du mouton Koundoum dans son habitat nat au Niger Hamadou I.¹,³, Marichatou H.³, Moula N.¹, Siddo S.¹, Moumouni I.³, Leroy P.¹, Antoine siaux N.¹,²  26. Perception des causes et les conséquences de la régression de l'effectif du mouton Koundoum du Niger par les éleveurs Hamadou I.¹,³, Marichatou H.³, Moula N.¹, Siddo S.¹,³, Leroy P.¹, Antoine-Maussiaux N.¹,²  27. Using conjoint analysis to estimate farmer's preferences for breeding ram traits in t Middle Niger Hamadou I.¹,³, Marichatou H.³, Moula N.¹, Siddo S.¹, Moumouni I.³, Leroy P.¹, Antoine siaux N.¹,²	p.43 urel p.44 -Maus- p.44 -he p.45 -Maus-
<ul> <li>process     Gatez C.¹, Ectors F.J.², Farnir F.³, Beckers J-F.¹, Deleuze S.⁴</li> <li>25. Etude des caractéristiques des élevages du mouton Koundoum dans son habitat nat au Niger     Hamadou I.¹,³, Marichatou H.³, Moula N.¹, Siddo S.¹, Moumouni I.³, Leroy P.¹, Antoine siaux N.¹,²</li> <li>26. Perception des causes et les conséquences de la régression de l'effectif du mouton Koundoum du Niger par les éleveurs     Hamadou I.¹,³, Marichatou H.³, Moula N.¹, Siddo S.¹,³, Leroy P.¹, Antoine-Maussiaux N.¹,²</li> <li>27. Using conjoint analysis to estimate farmer's preferences for breeding ram traits in t Middle Niger     Hamadou I.¹,³, Marichatou H.³, Moula N.¹, Siddo S.¹, Moumouni I.³, Leroy P.¹, Antoine siaux N.¹,²</li> <li>28. A new methodology to assess data availability, accessibility and form for risk analyst Humblet M.F.¹, Vandeputte S.¹, Mignot C.¹, Bellet C.², De Koeijer A.³, Swanenburg M.³,</li> </ul>	p.43 urel p.44 -Maus- p.44 the p.45 -Maus- sis p.45 Afonso

Third FMV Scientific Meeting - October 11, 2013

13

16. Detection of antibodies against Schmallenberg virus in wild boars, Belgium, 2010-2012

31.	The ORF25 gene family of Cyprinid herpesvirus 3 encodes non-essential structural glycoproteins: roles of the paralogues in the biology of the infection <i>in vitro</i> and <i>in v</i>	ivo p.47
	$ \label{eq:section}                                    $	
32.	Aujeszky's disease virus seroprevalence in wild boar, Southern Belgium, 2012  Jouant L. <sup>1</sup> *, Wayet J. <sup>2</sup> , Volpe R. <sup>1</sup> , Ceuleers V. <sup>1</sup> , Paternostre J. <sup>1</sup> , Massart L. <sup>3</sup> , Linden A. <sup>1</sup>	p.47
33.	The nod-like receptor homolog encoded by murid herpesvirus-4 <i>orf63</i> is important b not essential for virus replication and latency establishment <i>in vivo</i> Latif Muhammad B. <sup>1</sup> , Machiels B. <sup>1</sup> , Vanderplasschen A. <sup>1</sup> , Gillet L. <sup>1</sup>	ut p.48
34.	Bovine herpesvirus 4 glycoprotein L is a major target of antibody neutralization Lété C. <sup>1</sup> *, Machiels B. <sup>1</sup> , Vanderplasschen A. <sup>1</sup> , Gillet L. <sup>1</sup>	p.48
35.	<b>Ultrasound-guided epidural access in dog</b> Liotta A. <sup>1</sup> *, Sandersen C. <sup>2</sup> , Busoni V. <sup>1</sup> , Etienne A-L. <sup>1</sup> , Bolen G. <sup>1</sup>	p.49
36.	Economical evaluation of the impact of PRRS in a Walloon farrow-to-finish farm Mahu J.L. <sup>1</sup> , Thilmant P. <sup>1*</sup> , Laitat M. <sup>2</sup>	p.49
37.	<b>Dose dependent challenge of sheep with Schmallenberg virus</b> Martinelle L. <sup>1</sup> , Poskin A. <sup>2</sup> , Dal Pozzo F. <sup>1</sup> , Mostin L. <sup>3</sup> , De Regge N. <sup>2</sup> , Saegerman C. <sup>1</sup> , Cay A.B	<b>p.50</b>
38.	The Status of Indigenous Village Chicken Production in Democratic Republic of Cong	
	Moula N. 1,2, Luc D.D. 1, Hornick J.L. 1,2, Ruppol P. 2, Leroy P. 1,2, Antoine-Moussiaux N. 1,2	p.50
39.	Caractérisation de la race ovine Tazegzawth en Algerie: description morpho-biométr et détermination d'une formule barymétrique  Moula N. <sup>1,2</sup> , Philippe F.X. <sup>1</sup> , Luc D.D. <sup>1,3</sup> , Farnir F. <sup>1</sup> , Antoine-Moussiaux N. <sup>1,2</sup> , Leroy P. <sup>1,2</sup>	ique p.51
40.	Performances de productions de la poule locale kabyle Moula N. <sup>1,2</sup> , Philippe F.x. <sup>1</sup> , Ait Kaki A. <sup>3</sup> , Antoine-Moussiaux N. <sup>1,2</sup> , Farnir F. <sup>1</sup> , Leroy P. <sup>1,2</sup>	p.51
41.	Identification of Colistin resistant strains among pathogenic and non-pathogenic coliforms isolates of human and animal origin  Muylaert A. <sup>1*</sup> , Duprez JN. <sup>1</sup> , Mainil J. <sup>1</sup>	p.52
42.	Evaluation of the influence of weaning on changes in the composition of the piglets intestinal bacterial flora $ \text{Ngassam C}^1\text{., Leroy P.}^1\text{, Taminiau B.}^2$	p.52
43.	The IL-10 homologue encoded by cyprinid herpesvirus 3 is essential neither for viral replication <i>in vitro</i> nor for virulence <i>in vivo</i> Ouyang P. <sup>1</sup> , Rakus K. <sup>1</sup> , Boutier M. <sup>1</sup> , Reschner A. <sup>1</sup> , Leroy B. <sup>2</sup> , Ronsmans M. <sup>1</sup> , Fournier G. <sup>1</sup> , S. <sup>3</sup> , Costes B. <sup>4</sup> , Wattiez R. <sup>2</sup> , Vanderplasschen A. <sup>1</sup> *	p.53
44.	<b>Myeloperoxidase as an indicator of endometritis in the mare: preliminary results</b> Parrilla-Hernandez S. <sup>1,2</sup> , Franck T. <sup>3</sup> , Ponthier J. <sup>2</sup> , Mottart E. <sup>4</sup> , Deleuze S. <sup>2</sup>	p.53
45.	Comparison of ammonia and greenhouse gases emissions associated to fattening pickept either on fully or partly slatted floor  Philippe FX. <sup>1*</sup> , Laitat M. <sup>2</sup> , Wavreille J. <sup>3</sup> , Nicks B. <sup>1</sup> , Cabaraux JF <sup>1</sup>	js p.54
46.	Effect of a fibrous diet on growth performance, carcass characteristics and gut healt fattening pigs  Philippe FX. <sup>1</sup> *, Antoine N. <sup>2</sup> , Cabaraux JF. <sup>1</sup> , Cassart D. <sup>2</sup> , Mainil J. <sup>3</sup> , Nicks B. <sup>1</sup> , Wavreil Laitat M. <sup>5</sup>	p.54

associated with fattening pigs kept on deep litter Philippe FX. <sup>1</sup> *, Laitat M. <sup>2</sup> , Wavreille J. <sup>3</sup> , Nicks B. <sup>1</sup> , Cabaraux JF. <sup>1</sup>	p.55
<b>48.</b> Influence of the void percentage of the floor on ammonia and greenhouse gas emiss for group-housed gestating sows  Philippe F. X. <sup>1</sup> , Laitat M. <sup>2</sup> , Wavreille J. <sup>3</sup> , Nicks B. <sup>1</sup> , Cabaraux J. F. <sup>1</sup> *	ions p.55
<b>49. Influence of concentration on equine fresh semen conservation</b> Ponthier J. 1*, Blommaert D. 2, Parrilla S. 1, 2, van Den Berghe F. 1, Deleuze S. 1	p.56
<b>50.</b> Retinal function in horses with traumatic panuveitis and equine recurrent uveitis Premont J.E. $^{1*}$ , Ofri R. $^{2}$ , Bdolah-Abram T. $^{2}$ , Grauwels M. $^{3}$	p.56
<b>51.</b> Use of Staby® technology for development and production of DNA vaccines free of antibiotic resistance gene  Reschner A. 1*, Scohy S. 2, Vandermeulen G. 3, Daukandt M. 4, Jacques C. 2, Michel B. 2, Naur H. 5, Xhonneux F. 4, Préat V. 3, Vanderplasschen A. 1, Szpirer C. 2	<b>p.57</b> wynck
<b>52.</b> Persistent right aortic arch associated with an aberrant left subclavian artery arising from a patent ductus arteriosus in a puppy Rizza M. <sup>1</sup> , Claeys S. <sup>2</sup> , Billen F. <sup>3</sup> , Mc Entee K. <sup>3</sup> , Bolen G. <sup>1</sup>	9 p.57
53. Molecular characterization of <i>Clostrium difficile</i> strains from elderly care home resid	ents p.58
Rodriguez C. <sup>1</sup> , Taminiau B. <sup>1</sup> , Korsak N. <sup>1</sup> , Avesani V. <sup>2</sup> , Van Broeck J. <sup>2</sup> , Delmée M. <sup>2</sup> , Daube G	
<b>54.</b> Is the CXC-chemokine CXCL8 involved in the breed predisposition of West Highland White Terrier to canine idiopathic pulmonary fibrosis?  Roels E. <sup>1</sup> , Krafft E. <sup>1</sup> , Laurila HP. <sup>2</sup> , Rajamäki MM. <sup>2</sup> , Antoine N. <sup>3</sup> , Massart L. <sup>4</sup> , Clercx C. <sup>1</sup>	p.58
<b>55.</b> Comparison of four cartridge-type automated blood gas analyzers for arterial blood analysis in dogs  Roels E. <sup>1</sup> , Delvaux F. <sup>1</sup> , Farnir F. <sup>2</sup> , Billen F. <sup>1</sup> , Clercx C. <sup>1</sup>	gas p.59
<b>56. Event-based surveillance of equine West Nile fever, Spain</b> Saegerman C. <sup>1</sup> *, Alba Casals A. <sup>2</sup> , García-Bocanegra I. <sup>3</sup> , Dal Pozzo F. <sup>1</sup> , van Galen G. <sup>4</sup>	p.59
<b>57. Production laitiere de la lapine locale pendant les deux premieres lactations</b> Saidj D. <sup>1</sup> *, Salhi O. <sup>1</sup> , Moula N. <sup>2</sup> , Temim S. <sup>1</sup> , Ain Baziz H. <sup>1</sup>	p.60
<b>58.</b> The development of astrocytes in the sheep cerebellum Salouci M. <sup>1</sup> *, Antoine N. <sup>1</sup> , Mignon Y. <sup>1</sup> , Kirschvink N. <sup>2</sup> , Gabriel A. <sup>1</sup>	p.60
<b>59.</b> Typologie des préférences des éleveurs de zébu Azawak pour le choix de reproducte dans les zones d'Abalak, Filingué et Niamey (Niger)  Siddo S. <sup>1, 4</sup> , Moula N. <sup>1,2</sup> , Marichatou H. <sup>3</sup> , Moumouni I <sup>3</sup> ., Leroy P. <sup>1,2</sup> , Antoine-Moussiaux N. <sup>3</sup>	p.61
<b>60.</b> Critères d'appréciation et consentement à payer pour des reproducteurs améliorés de <b>zébu Azawak au Niger</b> Siddo S. <sup>1, 4</sup> , Moula N. <sup>1,2</sup> , Marichatou H. <sup>3</sup> , Hamadou I. <sup>3</sup> , Moumouni I. <sup>3</sup> , Leroy P. <sup>1,2</sup> , Antoinesiaux N. <sup>1,2</sup> *	p.61
<b>61.</b> Measurement of the estrogenic activity of migration products from plastic contact materials  Simon C. <sup>1</sup> , Oghena M. <sup>2</sup> , Covaci A. <sup>2</sup> , Van Hoeck E. <sup>3</sup> , Van Loco J. <sup>3</sup> , Elskens M. <sup>4</sup> , Demaeg Mertens B. <sup>3</sup> , Scippo M.L. <sup>1</sup>	<b>p.62</b> gt H. <sup>5</sup> ,
<b>62. Evasion of cytotoxic T cell response by </b> <i>Alcelaphine herpesvirus 1</i> <b> genome maintenar protein.</b> Sorel 0.1*, Myster F. <sup>1</sup> , Vanderplasschen A. <sup>1</sup> et Dewals B.G. <sup>1</sup>	nce p.62

47. Impact of the amount of straw on emissions of ammonia and greenhouse gases

63.	Growth optimization of <i>Bifidobacterium crudilactis</i> and <i>Bifidobacterium mongoliense</i> , new potential probiotic strains  Tanimomo J. <sup>2</sup> , Delcenserie V. <sup>1</sup> , Taminiau B. <sup>1</sup> , Daube G. <sup>1</sup> , Durieux A. <sup>2</sup>	, <i>two</i> p.63
64.	Environment factors affecting racing performances of Thoroughbred horses in Algeri	
	Tennah S. $^{1,2}$ , Kafidi N. $^{2,4}$ , Antoine-Moussiaux N. $^{1,3}$ , Michaux C. $^{1}$ , Leroy P. $^{1,3}$ , Farnir F. $^{1}$	p.63
65.	Evaluation morphométrique des chevaux pur-sang Arabe en Algérie: mensurations corporelles et proposition d'équations barymétriques $ \text{Tennah S.}^{1,2}, \text{ Kafidi N.}^{2,4}, \text{ Antoine-Moussiaux N.}^{1,3}, \text{ Luc D. D.}^{1,5}, \text{ Moula N.}^{1,3}, \text{ Michaux C.}^{1}, \text{ P.}^{1,3}, \text{ Farnir F}^{1*} $	<b>p.64</b> Leroy
66.	Genetic parameters of racing performance traits of Arabian horses in Algeria Tennah S. <sup>1,2</sup> , Kafidi N. <sup>2,4</sup> , Antoine-Moussiaux N. <sup>1,3</sup> , Michaux C. <sup>1</sup> , Leroy P. <sup>1,3</sup> , Farnir F <sup>1</sup> *	p.64
67.	Pedigree analysis in the Arabian horse in Algeria: estimation of inbreeding coefficien	
	Tennah S. $^{1,2}$ , Kafidi N. $^{2,4}$ , Antoine-Moussiaux N. $^{1,3}$ , Michaux C. $^{1}$ , Leroy P. $^{1,3}$ , Farnir F $^{1}*$	p.65
68.	Morphological and Molecular Characterization of Lymnaeid Snails and Their Potential Role in the Transmission of Fasciola spp. in Vietnam Thi Dung B. $^{1,2*}$ , Bertrand Losson B. $^2$ , Ngoc Doanh P. $^1$ , Tat The D. $^3$ , Thi Loan H. $^3$ , Caron Y. $^2$	p.65
69.	An attempt to eradicate PRRS in Wallonia (Belgium), a low density area of swine production $ \text{Thilmant P.}^1\text{, Laitat M.}^2$	p.66
70.	Hepatitis E virus infection in wild boars and humans in Belgium  Thiry D. <sup>1</sup> , Mauroy A. <sup>1</sup> , Saegerman C. <sup>2</sup> , Brochier B. <sup>3</sup> , Thomas I. <sup>3</sup> , Thiry E. <sup>1</sup> , Linden A. <sup>4</sup>	p.66
71.	Gestion des ressources génétiques ovines dans les élevages périurbains de Ouagadougou, Burkina Faso Tindano K. <sup>1,2</sup> , Moula N. <sup>1,3</sup> , Traoré A. <sup>4</sup> , Leroy P. <sup>1,3</sup> , Antoine-Moussiaux N. <sup>1,3</sup>	p.67
72.	Gestion des ressources génétiques ovines dans des élevages ruraux dans la région de Plateau Central du Burkina Faso Tindano K. $^{1,2}$ , Moula N. $^{1,3}$ , Traoré A $^4$ , Leroy P. $^{1,3}$ , Antoine-Moussiaux N. $^{1,3}$	u p.67
73.	Evaluation des performances zootechniques et estimation d'une formule barymétrique adaptée à la race Zébu Azawak au Nord du Mali  Toure A. <sup>1</sup> , Moula N. <sup>3</sup> , Kouriba A. <sup>2</sup> , Antoine-Moussiaux N. <sup>1,3</sup> Leroy P. <sup>1,3</sup>	је р.68
74.	Extraintestinal lesions associated with <i>Mycobacterium avium paratuberculosis</i> in wilcervids  Volpe R. <sup>1,4</sup> , Gregoire F. <sup>1</sup> , Wirtgen M. <sup>1</sup> , Paternostre J. <sup>1</sup> , Cassart D. <sup>2</sup> , Govaerts M. <sup>3</sup> , Linden A.	p.68
75.	Effect of biocides on murine norovirus and feline calicivirus, surrogates of human norovirus $ \hbox{Zonta W.}^1, \hbox{Mauroy A.}^1 \hbox{ and Thiry E.}^1 $	p.69

#### **Addendum**

**76. Evaluation of some production parameters in Kabyle local rabbit population p.69** Moula N.<sup>1,2</sup>, Michaux C.<sup>1</sup>, Luc D.D.<sup>1,3</sup>, Saidj D.<sup>4</sup>, Farnir F.<sup>1</sup>, Antoine-Moussiaux N.<sup>1,2</sup> and Leroy P.<sup>1,2</sup>

# 09:45 - Diversity of psychrophilic and psychrotrophic *Clostridium* sp. in cattle faecal samples collected at a slaughterhouse

Rodrigues A.<sup>1</sup>, Taminiau B.<sup>2</sup>, Daube G.<sup>3</sup>

Introduction: Globalization of meat exchanges led to a generalized application of conservation techniques assuring a longer shelf-life, such as chilling and vacuum-packaging. However, a type of spoilage of chilled vacuum-packaged meat is becoming a major problem for the meat industry. This spoilage phenomenon referred to as "Blown Pack Spoilage" can be caused by different species of psychrophilic and psychrotrophic clostridia such as *Clostridium estertheticum* and *Clostridium gasigenes*. Material and methods: Psychrophilic/trophic *Clostridium* colonies were isolated form bovine faecal samples and a species-specific PCR for *Cl. estertheticum* was conducted for identification. All the isolates identified as *Cl. estertheticum* were then compared genotypically by means of 16S gene sequencing. Results: Our study demonstrated that the PCR is not specific for Cl. estertheticum. Sequencing analysis showed a large diversity among isolates as other psychrophilic/psychrotrophic species belonging to the *Clostridium* genus were identified.

# 10:00 - Impact of latent murid herpesvirus 4 infection on the development of an anti-pneumovirus vaccine-induced respiratory immunopathology

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Gammaherpesviruses are a lymphotropic family of viruses which persist lifelong in their host by establishing and maintaining a latent infection. Murid herpesvirus 4 (MuHV-4) is a natural pathogen of wild murid rodents and provides a valuable gammaherpesvirus small animal model. Recently, it has been demonstrated that this virus significantly modulates the immune system and offers symbiotic protection to the host against heterologous pathogens. The human respiratory syncytial virus is a ubiquitous virus which induces lower respiratory infections and Th2 immunopathologies, as the particular vaccine-enhanced disease. Interestingly, this paradoxical disease has been successfully reproduced in a rodent host using the Pneumonia virus of mice (PVM). Here we decided to explore the impact of latent gammaherpesvirus infection on pneumovirus-induced Th2 immunopathologies. Briefly, Balb/c mice were either mock infected or infected by MuHV-4. One month after infection, the Th2-skewed challenge was induced by the subcutaneous inoculation of formalin-inactivated PVM antigens and a following intranasal infection with wild type PVM. Our results demonstrated that MuHV-4 latency significantly drives the Th2-skewed pathological response to a balanced Th1/Th2 physiological immune response. Notably, we detected significant lower levels of IL-4, IL-5, IFN-y and eosinophils in bronchoalveolar lavage fluids in MuHV-4 infected mice which were challenged than in mock infected mice. Interestingly, MuHV-4 infection did not reduce protection against PVM conferred by the vaccine. In conclusion, this study suggests that gammaherpesvirus latency induces profound immunomodulations which can confer protection to the host against immune disorders.

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# 10:15 - Preliminary etiological data on the epidemiology of sub-maxillary adenitis in Belgian slaughter pigs

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Lymphadenitis in slaughter pigs is reported worldwide at prevalence rates 0.33 to 1%, and leads through carcass condemnation to significant losses. Its infectious origin and associated health risk are mostly undefined, and include Mycobacterium bovis, the agent of zoonotic tuberculosis, M. avium complex (MAC) and Rhodococcus equi (Re). Unlike MAC and M. bovis, the zoonotic risk posed by Re in immunocompromised patients has been extended only recently to immunocompetent individuals. To determine lymphadenitis prevalence and causes in Belgium, 16211 pig carcasses were inspected over a 12-month period by a single meat inspector in two slaughterhouses, and lesions were found on 87 carcasses (0.54%) and processed for bacteriology and histopathology. 72 carcasses carrying no gross visible lesions were sampled alongside as controls. Samples were plated onto two Re selective media and two egg-based media for mycobacterial isolation, and cultures examined at days 5 and 26 for Re and at week 8 for Mycobacterium sp. Suspect colonies were identified through PCR amplification of the choE gene (Re) and of the IS901 and IS1245 (MAC). Re was found in 45 (51.7%), and M. avium subsp. hominissuis in 6 (6.9%) out of the 87 grossly lesioned carcasses found, whereas Re only was found in 16 out of 72 controls (22.2%). The casual isolation of M. avium subsp. avium in 5 pigs originating from 3 herds further extends the etiological range. Detailed genotyping of these isolates and human strains, alongside representative sampling in Belgian abattoirs, will allow to better define this emerging risk.

# 10:30 - Long term follow up in dogs with eosinophilic bronchopneumopathy treated with inhaled glucocorticosteroid therapy: preliminary results

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The treatment of dogs with idiopathic eosinophilic bronchopneumopathy (EBP) consists in long-term oral administration of prednisolone at the minimum effective dose. Unfortunately, chronic systemic glucocorticosteroid therapy may lead to iatrogenic hyperadrenocorticism. Therefore, inhaled steroids have been increasingly used, although the clinical response remains unknown in EBP. The aims of this study were to assess the clinical response to long-term inhaled steroid therapy with fluticasone (IST), and to investigate possible side effects including inhibition of pituitary-adrenal axis (PAA). Six dogs with EBP, diagnosed based on clinical, radiographical, bronchoscopical examinations and bronchoalveolar lavage fluid analysis, and treated with IST only were retrospectively recruited. At the diagnosis, duration of clinical signs varied from 2 to 12 months; a clinical severity index (CSI) was calculated (cough 0-3, retch 0-1, exercise intolerance 0-1) and varied between 3 and 5. At the recheck, CSI was calculated and an ACTH stimulation test was performed. CSI decreased in all dogs and varied from 1 to 3. Three dogs showed persistent cough. In the 3 other dogs, cough reappeared as soon as treatment was not regularly administered. In two dogs treated for more than 2 years, ACTH stimulation test revealed inhibition of PAA; only one dog had clinical iatrogenic hyperadrenocorticism. This preliminary study indicates long term IST does not appear to allow a proper management in all dogs with EBP. Besides, this therapy may induce inhibition of PAA. A prospective study including more dogs treated with IST and with regular control of the PAA is warranted.

#### 10:45 - Effect of omeprazole on canine cerebrospinal fluid composition

Girod M.<sup>1</sup>, Allerton F.<sup>1</sup>, Gommeren F.<sup>1</sup>, Tutunaru F.<sup>1</sup>, De Marchin J.<sup>2</sup>, Van Soens I.<sup>1</sup>, Peeters D.<sup>1</sup>.

The use of omeprazole has been advocated in dogs to reduce cerebrospinal fluid (CSF) production in conditions where an accumulation of CSF may be associated with intracranial hypertension. Two previous studies have shown that omeprazole significantly decreased CSF production in the short term in rabbits and dogs but the underlying mechanism remains unknown. In this study, we evaluated the effect of omeprazole administration on canine blood and CSF composition, including albumin quotient (QAlb: the ratio between CSF and serum albumin concentrations, a reliable parameter to evaluate CSF flow with decreasing CSF production resulting in an increased QAlb). Fifteen healthy dogs received omeprazole orally for 14 days. In each dog, CSF and blood were obtained before and after treatment. Erythrocyte and leukocyte counts, pH, lactate, glucose, total protein and electrolyte concentrations were measured in samples. CSF albumin concentrations were evaluated by immunoturbidimetric assay and high-resolution protein electrophoresis (HRE). There were no significant differences in the composition of CSF before and after omeprazole administration except for a mild increase in sodium (P=0.002). Omeprazole induced significant increase in blood pH (P<0.001). A strong linear correlation was found between albumin concentrations measured with immunoturbidimetry and HRE (P<0.0001, correlation coefficient = 0.86). Regardless of methodology, QAIb did not change significantly (P=0.918). The lack of change in QAIb suggests that there is no change in the CSF flow rate following oral therapy with omeprazole. Therefore, in this study, there is no evidence of scientific benefit of long-term omeprazole treatment to reduce CSF production in dogs.

# 11:30 - Haematology and plasma metabolites in horses offered linseed oil over a 4 months period

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Horses are offered diets high in concentrate during the training and racing seasons. Fat has been suggested as an alternative compound to cereals to provide energy and to decrease the starch level and associated disturbances. Oils increase the energy but also the essential fatty acids supplies. In this study, eight exercised adult horses were used during four months. The diet was made of 50% grass hay and 50% compound feedstuff. The control concentrate was composed of 48% of whole spelt, 48% of rolled barley, 3% of molasses and 1% of a mineral mixture. In the oil supplemented concentrate, 8% of barley was substituted by 8% of first pressure linseed oil. The hay and the compound feedstuffs were completely eaten within one hour after being offered. The linseed oil inclusion did not affect the plasma glucose and insulin concentrations. By contrast, there were reductions in plasma concentrations of urea and triacylglycerol. An increase in total cholesterol concentration was also observed. There were period effects on concentrations of plasma glucose, total cholesterol and insulin with a large increase in plasma insulin when month 4 was compared with month 1. In terms of haematology, the linseed oil inclusion reduced the erythrocyte counts, the haemoglobin content and the haematocrit but there were no period effects on haematology.

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## 11:45 - Exercise modifies the innate immune response in equine bronchial epithelial cells

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Lower airway diseases are common problems in sports and racing horses. In humans, exercise causes increased susceptibility to respiratory infections associated with down-regulated expression of Toll-like receptors (TLRs), co-stimulatory and antigen-presenting molecules. Whether such immunosuppression happens in horses following exercise has not been investigated yet. Because the airway epithelium is a major barrier against airborne infections with important immune functions, we aimed to assess the effect of exercise and training on innate immune responses of equine bronchial epithelial cells (EBEC). Eight horses were sampled by bronchoscopy at rest and 24 hours after a standardized exercise test (SET) both under an unconditioned state and following a 4-month training period. We cultured EBEC in vitro and compared their respective expression of TLR1-9 by qPCR and their cytokinic responses following exposure to various TLR ligands by ELISA. The expression of TLRs in EBEC was not modified by a SET or training. Nevertheless, EBEC from trained horses produced less TNF-alpha after treatment with ligands of TLR2 (FSL) and TLR3 (Poly(I:C)) compared with EBEC from unconditioned horses. Additionally, a single SET increased TNF-alpha significantly after treatment with ligands of TLR2 and TLR3 in EBEC from trained horses but not in EBEC from unconditioned horses. In contrast, neither a single strenuous exercise nor training had a significant effect on the production of IFN-beta in EBEC. In summary, although exercise does not impact on TLR mRNA expression, the secretion of TNF-alpha induced by TLR ligands is modulated in an opposite manner after acute and chronic exercise.

# 12:00 - Early indicators for exertional rhabdomyolysis in standardbred racehorses using high-resolution respirometry

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Reasons for performing study during racing, skeletal muscle mitochondria are the main producers of energy for contraction. Dysfunction of the oxidative phosphorylation (OXPHOS) system may alter performance and result in myopathy. Assessment of muscle oxidative capacity in racehorses might identify animals at risk. **Objectives:** To assess the applicability of high-resolution respirometry (HRR) for early detection of racehorses at risk of exertional rhabdomyolysis (ER). Study design: Prospective cohort study. Methods: At the start of a competitive racing season, 10 French standardbreds in training underwent a standardized exercise test with determination of speed at onset of lactate accumulation (VLA4) and postexercise serum creatinine kinase activity (CK), and muscle microbiopsy for HRR measurement of OXPHOS and electron transfer system (ETS) capacities with electrons fed into the ETS at different levels of the mitochondrial respiratory chain complexes. Associations between HRR parameters and occurrence of ER over the following racing season were analysed using univariate logistic regression. **Results:** Horses which developed ER during the study period (n=2) were those with lowest OXPHOS/ETS, highest Complex I/Complex I+II OXPHOS and highest Complex I OXPHOS/Complex II ETS ratio (perfect prediction of ER whit ratio of  $\leq 0.74$ ,  $\geq 0.60$  and  $\geq 0.715$  respectively). VLA4 for ER horses was within the range of non-affected horses. Postexercise CK was within normal limits for all horses in the study. Conclusions: and potential relevance OXPHOS and ETS capacities and derived ratios seem useful indicators of risk for ER whereas SET results VLA4 and CK were unremarkable in the horses subsequently affected by ER.

## 12:15 - Altered mitochondrial oxidative phosphorylation capacity in horses suffering from polysaccharide storage myopathy

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Introduction: Polysaccharide storage myopathy (PSSM) is a widely described cause of exertional rhabdomyolysis in many equine breeds. Recent studies identified a dominantly genetic defect in the skeletal muscle glycogen synthase (GYS1) enzyme at the basis of the Type 1 PSSM phenotype. The condition is characterized by increased skeletal muscle glycogen concentration, and abnormal polysaccharide accumulation in myofibers. Gene expression studies indicated down-regulation of some mitochondrial genes and we hypothesized that, in type-1 PSSM-affected horses, the energetic production through the oxidative phosphorylation (OXPHOS) in the mitochondria of myofibres might be impaired. Material and Methods: During the year 2012, 8 horses with a history of recurrent exertional rhabdomyolysis with increased serum creatine kinase activity were tested for the GYS1 mutation (test performed on EDTA whole blood). Muscle biopsies were collected on the gluteus medius and triceps brachii muscles of each horse and used for histological analysis and high resolution respirometry (HRR). Results: Four horses were tested positive to type-1 PSSM and were included in the study. Histology revealed accumulation of abnormal glycogen in both muscles. A severe depression of the maximal OXPHOS capacity was observed by HRR (minus 56% in the *gluteus medius* and minus 38% in the *triceps brachii*). Conclusion/Discussion: Our study shows for the first time a severe decreased OXPHOS capacity in type-1 PSSM-affected horses. PSSM is considered primarily as a glycogenosis but altered OXPHOS might also play a central role in the pathogenesis of this condition. Our fundamental understanding of the pathophysiology of PSSM will be improved by detailed diagnosis of mitochondrial dysfunction.

# 14:30 - A gammaherpesvirus uses alternative splicing to regulate its tropism and its sensitivity to neutralization

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Human gammaherpesviruses are associated with the development of several lymphomas and epithelial malignancies. The heterogeneity of these tumors reflects the ability of these viruses to route infection to different cell types at various stages of their lifecycle. While the Epstein Barr virus uses gp42 - Human leukocyte antigen class II interaction as a switch of cell tropism, the molecular mechanism that orientates tropism of rhadinoviruses is still poorly defined. Here, we used bovine herpesvirus 4 (BoHV-4) to further elucidate how rhadinoviruses regulate their infectivity. In absence of any gp42 homolog, BoHV-4 exploits the alternative splicing of its Bo10 gene, to produce distinct viral populations in function of the originating cell. While epithelial cells produce virions with high levels of the accessory envelope protein gp180, encoded by a Bo10 spliced product, myeloid cells express reduced levels of gp180. As a consequence, virions grown in epithelial cells are hardly infectious for CD14+ circulating cells, but are relatively resistant to antibody neutralization due to the shielding property of gp180 for vulnerable entry epitopes. In contrast, myeloid virions readily infect CD14+ circulating cells but are easily neutralized. This molecular switch could therefore allow BoHV-4 to either promote its dissemination into the organism or, on the opposite, its transmission between hosts.

# 14:45 - Experimental infection of cattle with bluetongue virus serotypes circulating in Europe

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From 1998 to 2006, Europe had to face the emergence of Bluetongue virus serotypes 1, 2, 4, 8, 9 and 16 (BTV-1, 2, 4, 8, 9, 16), including countries where the virus has never been detected before. These unprecedented outbreaks trigger the need to evaluate and compare the clinical, virological and serological features of the European BTV serotypes. In this study groups of calves were infected with European BTV serotypes. For each tested serotype, two groups of three male Holstein calves were used: one vaccinated (against BTV-8) and one non-vaccinated group. BTV-6 was evaluated through a co-infection with BTV-8 and the calves were not vaccinated. Clinical signs were quantified. Viral RNA was detected by serotype specific RTqPCR. Serological relationship was assessed and *In vitro* growth properties of the different serotypes were compared. Calves were slaughtered 35 days post infection and necropsied. Most of the infected animals showed very mild clinical signs. BTV-2, 4 and 6 viral RNA only reached low levels, when compared to other serotypes. Maximal viraemia level of the vaccinated calves ranged from 53 to 91 % of the non-vaccinated animals. In addition, being non-vaccinated led to a relative risk of 1.76 (1.02-3.04) of positive viral RNA detection in organs. A partial cross reactivity could be shown between BTV-1, and serum from BTV-16 and BTV-8/BTV-6 groups; also between BTV-8 versus BTV-1 serum. BTV-1 had a faster replication within the first 48 hours post-infection. The real consequences of these findings in the field cannot be stated so far.

# 15:00 - Bovine noroviruses in Belgium: from a molecular and evolutionary perspective

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Viruses genetically related to noroviruses, major agents of acute non bacterial gastroenteritis in humans, were detected in stools from cattle, logically raising the hypothesis of a zoonotic risk or a bovine reservoir. Here are reported the results of about 8 years of molecular and genetic studies designed to resolve that hypothesis in the Belgian epidemiological context. These studies helped the Public Health authorities to presently refute or attenuate the zoonotic risk associated to bovine noroviruses. No human norovirus sequence was detected in calf stools and phylogenetic relationships showed that genotype 2 strains were the more prevalent, raising hypothesis on their better host adaptation and reinforcing the low zoonotic risk associated to bovine noroviruses as their attachment factors are not expressed on human cells. Recombination events were detected and genetic comparisons between entire sequences detected on a thirty-years period allowed to highlight the genomic regions the more subjected to genetic evolution. Substitution rates were inferred by Bayesian methods. Interestingly, they notably showed that bovine noroviruses seemed to evolve slower than the human ones, allowing the development of a concept of different evolution driving forces depending on the genogroup/genotype in the genus *Norovirus*.

# 15:15 - The role of Alcelaphine herpesvirus 1 Semaphorin 7a-homologue secreted 115 kDa glycoprotein during malignant catarrhal fever

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Alcelaphine herpesvirus 1 (AlHV-1) is a gammaherpesvirus that persists asymptomatically in wildebeest. However, AIHV-1 transmission to a large number of ruminants, including cattle and the experimental rabbit model, results in the development of a lethal lymphoproliferative disease named malignant catarrhal fever (MCF). The A3 gene of AlHV-1 encodes a putative semaphorin-homolog protein termed AlHV-Sema. Semaphorins are glycoproteins characterized by a conserved amino-terminal Sema domain. The cellular homologue semaphorin 7A (Sema7A) is notably involved in T cell-mediated inflammatory responses. Here, we sought to determine whether AIHV-Sema shares similar functions with Sema7A and whether the viral protein has a role during MCF. First, we used Fc-tagged AIHV-Sema constructs and showed that AIHV-Sema is a 115 kDa secreted glycoprotein during viral infection in vitro. Next, the Fctagged AlHV-Sema recombinant protein was used in binding assays on different cell lines. Though binding could not be detected on murine macrophages, we detected specific binding of AIHV-Sema to the surface of several bovine cell lines, including macrophages and monocytes. Finally, we addressed the role of AlHV-Sema during viral infection and produced an A3-deleted viral strain and its revertant control strain. In vitro, the absence of A3 did not significantly affect virus growth. In vivo, although rabbits infected with the A3-deleted virus developed typical MCF lesions, MCF developed with a small delay compared to control groups. This observation was associated with a reduced inversion of CD8 over CD4 ratio and a reduced increase of IFN-y production in lymphoid tissues. Together, these results suggest that AIHV-Sema might play a role in the host response to AlHV-1 infection. Further analyses are currently in progress to determine the regulating functions of AIHV-Sema during viral infection.

## 15:30 - Milk fatty acids as influenced by energy metabolism in early lactating dairy cows

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The monitoring of energy metabolism in the early part of lactation of high producing cows is essential for the management of the herd. Blood and milk samples were obtained monthly on 61 cows on 4 occasions starting from calving. The fatty acids (FA) data sets were divided in categories based on  $\beta$ -hydroxibutyrate (BHB), plasma non esterified fatty acids (NEFA) and the ratio (C18:0 + C18:2)/C18:1 in the NEFA fraction as energy metabolism indicators. In the fat mobilizing cows, both the amounts and the proportions of the milk C6-C14 FA – neosynthesis in the mammary gland – were lower than in the non mobilizing cows. For example, 14.8 vs 17.6% P<0.01 in the FA ratio comparison, 15.3 vs 17.7% P<0.01 in the BHB classes and 15.1 vs 19.5% P<0.001 in the NEFA classes. On the other hand, the milk C18:1 was higher (P<0.001) in the mobilizing cows (14.4 vs 11.5 g/l in the FA ratio comparison, 14.2 vs 11.0 g/l in the BHB classes and 15.1 vs 10.1 g/l in the NEFA classes). It is concluded that milk fatty acids profile changed in proportions and in amounts when energy status is modified in early lactating cows. So milk fatty acids profile during early lactation could be an accurate indicator to manage the waiting period.

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# 15:45 - A splice-acceptor site variant in the bovine *PIGH* gene causes glycosylphosphatidyl inositol deficiency and lethal arthrogryposis syndrome

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In 2009, the heredo-surveillance platform in Belgian Blue Cattle Breed was contacted by breeders and veterinarians regarding the occurrence of newborn calves presenting lethal generalized arthrogryposis syndrome with macroglossy and impaired tooth eruption and in some cases cleft palate and lips, omphalocele and bilateral corneal clouding and fetal membranes hydrops causing early cull of the dams and extra economic losses for the breeders. We used haplotype-based association analysis to map the causative gene to a 2.2 Mb identical-by-descent interval on bovine chromosome 10. Selected cases were subjected to high-throughput (HT) genome-wide sequencing to reach full coverage of the region. After stringent filtering against a collection of polymorphisms present in unrelated control individuals, no evidently deleterious mutation stood out among the remaining handful of private mutations. Subsequent HT-RNA sequencing data analysis of a heterozygous mutant embryo highlighted a C to G transversion (211-10C>G) in the splice acceptor region of the phosphatidylinositol glycan anchor biosynthesis class H (PIGH) intron 1 as possibly relevant. Indeed, this mutation was shown to provoke total skipping of PIGH exon 2 in homozygous mutant tissues. Exon 2 being in frame, the corresponding mutant protein, 37% shorter, was missing its central part. PIG-H protein has been characterized as an essential member of the enzymatic complex catalyzing the initial step of glycosylphosphatidylinositol (GPI) biosynthesis involved in the anchoring of a subset of cell surface proteins. Mutations in other complex components are known to cause embryonic lethality in human and mice. Moreover, several characterized GPI-anchored proteins mutants in human and mice presented cranio-facial, skeletal and ocular abnormalities, macroglossia and hernia reminiscent of bovine arthrogryposis syndrome.

# 16:30 - Rational design of an attenuated recombinant vaccine against Cyprinid Herpesvirus 3 using BAC cloning mutagenesis and in vivo imaging system

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Cyprinid herpesvirus 3 (CyHV-3) is the causative agent of a lethal disease in common and koi carp. Since its emergence, in the late 1990s, CyHV-3 has caused severe economic losses worldwide creating a need for a safe and efficacious vaccine. Taking advantage of the recent cloning of CyHV-3 genome as an infectious bacterial artificial chromosome (BAC), we produced recombinant candidate vaccine strains by deletion of single gene. While producing such a recombinant for ORF134, we unexpectedly obtained a clone deleted for ORF56 and ORF57 in addition to ORF134 (as a consequence of an illegitimate recombination while removing the BAC cassette). Interestingly, this triple deleted recombinant replicated efficiently in vitro, exhibited an attenuated profile in vivo and induced an immune protection against a lethal challenge. To confirm that the triple ORF56-ORF57-ORF134 deletion was indeed responsible for the phenotype observed and to determine the contribution of ORF134 deletion in the attenuation; a double ORF56-ORF57 deleted recombinant and an independent triple ORF56-ORF57-ORF134 recombinant were produced and tested in vivo. These experiments revealed that ORF134 deletion did neither contribute significantly to the attenuation observed nor influence the adaptive immune response induced by the infection. Importantly, these experiments demonstrated that ORF56-ORF57 deletion was responsible for the attenuation observed. Next, to determine the contribution of each locus to the attenuated phenotype; two single deleted recombinants were produced for ORF56 and ORF57. In vivo testing of these recombinants suggested that most, if not all, the attenuation observed for ORF56-ORF57 double deleted recombinant is due to ORF57 deletion. Based on its safety-efficacy profile, the ORF56-ORF57 double deleted recombinant was selected as a candidate vaccine. Its tropism and replication were studied using a derived recombinant strain expressing luciferase and in vivo imaging system.

# 16:45 - The mechanism of behavioral fever induced by Cyprinid herpesvirus 3 in common carp (*Cyprinus carpio* L.)

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Fever in response to infectious or inflammatory agents is an ancestral mechanism of innate immunity existing in all vertebrates including fish. Cyprinid herpesvirus 3 (CyHV-3) is the causative agent of a lethal disease in common and koi carp. We demonstrated previously that CyHV-3 infection of carp induces the expression of behavioral fever. To study the mechanism of behavioral fever in carp during CyHV-3 infection we developed multi-chamber aquaria with temperature gradient (24°C-28°C-34°C) and informatics system to monitor the position of the fish. The results of our study demonstrated that: 1) CyHV-3 infection induced behavioral fever in carp. Infected fish migrated to the compartment with the highest temperature of water (34°C) and stayed there until control of the disease. 2) Behavioral fever induced by CyHV-3 is salutary. Migration of infected fish to 34°C resulted in the absence of mortality while a mortality of 100% and 80% was observed when the fish were maintained in control tanks at 24°C and 28°C, respectively. 3) PGE2 plays a role in development of behavioral fever. Inhibition of PGE2 production by COX1/2 inhibitor (Indomethacin) impaired the expression of behavioral fever. 4) Virus encoded genes can modulate behavioral fever. A series of experiment using CyHV-3 recombinant strains deleted for ORF12 (encoding a soluble TNF-a receptor homologue) or ORF134 (encoding a viral IL-10 homologue) demonstrated that deleted strains induced behavioural fever earlier than the control revertant strains. These results suggested that TNF-a could be one of the potential mediator of fever in fish. 5) Supporting this hypothesis, blocking of TNF-a inhibited behavioral fever. Anti-carp TNF-a antibodies blocked migration of infected fish to 34°C.

# 17:00 - Sensitivity and permissivity of *Cyprinus carpio* to Cyprinid herpesvirus 3 infection according to host ontogenesis

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Cyprinid herpesvirus 3 (CyHV-3) is the causative agent of a lethal disease in common carp (*Cyprinus carpio carpio*) and koi (*Cyprinus carpio koi*). In the present study, we investigated the sensitivity and the permissivity of common carp to CyHV-3 infection according to host ontogenesis. Each developmental stage (from hatching to juvenile stage) was infected by immersion in water containing the CyHV-3 recombinant LUC strain expressing firefly luciferase as a reporter gene. In vivo imaging system (IVIS) analyses performed 24 h and 72 h post-infection demonstrated that carp is sensitive and permissive to CyHV-3 infection from hatching onwards; however, both sensitivity and permissivity increased with ontogenesis. Permissivity of carp at all stages of development was further demonstrated by qPCR analyses, by immunostaining with a monoclonal antibody revealing replication and by the mortality induced. The results of the present study demonstrated that, in contrast to what has been claimed elsewhere, carp are sensitive and permissive to CyHV-3 infection at all stages of development.

# 17:15 - Laboratory validation of a lateral flow device for the detection of CyHV-3 antigens in gill swabs

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Cyprinid herpesvirus-3 (CyHV-3) induces the highly contagious koi herpesvirus disease (KHVD) and may result in significant economic losses to the ornamental and food-producing carp industry. Suspicion of KHVD is triggered by clinical signs and confirmed using laboratory techniques. The latter are labourand time-consuming, require specialised equipment and trained personnel. For rapid, on-site detection of CyHV-3, a lateral flow device (LFD) was developed using two monoclonal antibodies directed toward the viral glycoprotein ORF65. The LFD was highly specific with analytical and diagnostic specificities of 100%. Analytical sensitivity ranged between 1,25x10² and 2,40x10⁴ plaque forming units per ml for isolates originating from geographically distinct regions. In experimentally infected carp, CyHV-3 was detected as early as 4-5 days post infection. Diagnostic sensitivities of 52.6% and 72.2% relative to PCR were recorded, depending on the viral isolate used. When onset of mortality was taken as reference, diagnostic sensitivities increased to 67.0% and 93.3%. The diagnostic sensitivity for freshly found-dead animals was 100%, irrespective of the virus isolate used. Given the high specificity and ease-of-use for on-site detection of CyHV-3, the LFD was regarded fit for purpose as a first-line diagnostic tool for the identification of acute CyHV-3 infections in KHVD affected (koi) carp.

## **Posters**

#### **Posters**

#### 1. KNN-MDR Approach for Detecting Gene-Gene Interactions

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These last years have seen the emergence of a wealth of biological information. Facilitated access to the genome sequence, along with massive data on genes expression and on proteins have revolutionized the research in many fields of biology. For example, the identification of up to several millions SNPs in many species and the development of chips allowing for an effective genotyping of these SNPs in large cohorts have triggered the need for statistical models able to identify the effects of individual and of interacting SNPs on phenotypic traits in this new high-dimensional landscape. GWAS has allowed the identification of hundreds of genetic variants associated to complex diseases and traits, and provided valuable information into their genetic architecture. Nevertheless, most variants identified so far have been found to confer relatively small information about the relationship between changes at the genomic variants and phenotypes. A possible reason is that interactions between genomic variants play an important role. To tackle this subject, different statistical methods such as MDR (Multi Dimensional Reduction) have been proposed for detecting gene-gene interaction; their relative performances remain largely unclear, and their extension to situations combining many variants turns out to be challenging. So we propose a novel MDR approach using K-Nearest Neighbors (KNN) methodology (KNN-MDR) for detecting gene-gene interaction as a possible alternative, especially when the number of involved determinants is potentially high. The idea behind our method is to replace the status allocation used in classical MDR methods by a KNN approach. Simulations show that this strategy allows comparable performances in situations where few variants are involved, but increases power and accuracy for detecting gene-gene interaction when more mutations are interacting, which is an important result of our work.

**Key words:** gene mapping, interaction, machine learning.

# 2. Comparative study of 3 DNA extraction protocols for recovery of genomic microbial ADN from the Algerian traditional date product "Btana".

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The next-generation high-throughput DNA sequencing techniques applied to study the microbial diversity of food relied on DNA extraction efficiency for an accurate estimation of the true richness of the cultured and uncultured microbial community present. Therefore a comparative study of 3 protocols of DNA extraction from 11 samples of an Algerian traditional date product (Btana) was set up to evaluate their efficiencies in genomic microbial DNA recovery. Protocols were based on a commercial kit DNeasy (QIAGEN, Germany), and two modified CTAB extraction methods using polyvinyl pyrrolidone (PVP) and treatment with 5M NaCl solution (1: CTAB, 2: CTAB-DNeasy). Protocols were compared for quantity of DNA extracted using NanoDrop® ND-1000 Spectrophotometer and quality of DNA by 260/280 nm absorption ratio. The gDNA extracted was cheeked by PCR amplification of the region V7 to V8 of the 16S rRNA and visualized by electrophoresis on agarose gel (0.8%). Results showed that CTAB modified method1 provide the best DNA yield; however a purification with NucleoSpin® Kit (Clontech, UK) and dilution steps were mostly needed for amplifying the DNA template. DNeasy kit protocol gave an amplified high quality DNA, but poor yields were obtained from date samples.

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#### 3. Quality assessment of marketed eggs in Eastern Algeria

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The quality assessment of eggs can be based on their composition, freshness and price. It depends on many factors such as the breed, feed, age and storage conditions. The main objective of this study was to compare the eggs quality between industrial (IC) and local chicken (LC) in Bejaia, Jijel, Mila and Setif departments (Eastern Algeria). A total of 4748 eggs (LC: 1036 eggs; IC: 3712 eggs) were bought from three marketing channels, i.e. shops (LC: 416; IC: 768), public markets (LC: 578; IC: 765) and supermarkets (LC: 42; IC: 765). The proportion of stained eggs was lower in industrial eggs compared to local eggs (15.25 vs. 27.61%). The industrial eggs were heavier than local eggs (61.01 vs 53.28g) while shell weight and thickness are larger for industrial eggs (7.10 vs 6.30g; 0.381 vs 0.325mm), the shell percentage was lower than local eggs (11.60 vs 11.89%). The weight and the percentage of albumen were greater for industrial eggs (37.44 vs 29.69g; 61.34 vs 55.71%) but the albumen height and the Haught unit, as indicator of protein quality and egg freshness, were lower than local eggs (5.27 vs 5.93mm; 69.12 vs 77.80). The yolk parameters were at the advantage of local breeds. Yolk weight (16.47 vs 17.28g), yolk percentage (27.06 vs 32.44%), yolk color, determined by the DSM Yolk Color Fan method, (9.52 vs 10.94) and the yolk/albumen ratio were lower with industrial compared to local eggs (0.44 vs 0.58). Finally, the egg price of industrial chicken was lower than local breeds (7.67 vs 12.84 DA). These mentioned traits were significantly different between industrial and local breeds (P<0.0001). According to the national preference, the eggs of local hens present interesting quality criteria such as freshness, yolk/albumen ratio and yolk color.

Key words: Algeria, table eggs, eggs quality, eggs price, Marketed table eggs.

### 4. Mechanical Torsional Properties of Tibiae Following Modified Maquet Technique or Tibial Tuberosity Advancement

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**Introduction**: The purposes of this study were to quantify the variations in biomechanical torsional properties of the tibia following Modified Maquet Technique (MMT) or Tibial Tuberosity Advancement (TTA), and compare MMT versus TTA. **Materials and methods:** Twenty dogs were randomly assigned to MMT (n=10) or TTA (n=10) group. For each dog, one tibia was assigned for MMT or TTA and the contralateral tibia was used as a control. Tibiae were embedded in a polyester resin and tested up to failure at a constant  $1^{\circ}$ /s rate of internal rotation. Torsional strength, twist angle, rotational stiffness and energy absorbed at failure were calculated. **Results:** When compared to the control leg, torsional strength and energy absorbed at failure were decreased for MMT and TTA (p< 0.01). There was no difference between MMT and TTA for these 2 parameters. Rotational stiffness was decrease for TTA p=0.02, but not for MMT. The difference between MMT and TTA was significant. **Discussion/Conclusion:** Both techniques decrease the biomechanical torsional properties of the tibia. Extending the osteotomy of MMT in the proximal diaphysis does not result in a higher decreased torsional strength and energy absorbed when compared to drilling 2 holes in the proximal diaphysis with TTA. Decreased rotational stiffness with TTA may be due to the diaphyseal screw holes. Considering that clinically TTA is not commonly associated with tibial fracture, tibiae with MMT are likely to withstand physiological torsional loading *in vivo*.

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### 5. Identification of pregnancy-associated glycoproteins and alphafetoprotein in fallow deer (*Dama dama*) placenta

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This paper describes the isolation and characterization of pregnancy-associated glycoproteins (PAG) from fetal cotyledonary tissue (FCT) and maternal caruncular tissue (MCT) collected from fallow deer (Dama dama) pregnant females. After three consecutive extractions, soluble proteins were subjected to ammonium sulfate precipitations at 0-20%; 20-40% and 40-80% saturations. Fractions having the highest equivalent PAG/total protein (eqPAG/TP) ratios were submitted to affinity chromatographies by using Vicia villosa agarose (VVA) or anti-bovine PAG-2 (R#438) coupled to Sepharose 4B gel. Eluted proteins were characterized by means of monodimensional electrophoresis, Western blot and N-amino terminal microsequence. Four distinct fallow deer PAG (fdPAG) sequences were identified and submitted to EMBL-EBI database. Comparison of fdPAG with PAG sequences identified in other ruminant species exhibited 64 to 83% identity. Our results demonstrate the interest of VVA and bovine PAG-2 affinity chromatographies for the isolation of PAG molecules expressed in deer placenta. This is the first report giving four specific amino acid sequences of PAG isolated from feto-maternal junction (FCT and MCT) in Cervidae family.

#### Growth performance and meat quality of stress negative Piétrain pigs in the tropics: the case of Vietnam

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This study was carried out at the animal farm of Hanoi University of Agriculture, Vietnam to evaluate the growth performance and meat quality of stress negative Piétrain pigs. The study concerns the body weight (BW) of 116 pigs at 7.5 months of age and the ultrasound measurements on 83 live animals in order to determine backfat thickness, longissimus muscle depth and lean meat percentage (LM). Meat color (C.I.E L\*, a\* and b\*), peak shear force (SF), pH at 24h (pH24) and at 45 minutes (pH45) post mortem were measured on 31 meat samples. Meat chemical composition (dry matter, protein, lipids and ash) was determined on 23 meat samples. All the data were analyzed according to a linear fixed model including gender effect (female and intact males) and Halothane genotype (CC and CT). The interaction between gender and genotype was not observed and therefore it was ignored from the final model. The results show that BW and LM were not significantly different between genotypes (P>0.21) while females were heavier than males (101.11 vs. 93.85 kg, P=0.0011). LM of female and male were respectively 63.27 and 63.56%. Gender and genotype didn't affect meat quality and chemical composition (P>0.05) except pH24 (P=0.0011), a\* (P<0.0001) and lipids (P=0.0012) for gender effect. The pH45 (6.50 and 6.45 for females and males) and pH24 (5.34 and 5.49 for females and males) were in the range corresponding to a normal meat. The obtained results show that stress negative Piétrain pigs have high lean meat percentage and satisfactory meat quality under tropical climatic conditions in Vietnam.

**Keywords:** stress negative Piétrain pigs, growth performance, meat quality, tropics, Vietnam.

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### 7. Post-translational modification of the Myxoma virus chemokine-binding protein M-T7 by a virally encoded $\alpha$ -2,3-sialyltransferase

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Myxoma virus is a pathogenic Poxvirus that induces a lethal disease called myxomatosis in European rabbits. It is one of the very rare viruses that encodes an α-2,3-sialyltransferase that transfers sialic acid to glycoproteins and glycolipids. Very little information is available about the role played by this glycosyltransferase in the pathogenesis of the infection. Previous experiments showed that the enzyme, encoded by the M138L gene, is not essential for virus replication *in vitro* but is important in the *in vivo* pathogenesis of myxomatosis. The objective of this study is the identification of the viral and cellular proteins modified by the α-2,3-sialyltransferase. A two-dimensional differential gel electrophoresis revealed that a target of the enzyme is the viral chemokine-binding protein M-T7. This information was confirmed by western blots. Moreover, a mass spectrometry glycan analysis of purified M-T7 proteins revealed precisely the nature of the modifications introduced by the M138L gene product. As M-T7 is a known important virulence factor of the virus, the difference in M-T7 sialylation could therefore be responsible of the *in vivo* attenuation observed during the infection with the M138L knockout virus. In the future, these results could help us to better understand the pathogeny of myxomatosis in European rabbits. Moreover, they could also help us to decipher the importance of glycans in host-pathogens interactions.

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### 8. Strandings of the common porpoise (*Phocoena phocoena*) in the southern North Sea and modeling of backtrack drift.

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Since the end of the 1990's, a strong increase in the stranding of the common porpoise has been observed in the southern North Sea. Strandings are now the main source of information in terms of the composition of the populations of marine mammals. Our study aims to refine the representativeness of the strandings as an ecological indicator of the populations at sea. To do this, the information collected during the autopsies of 90 animals stranded in two major stranding peaks (from March 1st till May 31st 2006 and from March 20th till May 20th, 2013) on the Belgian coast and Nord-Pas-de-Calais was used. Furthermore, the backtrack drift of the carcasses was simulated allowing the areas where the death of the animal would have occurred to be determined. A large part of animals come from waters lining the Dutch, Belgian and North France coast. Furthermore, their likely areas of origin are very big, covering the Channel and the southern North Sea. This phenomenon could be partially explained by the current patterns, the tides and the marine winds, which would be the reason behind the strong density of stranding on our coasts. Finally, the progressive increase of the strandings since 1990 is confirmed by our results. It would be explained in particular by the shift of the population of porpoises in the North Sea to the South of the North Sea and by the higher incidence of by-catch since the beginning of the 2000's. On top of these two reasons, are added the characteristics of winds, tides and currents pushing carcasses towards our coasts.

#### 9. Thelazia callipaeda ocular infection in two dogs in Belgium

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Thelaziosis is caused by a parasitic nematode belonging to the genus *Thelazia* (*Spirurida*, *Thelazii-dae*). Eyeworms were retrieved from the left eyes of two dogs presented for unilateral ocular discharge in Belgium. Morphological and molecular identification were performed and the parasites were identified as *Thelazia callipaeda*. The history suggested that the infection had been acquired in South-Western France and Southern Italy where the disease has been observed regularly for the last 6 and 12 years, respectively. In these two regions, the disease is considered endemic and spreading. Under topical anaesthesia the parasites were removed mechanically. The dog was treated with one dose of spot-on dermal application of imidacloprid 10% and moxidectin 2.5% (Advocate®Spot on). A dexamethasone and chloramphenicol ointment was applied three times daily. At the control visit three weeks later the infection had resolved. To the best of our knowledge, this is the first complete case report of canine thelaziosis in Belgium. The risk of introduction of the parasite in Northern Europe and particularly in Belgium is discussed.

### 10. Three cases of *Parafilaria bovicola* infection in Belgium and a few recent epidemiological observations on this emergent disease.

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Parafilariosis is a vector borne parasitic disease caused by the development of the nematode *Parafilaria bovicola* in the subcutaneous and intermuscular connective tissues of cattle. On February 28th 2012, the so-called bleeding spots were observed in two heifers and one bull in a cattle herd close to Namur (Belgium). The animals had been treated in December with an application of ivermectin/closantel solution (Closamectin pour on®, Norbrook Lab) at the recommended dosage. Samples of serohaemorrhagic exudate and blood as well as skin biopsies were collected. Embryonated eggs of *Parafilaria bovicola* in the serohaemorrhagic exudate and high levels of creatine phosphokinase (CPK) were detected. Clinically affected animals were treated with injectable ivermectin (Ivomec®, Merial) at 200 µg/kg. Two epidemiological phone surveys were carried out in the south of Belgium (Wallonia) in order to estimate the geographical distribution of this condition since it was first described and published in 2009. A standardized questionnaire was used and the results were analysed. Most outbreaks were recorded in the provinces of Liege and Luxembourg. The initial source of infection is still unknown but this parasitic infection is clearly spreading from the initial Belgian outbreak site.

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### 11. Characterization of host factors involved in resistance/susceptibility to influenza A infection in Mx-negative mouse models

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DBA/2J mice and C57BL/6J are two extremes in terms of susceptibility to influenza A virus among Mx-negative mouse strains. Several research teams focused on the factors explaining this difference, mainly by genetic approaches using Recombinant Inbred Lines between those two strains. Several candidate-genes have been proposed, but it was not possible to determine their importance. We chose a phenotypic approach, by dissecting each stage of influenza A infection virus in mice of each line, aiming at identifying critical differences between C57BL/6J and DBA/2J. Preliminary observations suggest that either the viral infection of the airway epithelium of DBA/2J is more productive, either alveolar macrophages from C57BL/6J are more efficient in viral particles phagocytosis, or a combination of these two mechanisms. We isolated and cultured tracheal cells, pneumocytes and alveolar macrophages from both strains of mice to determine the permissiveness of the cells of the respiratory tree, quantify the specific receptors of influenza A virus and to compare the alveolar macrophage phagocytic abilities. We have demonstrated a greater presence of a2,3 receptors on alveolar macrophages and tracheal cells of DBA/2J and a higher in vitro viral amplification on DBA/2J respiratory cells.

#### 12. Detection of Mycobacterium celatum in wild boars in Southern Belgium

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Bovine tuberculosis (bTB) remains a major threat in livestock industry and presence of wildlife reservoirs of Mycobacterium bovis (M. bovis) is an obstacle to bTB eradication in domestic animals. In this context, surveillance of M. bovis in wildlife is strategic for the implementation of effective control measures. The aim of this study was to analyse wild boars lymph nodes for the presence of Mycobacterium spp. In field conditions, 230 hunter-killed wild boars were investigated in hunting areas spread over 4 provinces in Southern Belgium. Wild boars submandibular lymph nodes were collected for bacteriologic examinations (Ziehl-Neelsen (ZN) staining, histopathological examination and classical mycobacterial culture). Out of 230 submandibular lymph nodes, no acid fast-bacilli were visible with ZN staining but 49 (21%) showed suggestive macroscopic lesions at necropsy. All the mycobacterial cultures were negative for M. bovis but an atypical mycobacterium, M. celatum, was isolated from 4 wild boars isolates. This uncommon bacteria was also isolated in 2 cattle farms in the same region (M. Govaerts, personal observation). M. celatum is a slow growing mycobacterium which is potentially pathogenic in humans. But the presence of this nontuberculous mycobacterium may be challenging in *M. bovis* diagnosis. Indeed, M. celatum could complicate tuberculin skin testing in cattle and serologic screening in wildlife. In conclusion, we report the first detection of M. celatum in wild boars but further studies are needed to determine the impact of this mycobacterium in the surveillance strategies related to M. bovis.

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#### 13. Prevalence Of PRRS virus in Wallonia (Belgium)

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Porcine reproductive and respiratory syndrome (PRRS) causes major economical losses in pig farms. In different parts of the world, there is a growing consensus that PRRS virus (PRRSv) eradication should be performed on a regional basis, particularly in low density areas of pig production. The objective of the present study was to evaluate the apparent prevalence of PRRSv in Wallonia, a low density area of pig production (0.05 pig farms/km²). At this stage of the study, a phone survey has been addressed to 61/276 Walloon breeding pig owners. Fifty-eight answered to the questionnaire: 51 are responsible of a farrow-to-finish farm, 6 of a farrowing farm and 1 of a PRRS-free boar station. In 35% of the tested farms, sows are vaccinated with a modified live virus (MLV) PRRS vaccine (n=11) or with a killed PRRS vaccine (n=9). In two farms, both sows and piglets are vaccinated with a MLV PRRS vaccine. When the pig owner did agree and if no vaccination was carried out on sows or on piglets, a serological Elisa test was performed in 10 breeding pigs and/or 5 feeder pigs >70kg (or in all available sera if a smaller number of pigs was present). Globally, at herd level and at animal level, the apparent prevalence were respectively 35.2% (95% CI: 23.0-47.4%) and 23.4% (95% CI: 20.1-26.6%).

#### 14. Effects of Q-fever vaccination in Belgian goat farms.

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Q fever is a zoonosis occurring worldwide and caused by *Coxiella burnetii*. In Belgium, the Ministerial Decree of May 11, 2011 establishes some control measures to be applied to ovine and goat milk farms. Among these, compulsory vaccination of PCR positive farms is implemented.

The aim of this work is to investigate the effects of Q-fever vaccination on *C. burnetii* circulation among Belgian goat farms. Sampling is carried out between 9 to 11 months after the occurrence of the last annual vaccination, on a randomly selected animal population of the farm. Three groups of goats are sampled according to their age (1, 2 and older than 2 year-old). Serum, milk, vaginal and rectal swabs are collected, as well as bulk tank milk (BTM), air collected inside the farm and at the proximity of the manure heap, and faeces on the stable floor by the «overshoes» method. Antibodies against *C. burnetii* are detected in serum, individual milk and BTM. The excretion of the bacterium is evaluated from individual animal samples (milk, vaginal and rectal swabs) and from the BTM, the air samples and the overshoes.

At present three goat farms are involved in the study (Province of Antwerp, East Flanders and Hainaut). Despite vaccination, *C. burnetii* DNA was found in the BTM of the three farms, and in environmental samples (air samples and faeces with «overshoes» method). The ELISA performed on the individual serum showed that seronegative animals of different ages were present among the vaccinated goat population.

### 15. Risk of introduction of alphaviruses responsible for American equine encephalitides in Belgium

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Arthropod-borne viruses are a threat for human and animal healths in regards with their dissemination out of their endemic area. The arboviruses reviewed here belong to the family *Togaviridae* genus *Alphavirus* and are small enveloped positive sense RNA viruses. They are considered as exotic equid pathogens in Europe and can cause severe diseases in humans in the context of an epidemic. Arboviruses have complex epidemiologic features characterised by interactions between viruses, vectors, reservoir or susceptible host species, and environment. A bibliographic search was performed to identify the mean factors that influenced past outbreaks in America and the presence of potential vectors/vertebrate hosts that could play a role in the transmission cycle in Belgium. Three equine arboviruses, currently considered as the main current threats of emergence/introduction in Western Europe, were chosen as model for this study: Eastern equine encephalitis virus (EEEV), Western equine encephalitis virus (WEEV) and Venezuelan equine encephalitis virus (VEEV). In conclusion, taking into consideration the globalisation (increase of international exchanges) and climate warming, the analysis of the different features of the arbovirus cycles are essential to a balanced risk expertise in the Belgian context.

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#### Detection of antibodies against Schmallenberg virus in wild boars, Belgium, 2010-2012

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In the summer/fall of 2011, a nonspecific febrile syndrome characterized by hyperthermia and drop in milk production with occasional reports of watery diarrhea and abortion was reported among dairy cows on farms in northwestern Europe. Further, in November 2011, an enzootic outbreak of malformed neonates emerged in several European countries, with stillbirth and birth at term of lambs, kids and calves with neurological signs or malformations of the head, spine, or limbs. Both syndromes were associated with the presence in the blood (adults) or in the central nervous system (newborns) of a new Shamonda/Sathuperi-like orthobunyavirus, provisionally named Schmallenberg virus (SBV) after the town in Germany where the first positive clinical samples were identified. Defining as precisely as possible the host range of the newcomer is a key point to predict the outcome of the emergence of SBV disease in Europe. In this respect, it must be pointed out that orthobunyaviruses infect more animal species than those in which the foetus is damaged. Recently, serological evidence for SBV infection in wild ruminant species (Cervus elaphus and Capreolus capreolus) was reported (Linden et al., 2012). In the present study, the objective was to seek after serological evidence of SBV infection among wild boars living in a geographical area where exposure to infected insect vectors was high in 2011, as judged from the very high seroprevalence reported among cattle in that region. About 700 animals were sampled during the 2010-2012 hunting seasons. All serum samples collected during the fall of 2010 were seronegative. On the contrary, apparent seroprevalence among wild boars in 2011 was ~27% and started to decline in 2012 (~11%). Acquired immunity against the new virus was thus already very high in the wild boar populations sampled in the fall 2011, suggesting that the new virus had quickly spread throughout the region since its emergence about 250 km northeast in the late summer 2011. The drop in seroprevalence recorded in 2012 suggests that the virus was no more circulating in the region.

### 17. Health screening to identify opportunities to improve preventive medicine in cats and dogs: focus on nutrition status

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Introduction: A free health screening was organized in 2011 by the Small Animal Veterinary Association (SAVAB) to emphasize the importance of preventive healthcare amongst pet owners. The purpose of this paper is to present the main results of the screening in terms of participation, animal health status and nutrition-related problems. Animals, material and methods: Vet practices were invited to register their participation on a voluntary base. By doing so, they were agreeing to allow pet owners to present their pet for a free health check (without vaccination, treatment or diagnosis procedures) to assess their pet's health status during the month of February 2011. The data obtained from the vet were recorded and analysed. **Results and discussion:** A total of 5305 health check forms (56 % dogs, 44 % cats) were analysed. Sixty-six % of dogs and 34 % of cats were considered to be « medicalized » (at least 1 visit during the previous year). For 83 % of cats, the major diet component was a commercial food, 16 % were fed mixed diets (commercial and home-made) and 0.8 % was fed homemade diets. Most dogs (65 %) were fed commercial food, 30 % mixed diets and 5 % homemade diets. Sixteen % of dogs were not weighed nor scored for BCS, 22 % and 19 % of cats, respectively, were not weighed nor scored for BCS during the health check. The high percentage of overweight dogs (34 %) and cats (36 %) was a key health issue identified. In a general way, preventive care was low in cats. Conclusion: Opportunities for increased quality of care are high. Health nutrition-related problems can benefit from preventive measures and clear recommendations including application of the WSAVA nutritional guidelines (Freeman et al., 2011).

### 18. Validation of a LC-MS analytical method for the measurement of aldehydes in meat and oil

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Due to the presence of polyunsaturated fatty acids, omega-3 rich foodstuffs are sensitive to oxidation and can generate aldehydes as degradation products during their storage and/or cooking. Some of the aldehydes formed are known to be toxic to human health. Therefore, a HPLC-MS method has been developed to evaluate the concentration of nine aldehydes in food samples: malondialdehyde, 4-hydroxy-2-nonenal (4-HNE), 4-hydroxy-2-hexenal (4-HHE), crotonaldehyde, acrolein, hexanal, 2,4-nonadienal, 2,4-decadienal and benzaldehyde. For now, the extraction and detection have been optimised in meat and oil. To assess the performance of the developed method, a method validation is necessary. The method validation will be conducted according to the criteria and procedure described in Commission Decision 2002/657/EC. This Decision recommends evaluating: specificity, trueness, recovery, ruggedness, stability, repeatability and within laboratory reproducibility. Those parameters will be evaluated during experiments planned on different days with blank samples and blank samples spiked at different levels of concentration. Stability studies of a reference material (meat, oil) and extracts will be conducted under various storage conditions and with/without the presence of an antioxidant. The concentration of each aldehyde measured in the reference material will be used to implement a control chart.

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### 19. Tolerance and anesthetic effects of 1% tetracaine hydrochloride and 0.4% oxybuprocaine hydrochloride on healthy feline cornea.

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Purpose: To evaluate the tolerance and duration of corneal anesthesia of two commercially available topical anesthetics in nine healthy European shorthair cats. Methods: In study 1, each cat received a drop of 1% tetracaine hydrochloride (Tet1%; Minims® Tetracaine Hydrochloride 1% ophthalmic solution, Bausch & Lomb, Brussels, Belgium) in one eye and 0.4% oxybuprocaine hydrochloride (Oxy0.4%; Minims® Oxybuprocaine Hydrochloride 0.4%, Théa pharma, Wetteren, Belgium) in the other eye. Signs of ocular irritation were recorded. In study 2, cats were randomly allocated to treatment order in a 2x2 (period x treatment) crossover study. Treatments consisted of topical application of ophthalmic Tet1% or Oxy0.4% to both eyes. Corneal sensitivity was determined by evaluating the corneal touch threshold (CTT) using a Cochet-Bonnet aesthesiometer (Luneau®, Chartres Cedex, France), before corneal application, 1 and 5 minutes after corneal application and at 5-minutes intervals thereafter until pretreatment CTT was reached. Data were statistically analyzed using mixed model methodology. Successive measurements made at equally-spaced timestamps on the same animal were correlated using an autoregressive structure (P<0.05). Results: Tet1% was better tolerated than Oxy0.4% and did not induce any side effects. Corneal sensitivity was significantly reduced for both Oxy0.4% and Tet1%, starting 1min up to 30min after instillation. A maximal anesthetic effect was observed 5min after application in 100% of the eyes treated with Tet1%, and only in 77% of the eyes treated with Oxy0.4%. Conclusions: 1% tetracaine eye drops are better tolerated as a local anesthetic than 0.4% oxybuprocaine for the same duration of corneal anesthesia.

### 20. Atypical presentation of an orbital osteosarcoma in a two year old dog: a case report and literature review

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In general, orbital neoplasms occur in older dogs and cause exophthalmos, prolapse of the third eyelid and pain upon opening the mouth. In this case, a 2-year-old female Bernese mountain dog initially presented with a severe chronic bilateral follicular conjunctivitis unresponsive to topical steroids. An obstruction of the lacrimal duct was then observed, followed by a prolapse of the nictitating gland 3 weeks later. Clinical work-up included complete ophthalmic examination, orbital ultrasound, x-rays of the skull, CT-scan of the head and retropharyngeal endoscopy. Ultrasonography of the eye and its adnexa showed a severe dilation of the lacrymal duct and an osteolysis of the left lacrymal bone. CT-scan showed an aggressive mass in the left retrobulbar region and left nasal cavity with bone production and destruction involving the left orbit, the left nasal cavity and the left maxillary bone. Biopsies with the help of retropharyngeal endoscopy revealed the hard consistency of the mass. Histopathological diagnosis was consistent with a well differentiated osteosarcoma. This well-documented case report describes a young dog with an unusual clinical presentation of an orbital osteosarcoma and is presented with a review of literature on orbital neoplasms.

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### 21. Ultrasonographic percutaneous anatomy of the atlanto-occipital region and indirect ultrasound-guided cisternal puncture in the dog and the cat

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Cisternal puncture in dogs and cats is commonly carried out. This poster describes the percutaneous ultrasound anatomy of the cisternal region in the dog and the cat and an indirect technique for ultrasound-guided cisternal puncture. This study was conducted in 4 stages: 1) cadaver study to establish detailed sonographic anatomy in comparison with cross anatomical sections, 2) to examine the feasibility of atlanto-occipital region sonography in live animals, 3) cadaver study to test the indirect US-guided technique, 4) in vivo study to apply the US-guided technique on clinical patients. The cisterna magna (CM) appeared as an anechoic band dorsal to the spinal cord. The spinal cord was visualized as a hypoechoic structure, tubular or oval depending on views, outlined by two thin echoic lines. The anatomic landmarks for the ultrasound-guided puncture are the CM, the spinal cord, the two occipital condyles on transverse images, the external occipital crest and the dorsal arch of the first cervical vertebra on longitudinal images. A transverse and a longitudinal image of the CM were obtained with the probe placed in the 2 perpendicular positions. While inserting the needle at the intersection of the 2 lines traced based on these images, the CM was successfully reached in all animals with the first attempt. Using these ultrasound anatomic landmarks, an indirect ultrasound-guided technique for cisternal puncture is applicable in the dog and the cat.

### 22. Ultrasonography of the collateral ligaments of the distal interphalangeal joint in horses: technique and reference images

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This study evaluates the portion of the collateral ligaments (CLs) of the equine distal interphalangeal joint (DIPJ) assessable by ultrasound (US) and describes US technique and reference images of the CLs in a series of normal forelimbs. Transverse and longitudinal US images were obtained on 5 sound horses and on 25 equine cadaver forelimbs. On 6 limbs, a needle was placed under US-guidance at the distal limit of visualization of each CL and the portion of CL visible at US was evaluated on computed tomographic (CT) images. The proportion of CL visible at US examination was more than 50% of the total ligament length in 9 of the 12 CLs assessed by CT. The normal CLs appear as oval structures located abaxially to the fossae of the middle phalanx in the transverse sections, obtained at the level of the coronary band. A centro-dorsal hypoechoic image appears with increasing proximodistal probe inclination, demonstrating different fiber orientations within the ligament. Two main fascicles, a deep and a superficial, distally divergent, are visible on longitudinal images obtained in the central part of the ligament. Awareness of the estimated portion of DIPJ CLs visible at US and detailed knowledge of the US technique and CLs morphology are essential to efficiently use US examination on clinical cases.

### 23. Circulation of Schmallenberg virus in Belgian red and roe deer populations Garigliany M.<sup>1\*</sup>, Volpe R.<sup>1</sup>, Paternostre J.<sup>1</sup>, Desmecht D.<sup>1</sup>, Linden A.<sup>1</sup>

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Schmallenberg virus (SBV) is a recently discovered vector-borne Orthobunyavirus targeting ruminants. SBV was responsible for a large outbreak in European domestic ruminant populations in 2011 and 2012. The infection of adults was associated with a mild disease, but the virus was shown to cross the placental barrier, causing a hydrocephaly/arthrogryposis syndrome in calves and lambs. After its occurrence in 2011 in Germany, SBV quickly spread across Europe and the current seroprevalence is over 90%. To assess the susceptibility of wild ruminants to the infection, a total number of 547 and 494 sera, from red (Cervus elaphus) and roe deer (Capreolus capreolus), respectively, were collected during the hunting seasons 2010 to 2012 and tested for the presence of anti-SBV antibodies. While no samples from 2010 revealed to be positive, about two-thirds of red deer and half of roe deer sampled in 2011 were seropositive. In 2012, the seroprevalence dropped to 33% in red deer and remained stable in roe deer. The high seroprevalence rates found in both species in Belgium shows that wild ruminants are susceptible to the infection by SBV. If the infection of deer was associated to a hydrocephaly/arthrogryposis syndrome similar to that observed in domestic ruminants is still unknown. The decrease in the seroprevalence observed in red deer in 2012 might be the result of the turn-over in the red deer population and reflect an absence of virus circulation in 2012. Further investigations in the upcoming years will help to enlighten this point.

### 24. Vitrification of immature equine oocytes: investigation of volume variations during the process

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Vitrification is a cryopreservation method based on an increase to infinity of viscosity (no crystallization) obtained after short time exposures to high concentration of cryoprotectants (CPs) associated with very fast cooling and warming. Crystallization of intracellular water induces disorganisation of cell organites and cell death. For these reasons, when oocytes are vitrified, the dehydration and CPs incorporation have to be optimized for a better cell survival. Actually the large size, the low membrane permeability and the high lipid content in the equine oocyte will interfere with the transmembrane flows of molecules. This study was conducted to evaluate denuded oocyte volume variations during two protocols of vitrification based on dimethyl sulfoxyde, ethylene glycol and sucrose. Base solution of the first one was a usual saline solution (PBS), and for the second one a commercialized solution for slow freezing of equine sperm and containing glycerol and egg yolk plasma (INRA-Freeze®). Oocyte volume was extrapolated from surface measurements of microscopic photographies taken every 30 seconds during expositions to CP solutions. We observed a rapid size reduction immediately after immersion into the solution illustrating the osmotic effect (outflow of water) followed by a small recovery of volume corresponding to a partial inflow of CPs across the membrane. In conclusion, oocyte volume decreased significantly during the two protocols (about 28%), whereas reduction was significantly more important with the first solution (P=0.04). The lower membrane permeability observed with the second solution could be explained by the presence of glycerol or egg yolk plasma in the medium.

### 25. Etude des caractéristiques des élevages du mouton Koundoum dans son habitat naturel au Niger

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La diffusion des races animales à haut rendement et l'uniformisation des modes d'élevage vont de pair avec un abandon des races moins productives valant par leur adaptation au milieu. Ainsi, sur les bords du fleuve Niger, les effectifs de mouton Koundoum, principal mouton à laine de la région, régressent. Face à cette érosion, l'Université de Niamey a initié un projet de conservation. Pour comprendre le contexte d'élevage de la race, une enquête a été menée, caractérisant les élevages ovins dans l'habitat naturel du Koundoum. 104 éleveurs ont été interrogés dans 4 communes sur les rives gauche et droite du fleuve Niger à 113 km de Niamey (Kollo, Tillabery, Say et Tera). Les éleveurs enquêtés avaient entre 27 et 90 ans avec une moyenne de 63 ans et sont en majorité affiliés à des groupements villageois. L'effectif moyen des troupeaux ovins dans les élevages enquêtés est d'environ 11 têtes. Exploitant principalement le pâturage naturel, 85,6% des éleveurs pratiquent la complémentation alimentaire par les résidus de récolte. La monte naturelle constitue l'unique mode d'accouplement des ovins. Les soins vétérinaires se limitent au déparasitage et quelques traitements indigènes. Des abris de nuits sont utilisés. Le manque d'implication de jeunes dans l'élevage du mouton Koundoum signe l'érosion de la race. L'importante affiliation des éleveurs à des groupements est favorable à l'organisation du projet de conservation.

Mots clés : biodiversité, élevage, caractérisation, mouton Koundoum, conservation, Niger.

### 26. Perception des causes et les conséquences de la régression de l'effectif du mouton Koundoum du Niger par les éleveurs

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En Afrique, la diversité des animaux domestiques se maintient grâce aux communautés agro-pastorales traditionnelles. Les mutations que connaissent ces communautés résultent en une érosion de la diversité génétique dont elles sont les gardiennes. En réponse aux menaces pesant sur la diversité biologique, une convention pour sa protection a été signée en 1992 par plusieurs pays, dont le Niger, dès lors engagé dans la conservation de la diversité génétique de ses races d'élevage. L'une de ces races, le mouton Koundoum, est menacé de disparition. L'objectif de ce travail est d'étudier les raisons et conséquences de ce déclin, à travers des entretiens semi-structurés auprès de 104 éleveurs de moutons dans l'habitat naturel du Koundoum. Les explications avancées pour la diminution des effectifs de moutons Koundoum sont les croisements non-contrôlés (25,00%), le manque de pâturages sur les bords du fleuve (19,23%) et la mortalité liée au parasitisme (15,38%). Cette disparition était considérée comme dommageable pour la biodiversité nationale par 7,69% des éleveurs.14,42% des éleveurs pensent que c'est une perte de valeurs culturelles et 9,62% y voient un risque de perte d'une race adaptée à leur terroir. 42,31% des éleveurs perçoivent d'abord cette disparition comme une perte de ressources financières. Les réponses des éleveurs renvoient à deux phénomènes distincts : la subsitution entres races et la diminution de l'effectif ovin total dans la zone. Les deux phénomènes appellent des stratégies de conservation différentes, in situ et ex situ. Celles-ci devraient être intégrées dans un programme global impliquant éleveurs, scientifiques et autorités politiques.

Mots clés: Perception, biodiversité, érosion génétique, éleveurs, mouton Koundoum, Niger.

### 27. Using conjoint analysis to estimate farmer's preferences for breeding ram traits in the Middle Niger

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Many methods have been developed in economics to measure consumer preferences. In general, these methods are either based on observation of behavior on the market (revealed preferences), or based on the statement of the intentions and preferences of consumers (stated preferences). Conjoint analysis used in this study falls into this latter. The objective of the study is to determine the most important attributes and attribute levels of a breeding ram for farmers in order to integrated them as part of conservation schemes of the Koundoum sheep. The attributes and their levels were determined through eleven focus groups, gathering tem persons each. These have revealed that the important characteristics of rams for farmers are the type of coat (wool/hair), coat color (bicolor/white/other) size(small/large), ear length(short/long). Based on these attributes and price (25, 35 or 45 thousands FCFA), a conjoint analysis questionnaire was established and applied to 168 breeders, using drawings of rams with different combinations of attributes' levels. Twenty choices were asked to interviewees. Each choice opposed two combinations and let the possibility to opt-out (avoid making a choice). The type of coat showed the highest weight in the final choice, with 32.34%. The dress was second (26.30%) and the length of the ears third (20.62%). Size and price showed the lowest weight with 10.90% and 9.85%, respectively. Levels' preferences showed that the choice of farmers go to a haired, white, large ram with long ears. The negative utilities shown by wool and small size appear as a measure of the incentives for Koundoum's abandonment. Per cons, white colour being appreciated, the existence of white Koundoum sheeps can be an asset to ease the rehabilitation of this breed in its natural habitat.

**Keywords**: Conjoint Analysis, preferences, breeders, breeding rams, Niger.

### 28. A new methodology to assess data availability, accessibility and form for risk analysis

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Risk assessments are mostly carried out based on available data, which do not reflect all data theoretically required by experts to answer risk questions. The present study aimed at developing methodology to assess data availability, accessibility and form, based on a codification system. This is explained
using an exotic viral disease, Venezuelan equine encephalomyelitis (VEE) as case study. The specific
data required were organised as a generic exhaustive list of data (types). A direct and an indirect survey
allowed elaborating an inventory of data sources. A coding system with reference to data availability, accessibility and form was elaborated. A parallelism was highlighted between data availability and accessibility. Most available data were found in HTML and PDF files, thus not raw tabulated data, which are more
suitable for data handling, leading to slower progress in risk assessments that require such data. The
form of data plays a key role in the feasibility and rapidness of data management and analysis, through
a prompt compilation, combination and aggregation in working databases. Harmonized and standardized
data collection systems in animal and public health would provide useful and reliable data and allow assessing data gaps through comparative studies.

### 29. Bacterial diversity and its evolution during storage of fresh beef from different origins under different atmosphere and temperature conditions

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The purpose of this study was to evaluate the bacterial diversity and its evolution during storage of fresh beef, depending on its origin, packaging and storage temperature. Two batches of three vacuum packed striploins from United Kingdom and Belgium were obtained from a food wholesaler located in the Walloon Region. Fifteen days after slaughter, the striploins were sliced and individually kept under vacuum for 30 days: i) at  $-1^{\circ}$ C; ii) at  $+4^{\circ}$ C and iii) at  $-1^{\circ}$ C for 15 days and then at  $+4^{\circ}$ C for 15 days. The bacterial diversity was evaluated by metagenomic approach 15, 30 and 45 days after slaughter. Furthermore, each 15 days part of the vacuum packed striploin slices were repacked under modified atmosphere ( $70^{\circ}$ 0  $_2$ /30% CO $_2$ ), stored at  $+4^{\circ}$ C for 2 days and at  $+8^{\circ}$ C for 5 days, and then analyzed. Metagenomic analysis revealed a selection of the initial flora depending on atmosphere and temperature conditions. The development of *Lactobacillus algidus* was favored in samples preserved under vacuum at  $-1^{\circ}$ C, while a predominance of *Lactococcus piscium* was observed for samples stored at  $+4^{\circ}$ C. Moreover, storage under modified atmosphere favored the development of *Leuconostoc gasicomaticum*. These microorganisms have already been isolated from beef, but no study has evaluated their role in food conservation. The next step of this study will be to isolate and characterize strains of *Lactobacillus algidus* from meat and to assess their bioprotective potential.

#### 30. Fatal plastic impaction in a minke whale (Balaenoptera acutorostrata)

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On March 10, 2013, a minke whale was found dead on the beach of Nieuwpoort, Belgium. The animal was necropsied the day after and sampled following a standard procedure. The whale was a juvenile male of 3.4 m and an estimated weight of 400 kg. It was very fresh (conservation code 2) with hemorrhagic skin abrasions suggesting that it had stranded alive. The nutritional condition was poor (severe emaciation) with vertebral processes prominently discernible and a concave aspect of the dorsal muscular masses indicative for severe amyotrophy. The blubber thickness measured 24 mm. The main findings were anemia, subcutaneous edema, hydropericardium and hydroperitoneum (ascite) and edema around coronary vessels. Both lungs were uniformly congestive with a severe pulmonary edema. The size of the stomach was reduced and the gastric lumen in the fundus was filled with four compacted plastic items (400 g wet weight). The plastics bags were clogged tightly together at the junction with the third stomach, occluding the pylorus and responsible for an obstruction or impaction. The severe emaciation is believed to be associated with the gastric impaction responsible for a prolonged starvation. In baleen whales, ingestion of plastics has been described in a Bryde's whale (*B. edeni*) from Australia and in a minke whale from France, but in both cases, no associated consequences were reported and then this represents the first case of death due to litter ingestion in a baleen whale.

# 31. The ORF25 gene family of Cyprinid herpesvirus 3 encodes non-essential structural glycoproteins: roles of the paralogues in the biology of the infection *in vitro* and *in vivo*

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Cyprinid herpesvirus 3 (CyHV-3) genome encodes a family of structural transmembrane glycoprotein called ORF25 family. The ORF25 gene family is composed of 6 paralogous sequences: ORF25, ORF26, ORF27, ORF65, ORF148 and ORF149. ORF26 was described as a pseudogene. ORF25, ORF65, ORF148 and ORF149 were classified as structural envelope glycoproteins. ORF27 was first described as a pseudogene, but after sequencing and comparison of laboratory strains and field strains, the hypothesis was raised that the laboratory strains could have acquired deletion in the ORF27 as a consequence of its possible negative effect on viral growth in vitro. The goal of the present study was first to produce a recombinant CyHV-3 strain expressing the wild type ORF27 and to assess how this modification could affect the structural proteome and viral replication in vitro. Using a mass spectrometry approach, proteins encoded by these recombinant strains were identified based on the analyses of semi-purified virions. A protein corresponding to ORF27 was detected in the field strain M3 and in both recombinant strains while it was not detected in the lab strain. The ORF27 recombinants were produced and their growth compared to control strains using plaque size assay and multistep growth curves. The results obtained suggest that wild type ORF27 does not regulate negatively viral replication in vitro, at least in the experimental conditions tested. However, futher experiments are required to confirm this conclusion and to identify the roles of the ORF25 family in the biology of CyHV-3 infection in vivo and in vitro.

#### 32. Aujeszky's disease virus seroprevalence in wild boar, Southern Belgium, 2012

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Sus scrofa is a largely distributed wild species in Southern Belgium. As wild boars share a variety of pathogens with farm animals, they theoretically constitute a source of contamination. From October 2012 to January 2013, an active surveillance program was carried out throughout of Wallonia. 596 blood samples were drawn from hunter-killed wild boars. The corresponding sera were screened for the presence of antibodies targeting the membrane glycoprotein-B of ADV using a competition ELISA. Descriptive characteristics of the cohort sampled are the following: 142 adults (> 2 yr old), 125 subadults (1-2 yr old, 252 juveniles (6-12 mo old) and 70 piglets (< 6 mo old). Global seroprevalence was 18.3% (IC 95%: 15.2 – 21.4). There was no significant difference in seroprevalence between males and females or between the different months of sampling. The age of the boar had a significant effect, the apparent seroprevalence observed being higher in adults than in subadults, juveniles and piglets. The location of sampling had a significant effect on the seroprevalence. The apparent prevalences were higher in the provinces of Hainaut, Namur and Luxemburg than in the province of Liege. The results gathered here are consistent with an endemic circulation of ADV in the wild boar population living in Southern Belgium.

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### 33. The nod-like receptor homolog encoded by murid herpesvirus-4 *orf63* is important but not essential for virus replication and latency establishment *in vivo*

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The role of the innate immunity is central in the immune system of the body. Therefore, pathogens have had to develop strategies in order to evade innate immune system. A recent study showed that the open reading frame 63 (ORF-63) of the Kaposi's Sarcoma Associated Herpesvirus (KSHV) can halt NOD-Like Receptor (NLR) recognition and inflammasome activation in vitro. Because KSHV has no well-established in vivo infection model and ORF-63 is a tegument protein which is conserved among all gammaherpesvirinae, related animal viruses such as Murid herpesvirus-4 (MuHV-4) allow us to tackle the same fundamental question in a more accessible form. In this study, computational analysis showed that ORF-63 of MuHV-4 shares homology with ORF-63 of KSHV and isoforms of mouse NLRP1. Expression of ORF-63 showed that this protein is mainly localized in the cytoplasm. To define the role that ORF-63 plays in gammaherpesvirus infections, we disrupted its coding sequence. Despite a severe growth deficit, MuHV-4 lacking ORF-63 (ΔORF-63) was still viable. Deletion of ORF-63 did not affect expression of adjacent genes, ORF-62 and ORF-64. In vivo experiments revealed that deletion of ORF-63 caused a significant in vivo viral replication defect in the lung. However, infectious center assays performed on spleen samples showed that MuHV-4  $\Delta$ ORF-63 viruses established delayed but equilvalent latency loads as compared to wild type and revertant viruses. In future, complete understanding of the functional significance of ORF-63 in biological cycle of MuHV-4 will help us to understand the virus cycle and to define new anti-viral targets.

### 34. Bovine herpesvirus 4 glycoprotein L is a major target of antibody neutralization

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The neutralization of enveloped virions typically involves blocking cell binding or membrane fusion (Knossow & Skehel, 2006). The core fusion machinery of mammalian herpesviruses comprises glycoproteins B, H and L (gB, gH and gL). gH and gL form a heterodimer with a central role in viral membrane fusion. In this study, in order to determine how gL contributes to the viral in vivo cycle and how this protein is important for neutralization, we disrupted its coding sequence in bovine herpesvirus-4 (BoHV-4). A lack of gL had no impact on the establishment and maintenance of BoHV-4 latency, indicating that host colonization occured. Then, we investigated the importance of anti-gL antibodies in the neutralization potential of anti-BoHV-4 immune serum. We tested this by neutralizing wild-type BoHV-4 virions with sera from rabbits infected with WT or gL deficient virions. While WT immune sera neutralized WT virions, gL- sera neutralized WT virions poorly although they display similar levels of anti-BoHV4 antibodies. These results suggest therefore that qL-dependent epitopes are the main targets for neutralization of BoHV-4 virions.

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#### 35. Ultrasound-guided epidural access in dog

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Introduction: Injections in the epidural space are commonly performed in veterinary medicine. To date, in dog ultrasound-guided epidural injection technique has not been described and herein our aim was to assess this technique. Materials and methods: A cross sectional anatomic atlas of the lumbosacral region and ex-vivo ultrasound images were obtained in two cadavers to describe the ultrasound anatomy of the region and to identify the landmarks. Eighteen cadavers were used to establish two different variations of an ultrasound-guided technique, using spinal needles or epidural catheters. The technique was then performed in three cadavers in situations, considered similar to the daily clinical activity. Contrast medium was injected and a computed tomography examination of the region was performed to assess the success of this technique. Results: The anatomic landmarks used to carry out the procedure were the seventh lumbar vertebra, the iliac wings and the first sacral vertebra. The target for the needle placement was the vertebral canal of the lumbosacral space, visualized in a parasagittal plane, which displayed a trapezoid-shaped echogenic image and the insertion of the spinal needle or epidural catheter was guided and followed in real time, until it reached the vertebral canal. Discussion: Our US features of the anatomic landmarks were similar to those reported in previous study describing the caudal lumbar region. US-parasagittal images can be used to localize and to obtain a lumbosacral epidural access. In conclusion, we described the feasibility of an ultrasound-guided epidural access technique in dog.

### 36. Economical evaluation of the impact of PRRS in a Walloon farrow-to-finish farm

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In a way to evaluate the economical effects of PRRS in a farrow-to-finish farm, a software program was used to analyze the production costs of weaned, grower and finisher pigs. Data were collected between 2004 and 2008 in two farms where Belgian Negative Landrace sows were bred with Piétrain boars. While farm B continued to be free from PRRS, farm A became positive in June 2006. To compare the production costs between the two farms considering the effects of PRRS without environmental effects, data obtained in 2006, 2007 and 2008 were corrected with differences already observed in 2004 and 2005. The production costs of weaned piglets (7-8 kg), growers (20 kg) and finishers (114 kg) were respectively 0.64, 6.45 and 20.13 EUR higher in farm A. Additional costs during the post-weaning and the fattening periods were essentially associated to lower performance: lower average daily gain and higher feed conversion ratio.

#### 37. Dose dependent challenge of sheep with Schmallenberg virus

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Schmallenberg virus (SBV) has been identified in Germany in November 2011. It belongs to the family Bunyaviridae, genus Orthobunyavirus. We implemented this study to assess the relationship between inoculum dilution and viraemia in sheep. Twelve "Mourerous" ewes, about one year old, were separated in 4 groups of 3 animals each, and were inoculated subcutaneously with 1ml undiluted infectious serum, 1/10, 1/100 and 1/1000 diluted serum following their respective group. Viral RNA was detected using a RTqPCR targeting the S segment. The induction of SBV specific neutralizing antibodies was evaluated by a seroneutralization test. Ewes were euthanized 10 days post infection (dpi) and necropsied. None of the animals showed any clinical signs or temperature rise. Mean viraemia duration was overall 6.6 ( $^{+}$ /- 1.2) days in the undiluted serum group and 5.2 ( $^{+}$ /- 0.8) days in the 1/10 serum group. In the 1/100 only one animal had a detectable viraemia and SBV RNA could only be detected at 3 dpi in the serum of one ewe infected with the 1/1000 serum. Maximal viraemia was detected in EDTA blood and serum at 3.9 and 4.3 dpi respectively. A clear increase in neutralizing antibodies titres could be found, starting at 5 and 6 dpi for the undiluted and 1/10 serum groups respectively. SBV RNA could be found regularly in spleen, prescapular and mesenteric lymph nodes. Viraemia earliness, level, and persistence, appeared to be directly dependent on the inoculated dose of SBV. Viraemia length in this study is consistent with recent experimental and field data.

### 38. The Status of Indigenous Village Chicken Production in Democratic Republic of Congo

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Local chicken breeds contribute significantly to the world production of meat and eggs and represent 80% of the world poultry population. However, the majority of these breeds had not been recorded and studied. In the Democratic Republic of Congo (DRC), poultry keeping is an important livelihood opportunity for the poors. To improve the local poultry sector, many problems are necessary to be resolved. The main objectives of this work were to evaluate the chicken production system in DRC (Kinshasa and Bas-Congo), its contribution to the food security and to improve income of breeders and to identify chicken production related constraints. The results revealed that the traditional production was dominant in the chicken production system, where the local chicken was managed mainly on scavenging with a poor supplementation grains and household food stuff. The poultry contributed significantly to the livelihoods of poor households; economically as an initial capital; as a protein source and for disposable income and exchange purposes; and socio-culturally for hospitality and exchange of gifts to strengthen social relationships. Limiting factors are animal diseases, food resource, housing conditions and predators. Thus, the economic and nutritional conditions of households can be improved by developing the local production potential. In order to reach this objective, a special attention should be paid to the food quality and management practice in general, including access to veterinary care. The genetic improvement is very important to protect local poultry breeds and increase their productivity in respecting the local breeding context.

**Key words:** Democratic Republic of the Congo, constraints, indigenous chicken, socio-economic; smallholder, village chicken production.

#### 39. Caractérisation de la race ovine Tazegzawth en Algerie: description morphobiométrique et détermination d'une formule barymétrique

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Le mouton Azegzaw est de robe blanche avec des taches noires à reflets bleuâtres sur le chanfrein, le pourtour des yeux et le lobe des oreilles. Non répertoriée par le standard des races ovines algériennes, cette race présente pourtant des qualités intéressantes d'adaptation aux régions montagneuses de Kabylie, ce qui justifie la mise en œuvre d'un programme de conservation de ce patrimoine génétique original. L'objectif de cette étude, est (1) de caractériser la morpho-biométrie de moutons de race Tazegzawth et (2) de déterminer des équations barymétriques destinées à l'estimation du poids vif (PV) au départ des mensurations corporelles (périmètre thoracique: PT; longueur du corps: LDC; hauteur au garrot: HG; hauteur à la croupe: HC). Au total, 89 sujets adultes (12 mâles, 77 femelles) sont concernés par cette étude. Les mensurations corporelles moyennes observées sont: PV: 41,53±0,53 kg (mâle: 46,71kg et femelle: 40,72kg); PT: 73,52±0,45 cm (mâle: 78,37cm et femelle: 72,77cm); LDC: 89,10±0,44 cm (mâle: 93,82cm et femelle: 88,37cm); HG: 71,40±0,43 cm (mâle: 76,06cm et femelle: 70,68cm); HC: 70,14±0,42 (mâle: 74,80cm cm et femelle: 69,42). Les formules retenues pour l'estimation du PV sont: Tous sexes confondus:  $PV=-0.025 \text{ HG}^2 + 4.78 \text{HG}-169.88 (R^2=0.87)$ ; Femelles:  $PV=-0.038 \text{HG}^2$ -6,58HG-232,39 ( $R^2$ =0,84); Mâles : PV = -0,16LDC<sup>2</sup>+0,21 HC<sup>2</sup>+284,92 ( $R^2$ =0,99). En dépit de l'échantillon réduit dans l'étude, celui-ci a révélé des caractéristiques relativement homogènes. La détermination d'une formule barymétrique pourra venir en soutien d'une sélection incluant un objectif de croissance.

Mots clés: Biodiversité, morpho-biométrie, barymétrie, race ovine Tazegzawth, Kabylie, Algérie.

#### 40. Performances de productions de la poule locale kabyle

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Cette étude a pour objectif d'évaluer le potentiel productif de la poule locale Kabyle en caractérisant d'une part les performances de croissance, et d'autre part les performances de ponte. Le suivi de la croissance des poulets a été effectué sur 204 poussins durant 17 semaines d'élevage (de mars à juin 2011). A 16 semaines d'âge, le taux de mortalité était de 9,31% et l'indice de consommation était de 6,78. La modélisation de la courbe de croissance au moyen des équations de Gompertz a permis d'obtenir les relations suivantes: pour les mâles: y = 2409 × exp (-3,68 × exp(-0,0260 × t)); pour les femelles: y = 1835 × exp (-3,71 × exp(-0,0294 × t)); avec y, le poids corporel en grammes et t, l'âge des animaux en jours. Les performances de ponte ont été étudiées chez 32 poules durant 52 semaines (d'août 2011 à août 2012). En moyenne, la ponte débute à l'âge de 166 jours à un poids corporel de 1334g. La poule pond en moyenne 173 œufs par an d'un poids moyen variant avec l'âge de la poule, de 44 à 53g. Au vu de ces résultats, il apparaît que la poule Kabyle constitue une race locale prometteuse tant pour ses performances de croissance que de ponte. A partir des données obtenues, il pourrait être envisagé de développer un programme de sélection afin d'améliorer encore davantage son potentiel productif.

Mots clés: Algérie, poule locale, croissance, Gompertz, ponte.

#### 41. Identification of Colistin resistant strains among pathogenic and nonpathogenic coliforms isolates of human and animal origin

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The increasing antibiotic resistance has led to renew interest in old antimicrobial substances. Thenceforth, during the last decade, colistin became a new therapeutic option in human medicine because of the emergence of multidrug resistant gram-negative bacilli like Pseudomonas aeruginosa, Acinetobacter spp. and carbapenemase-producing Enterobacteriaceae and because of the difficulty to find new antibiotic families active against these multiresistant bacteria. With an excellent intrinsic activity against Escherichia coli and Salmonella enterica, a low prevalence of acquired resistance and a poor absorption after oral administration, colistin is often used for the prevention and the treatment of neonatal or weaning-associated diarrhoea in food producing animals. Previously, colistin acquired resistance occurred occasionally in veterinary medicine but nowadays the problem is becoming more common and reports about this phenomenon in different bacteria species are increasing. The purpose of this work is to study the prevalence of colistin resistant strains in a collection of pathogenic and non-pathogenic coliforms of human and animal origin. Henceforth, the aims of the study are to test these isolates by a pre-screening method to find all the clinical resistant strains and to determine the MIC of the strains selected at the first step. A strain is considered to have acquired resistance when the MIC is higher than 2 µg/ml. Therefore, a colistin resistance pre-screening test is being carried out using a dilution method of this antibiotic concentration in Isosensitest agar. Then the strains growing on agar with 2 µg/ml of colistin will be tested by the Etest method to precisely determine the MIC. Work is currently ongoing.

### 42. Evaluation of the influence of weaning on changes in the composition of the piglets intestinal bacterial flora

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The objective of the study concerns the evaluation of changes in bacterial populations in the gut flora of piglets (n=5) around the time of weaning period. The samples of fecal material were collected from pigs at the experimental farm of Sart Tilman and this had enabled us to achieve our investigations by metagenomic analysis. It appears that at birth, the gut of pigs are colonized by *Escherichia coli* and *Streptococcus* that will quickly give way to other bacteria (*Bacteroidetes* and *Clostridiales*) which will also be subsequently disturbed during withdrawal. Weaning is also accompanied by a general decrease in the total bacteria population of the flora in the early days, but it will quickly regain its balance after a few days. In addition, there will be a new colonization by other bacteria like *Escherichia ssp.* that can cause diarrhea. This diarrhea may persist in some cases and cause significant mortality in farms by toxigenic strains of *Escherichia coli*.

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### 43. The IL-10 homologue encoded by cyprinid herpesvirus 3 is essential neither for viral replication *in vitro* nor for virulence *in vivo*

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Cyprinid herpesvirus 3 (CyHV-3), a member of the family Alloherpesviridae, is the causative agent of a lethal disease in common and koi carp. CyHV-3 ORF134 encodes an interleukin-10 (IL-10) homologue. The present study was devoted to this ORF. Transcriptomic analyses revealed that ORF134 is expressed as a spliced gene belonging to the early-late class. Proteomic analyses of CyHV-3 infected cell supernatant demonstrated that the ORF134 expression product is one of the most abundant proteins of the CyHV-3 secretome. To investigate the role of ORF134 in viral replication in vitro and in virulence in vivo, a deleted strain and a derived revertant strain were produced using BAC cloning technologies. The recombinant ORF134 deleted strain replicated in vitro comparably to the parental and the revertant strains. Infection of fish by immersion in water containing the virus induced comparable CyHV-3 disease for the three virus genotypes tested (wild type, deleted and revertant). Quantification of viral DNA by real time TaqMan PCR (in the gills and the kidney) and analysis of carp cytokine expression (in the spleen) by RT-qPCR at different times post-infection did not revealed any significant difference between the groups of fish infected with the three virus genotypes. Similarly, histological examination of the gills and the kidney of infected fish revealed no significant differences between fish infected with ORF134 deleted virus versus fish infected with the control parental or revertant strains. All together, the results of the present study demonstrate that the IL-10 homologue encoded by CyHV-3 is essential neither for viral replication in vitro nor for virulence in common carp.

## 44. Myeloperoxidase as an indicator of endometritis in the mare: preliminary results

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Diagnosis of endometritis in the mare is routinely based on the presence of polymorphonuclear cells (PMNs) on endometrial smears. Studies show a relation between PMNs and myeloperoxidase (MPO), an enzyme released by PMNs during degranulation or after cell lysis, in many fluids and tissues. The aims of this study were to assess the presence and concentration of MPO in the mare's uterus, and to investigate its relation with PMNs. Thirty-six cycles from 28 mares (ages ranging from 6 to 22 years) were used. Endometrial cytological samples were obtained with a small volume uterine flush and either a uterine cotton swab or a cytobrush, when a follicle >35mm was observed by ultrasound. The smears were stained with Diff-Quick® and one or more PMNs per field (400x) was diagnosed as endometritis. The supernatant of the flushes was used to measure MPO concentration with a specific equine MPO ELISA assay. Our results showed the presence of MPO in the equine uterus during oestrus (mean=2839±2785). MPO concentrations were significantly (p<0.05) higher in samples with positive cytologies. Occasionally, some samples with negative cytologies showed high MPO concentration, but the opposite was never observed. Clinical signs of endometritis are not always present, or they may be delayed. An early diagnostic improves the quality of treatment. Our results show that high quantities of MPO in endometrial samples indicate the presence of PMNs. However, further studies are needed to determine if MPO concentration could be routinely used as a tool of early detection of endometritis.

### 45. Comparison of ammonia and greenhouse gases emissions associated to fattening pigs kept either on fully or partly slatted floor

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The aim of this study is to compare the emissions of ammonia (NH $_3$ ) and greenhouse gases (nitrous oxide, N $_2$ O; methane, CH $_4$ ; carbon dioxide, CO $_2$ ) during the fattening of pigs kept either on fully or partly slatted floor. Two successive batches of 24 fattening pigs (Piétrain X Belgian Landrace) were divided into two groups housed in two separated pens of 9m $^2$  of surface area (0.75m $^2$ /pig) and fitted with either a fully or a partly slatted floor, with the slurry pit only under the slatted parts. Experimental rooms were automatically ventilated with continuous recording of ambient temperatures and ventilation rates. Gaseous concentrations were measured by photo-acoustic detection during 3 periods of 6 consecutive days throughout each fattening period. Housing system with partly slatted floor did not reduce NH $_3$ -emissions (on average 6.6g NH $_3$ /pig.day for both slat type) but are associated with reduced emissions of greenhouse gases (0.22 vs. 0.28g N $_2$ O/pig.day; 4.44 vs. 4.89g CH $_4$ /pig.day; 1.29 vs. 1.48kg CO $_2$ /pig.day).

### 46. Effect of a fibrous diet on growth performance, carcass characteristics and gut health of fattening pigs

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Two successive batches of 24 fattening pigs were divided into two groups fed ad libitum either a standard diet based on cereals (STD, 19% non-starch-polysaccharides, NSP) or a fibrous diet based on sugar beet pulp (HFD, 31% NSP). Diets were isoenergetic (2225 kcal/kg net energy) and isoproteic (16% crude protein). Pigs were fattened from 35 to about 110 kg. The average daily gain was reduced by 10%with HFD (788 vs. 877 g/day, P<0.001), as possible result of decreased feed intake (2.26 vs. 2.47 kg/ day, P>0.05) and deteriorated feed conversion ratio (2.88 vs. 2.82, P>0.05). The carcass traits associated with HFD showed reductions of dressing percentage (75.8 vs. 78.9%, P<0.001), muscle thickness (61.7 vs. 66.2 mm, P<0.05) and backfat thickness (11.3 vs. 13.9 mm; P<0.05). At the end of the fattening period, fecal bacteria counts associated with HFD showed higher results for Lactobacillus (10.21 vs. 9.84 log10 of cfu/g of faeces dry matter, P<0.05) and Bifidobacterium (9.47 vs. 8.87, P<0.01) but lower results for Enterobacteriaceae (4.83 vs. 5.97, P<0.001). The stomach weight and the total digestive tract weight of pigs fed HFD was higher than that of pigs fed STD (0.73 vs. 0.51 kg, P<0.001, and 4.89 vs. 4.28 kg, P<0.05, respectively). Gastric lesion score was decreased with HFD (0.93 vs. 1.55, P<0.05). It may be concluded that HFD impaired growth performance and carcass traits but may contribute to better gut health. The source and amount of fiber should be further explored to combine favourable effects on health and performance.

### 47. Impact of the amount of straw on emissions of ammonia and greenhouse gases associated with fattening pigs kept on deep litter

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The aim of this essay is to study the effect of the amount of straw on emissions of ammonia ( $\mathrm{NH}_3$ ) and greenhouse gases (nitrous oxide,  $\mathrm{N}_2\mathrm{O}$ ; methane,  $\mathrm{CH}_4$ ; carbon dioxide,  $\mathrm{CO}_2$ ) during the fattening of pigs kept on deep litter. Two successive batches of 30 fattening pigs (Piétrain x Belgian Landrace) were divided into 3 groups kept in separated pens of  $12.6\mathrm{m}^2$  of surface area ( $1.26\mathrm{m}^2/\mathrm{pig}$ ). At the beginning of each fattening period, 250kg of whole wheat straw were used to constitute the initial bedding. Thereafter, straw were supplied once a week in each pen to amount 500, 750 and 1000 kg of straw at the end of each fattening period (after about 100 days), in the 3 pens respectively. Experimental rooms were automatically ventilated with continuous recording of ambient temperature and ventilation rates. Gaseous concentrations were measured by photo-acoustic detection during 3 periods of 6 consecutive days throughout each fattening period. Increasing the amount of straw from 0.5 to  $1.0\mathrm{kg/pig.day}$  allowed to decrease  $\mathrm{NH}_3$ - and  $\mathrm{N}_2\mathrm{O-emissions}$  by 24 and 31% respectively, but increased the  $\mathrm{CH}_4$ -emissions by 90%.  $\mathrm{CO}_3$ -emissions seemed unaffected by the amount of applied straw.

### 48. Influence of the void percentage of the floor on ammonia and greenhouse gas emissions for group-housed gestating sows

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According to EU legislation, group-housed gestating sows must have a minimum of  $2.25~\text{m}^2$  floor area per sow with at least  $1.3~\text{m}^2$  of continuous solid floor of which a maximum of 15% is reserved for drainage openings. Furthermore, if slatted floors are used for the remaining floor area, the maximum width of the opening must be 20~mm and the minimum slat width must be 80~mm. The aim of this work was to investigate the influence of void percentage of the floor on gas emissions (nitrous oxide- $N_2O$ , methane- $CH_4$ , carbon dioxide- $CO_2$ , ammonia- $NH_3$  and water vapour- $H_2O$ ). Two trials were carried out. For each trial, three successive batches of ten Belgian Landrace gestating sows were used. Each batch was divided into two homogeneous groups randomly allocated to a treatment: 15% or 9% of void percentage of the floor for the first trial and 9% or 6% for the second trial. The groups were kept separately in two similar rooms. The pens were equipped with five individual feeding stalls with rear gates allowing or not permanent access to the stalls outside of feeding times. In the first trial, the void percentage of slatted-floor and thus the floor fouling influence the  $NH_3$ - and greenhouse gases-emissions, with lower emissions observed with an increased void percentage from 9~to 15%. In the second trial, the void percentage (9%~vs. 6%) did not significantly influence  $NH_3$ -,  $CH_4$ -,  $CO_2$ - and  $H_2O$ -emissions.  $N_2O$ -emissions were 8% greater with the highest void percentage and, in consequence,  $CO_3\text{eq}$ -emissions were 5% greater.

#### 49. Influence of concentration on equine fresh semen conservation

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Low volume deep horn insemination with highly concentrated fresh semen has become popular in the mare. However, only few data are available about conservation of highly concentrated equine semen. Five stallions were collected four times. Concentration and motility were determined in raw semen. Volumes of semen containing 110, 440 and 880 millions spz were extended (1v/4v) with INRA96 and centrifuged (1000g, 20 minutes) with cushion medium and the sperm-rich pellet re-suspended in 1 ml of supernatant. Total (TM) and progressive motility (PM) were determined for each sample after 8 and 24 hours at 20°C. As TM and PM in raw semen were stallion dependent, Percent of Conservation of Progressive (PCPM) or Total Motility (PCTM) were studied. Differences in the maintenance of motility were determined using Friedmann test and Dunn's post-test. Spermatozoa recovery rate (RR) after centrifugation were respectively 64.11±28.05%, 98.65±27.28% and 99.90±27.37%. RR was lower in low concentration samples (p<0.001). Mean final conservation concentrations were 70.45±30.59, 434.82±120.02 and 879.97±241.15 millions spz/ml. PCTM was decreased in 880x10 millions/ml samples after 8 and 24h of conservation (p<0.001) when compared to other concentrations. PCPM was lower after 24 hours within highest concentration sample (p<0.001), when compared to lower concentrations. Our data show that semen conservation with high concentration is rapidly deleterious for total motility, whereas progressive motility decrease between 8 and 24 hours when compared to non-concentrated semen. However, progressive motility is the only factor that has been associated to fertility, suggesting insemination with highly concentrated semen should be performed within 8 hours.

### 50. Retinal function in horses with traumatic panuveitis and equine recurrent uveitis

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Purpose: Use the electroretinogram (ERG) to assess changes in retinal function in horses with traumatic panuveitis (TU) and equine recurrent uveitis (ERU). Methods: Dark-adapted rod, mixed rod-cone, and cone responses were recorded in standing horses. Patients were sedated with acepromazine 0.1mg/ kg intramusculary one hour before a detomidine (0.022mg/kg/hr) and morphine (0.05mg/kg/hr) intravenous constant rate infusion. Study included 12 control horses, 4 TU horses (4 eyes), and 9 ERU horses (anterior uveitis only, 4 eyes; chronic panuveitis, 7 eyes). Affected horses were treated medically for 3 weeks and were visual at the time of testing. A three-way comparison between groups was conducted using the Kruskal-Walis test; in case of significant differences, pairwise comparisons (Mann-Whitney) were conducted to determine which pairs of groups were different. Results: Implicit times of cone and mixed rod-cone responses were significantly longer in eyes with ERU panuveitis compared to age-matched controls. In ERU horses, implicit times of the cone photopic and flicker responses were significantly longer in eyes with panuveitis than in eyes with anterior uveitis only. No significant differences were detected between normal and TU eyes, nor between panuveitis-affected eyes in the TU and ERU groups. When comparing both eyes of the same individual in all groups, b-wave amplitudes of the dark-adapted rod response were significantly lower in all ERU- affected eyes. Conclusions: Only mild reduction in scotopic and photopic responses were identified in eyes of horses with ERU, suggesting that in equine uveitis, treated visual eyes have only moderate changes in retinal function.

### 51. Use of Staby® technology for development and production of DNA vaccines free of antibiotic resistance gene

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The appearance of new viruses and the cost of developing certain vaccines require that new vaccination strategies now have to be developed. DNA vaccination seems to be a particularly promising method. For this application, plasmid DNA is injected into the subject (man or animal). This plasmid DNA encodes an antigen that will be expressed by the cells of the subject. In addition to the antigen, the plasmid also encodes a resistance to an antibiotic which is used during the construction and production steps of the plasmid. However, regulatory agencies (FDA, USDA and EMA) recommend to avoid the use of antibiotics resistance genes. Delphi Genetics developed the Staby® technology to replace the antibiotic-resistance gene by a selection system that relies on two bacterial genes. These genes are small in size (approximately 200 to 300 bases each) and consequently encode two small proteins. They are naturally present in the genomes of bacteria and on plasmids. The technology is already used successfully for production of recombinant proteins to achieve higher yields and without the need of antibiotics. In the field of DNA vaccines, we have now the first data validating the innocuousness of this Staby® technology for eukary-otic cells and the feasibility of an industrial production of an antibiotic-free DNA vaccine. Moreover, as a proof of concept, mice have been successfully vaccinated with our antibiotic-free DNA vaccine against a deadly disease, the pseudorables (induced by Suid herpesvirus-1).

### 52. Persistent right aortic arch associated with an aberrant left subclavian artery arising from a patent ductus arteriosus in a puppy

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Introduction: Persistent right aortic arch (PRAA) represents over 90% of described vascular ring anomalies and can be accompanied by a left ligamentum arteriosum or a patent left ductus arteriosus (approximately 10% of patients) that causes oesophageal entrapment and secondary sub-obstruction. When congenital vascular ring anomalies are suspected, thoracic radiographs and computed tomography angiography (CTA) of the thorax are recommended. Materials and methods: A 3.5-month-old, male French Bulldog was presented for regurgitation and vomiting since weaning. **Results:** Radiographs revealed severe oesophageal dilation cranial to the heart base and a ventral and left-sided tracheal displacement. On CTA, a PRAA was observed. An aberrant right subclavian artery (ARSA) was identified originating from the PRAA next to the brachiocephalic trunk. A patent left-to-right patent ductus arteriosus (PDA) was detected between the aorta and the pulmonary trunk. An aberrant left subclavian artery (ALSA) originated from this PDA. The oesophagus was compressed between the PDA and the trachea and was dilated cranially to this narrowing. Discussion: Seven types of vascular ring anomaly are described in the literature. In this patient, the vascular ring anomalies are a novel variant of the defined types because an ALSA originating from a PDA has not been described previously. The severe compression of the esophagus with severe dilation cranial to the heart was caused by the PDA. In conclusion, CTA is necessary to determine which vascular anomalies are present and to identify which of these anomalies is responsible for the clinical signs. This technique enables accurate pre-operative planning.

### 53. Molecular characterization of *Clostrium difficile* strains from elderly care home residents

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Clostridium difficile is the primary cause of nosocomial diarrhoea and pseudomembranous colitis after use of antibiotics. Production of toxins A and B are the main virulence factors responsible for its pathogenicity. The aim of this study was to characterize C. difficile strains isolated from faeces of elderly care home residents. Isolates were tested using multiplex PCR, cytotoxic immunoassay, PCR ribotyping and the molecular genetic GenoType CDiff test system. A total of 4 different PCR ribotypes were identified, including PCR ribotype 020 and PCR ribotype 027. Only one PCR-ribotype was negative for the cytotoxicity assay and for all toxin genes while all of the other types were positive for both of toxin genes tcdA and tcdB. In addition, type 027 contained cdtA gene encoding for the binary toxin and 39 bp deletion in the regulator gene tcdC, which is associated with an increased production of toxin and hyper-virulent C. difficile strains. All of PCR ribotypes 027 isolated and the non-toxigenic PCR ribotypes UCL36 had a mutation in the Gyr1A gene. This mutation is related with moxifloxacin resistance. The results obtained in this study present evidence of the presence of toxigenic and hyper-virulent C. difficile isolates in a Belgian nursing home.

### 54. Is the CXC-chemokine CXCL8 involved in the breed predisposition of West Highland White Terrier to canine idiopathic pulmonary fibrosis?

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Canine idiopathic pulmonary fibrosis (canine IPF) is a progressive interstitial lung disease of unknown aetiology and pathophysiology, mainly described in middle-aged to old West Highland white terriers (WHWT). CXCL8 is a proinflammatory chemokine probably involved in the pathogenesis of human IPF, where it appears to be a diagnostic and prognostic biomarker in serum and BALF. In dogs, little is know about the role of CXCL8 in the pathogenesis of IPF. The aim of the present study was to compare serum CXCL8 levels in healthy WHWT versus WHWT with IPF and healthy dogs from terrier breeds other than WHWT and from non-terrier breeds. Ten WHWT with IPF and 71 healthy dogs from different breeds entered the study. Serum CXCL8 concentrations were determined by ELISA. Results between IPF and healthy WHWT, and between healthy dogs of different breeds were compared using a global linear model (SAS<sup>®</sup> software) incorporating the effects of covariates age and gender;  $p \le 0.05$  was chosen as level of significance. No difference in serum CXCL8 levels was detected between WHWT with IPF and healthy WHWT. However, serum CXCL8 concentration was significantly higher in healthy WHWT compared to each of the other groups of healthy dogs. Results of the present study show that serum CXCL8 concentration is high in both healthy and IPF WHWT compared to healthy dogs from other breeds. Therefore, CXCL8 (1) cannot be used as a serum diagnostic biomarker for IPF; (2) might be related to the breed predisposition of the WHWT for IPF.

### 55. Comparison of four cartridge-type automated blood gas analyzers for arterial blood gas analysis in dogs

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Different cartridge-type blood gas analyzers are used to perform arterial blood gas evaluation in clinical units. However, little is known about how the results compare between different analyzers. Arterial blood samples were prospectively sampled from 36 dogs and was used to evaluate the analytical performances for pH and partial pressures of oxygen (PaO<sub>2</sub>) and carbon dioxide (PaCO<sub>2</sub>) values of 4 analyzers: Cobas b-123 POCsystem, IRMA TRUPoint, Idexx Vetstat and ABL80 FLEX. The order of analysis on each analyzer was random. Additionally, measures were repeated 5 times successively on a same sample, to calculate intra-analyzer variance for each measured parameter. Results obtained with the different analyzers were compared using a global linear model (SAS<sup>®</sup> software);  $p \le 0.05$  was chosen as level of significance. Values are given as lsqm ± standard error. Values of pH and PaCO, were not significantly different between analyzers. PaO, (mmHg) was significantly higher when measured with Idexx  $(84.8 \pm 2.1)$  and IRMA  $(83.8 \pm 2.4)$  than with Cobas  $(74.3 \pm 2.0)$  and ABL80  $(72.2 \pm 2.3)$ . There was no significant difference in variance between the 4 analyzers, for any of the measured parameters. PaO<sub>2</sub>, but not PaCO,, greatly differed between the 4 analyzers; therefore results obtained routinely in clinical patients should be interpreted in function of reference values established for each machine. Besides, blood gas results measured in patients cannot be compared when obtained from different analyzers, for either diagnostic or follow up purposes.

#### 56. Event-based surveillance of equine West Nile fever, Spain

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West Nile fever (WNF) is a worldwide viral zoonotic infection caused by a mosquito-borne *Flavivirus* of the *Flaviviridae* family. The current conception of WNF epidemiology in Europe combines an enzootic viral circulation in tropical Africa with seasonal introductions of the virus by migratory birds. However, recent studies also suggest possible enzootic WNV circulation in European countries (Chaskopoulou et al., 2011; Monaco et al., 2011). According to a recent comparative study (Chevalier et al., 2011), the passive (now called event-based) surveillance of horses by equine veterinary specialists appeared to be the most cost-effective system in the European context of WNF. Clinical data issued from an event-based epidemiosurveillance through August 2007 and December 2011 on horses in Spain were statistically compared and used to develop a diagnostic decision tree, both with the aim to improve early clinical detection of WNF in horses. Although clinical signs were variable in WNF horses, several clinical signs were identified to distinguish between WNF and neurological disease but WNF-negative (photophobia, affection of cranial nerves, nasal discharge and paralysis). Clinical examination of neurologically affected horses could potentially provide important clues for early clinical detection of WNF. The study of the clinical pattern of WNF in horses is of paramount importance to enhance awareness and understanding and to optimize surveillance designs for clinical detection of WNF.

### **57.** Production laitiere de la lapine locale pendant les deux premieres lactations Saidj D. 1\*, Salhi O. 1, Moula N. 2, Temim S. 1, Ain Baziz H. 1

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La cuniculture en Algérie a connu une importante évolution au cours de ces dernières années.Le développement économique et durable de ce sous secteur d'élevage nécessitera une amélioration conjointe de la valeur génétique des races locales caractérisées par la faiblesse de leur croissance et des pratiques d'élevage jugées rudimentaires. L'objectif de cette étude est d'évaluer la production laitière de quinze lapines locales lors des deux premières lactations. Les lapines suivies pendant une période de 3 semaines, sont pesées avant et après la tétée et la mesure de la perte de poids au cours de la tétée a fournit une estimation de la quantité de lait ingérée par les lapereaux. Les résultats montrent pour la première lactation, une production laitière moyenne de 2557,5±447,8g avec un pic de production au 18ème jour et un ingéré alimentaire de 5438,2±855,7g par lapine. Pour la 2ème lactation, la production laitière est de 2610,7±752,3g avec un pic de production au 19ème jour. et une quantité d'ingéré de5623,0±916,6g. Le poids des lapines varie au cours de la reproduction. Il passe de 2904,6±360g lors de la 1ère mise bas à 2823,3±266,9g lors de la 2ème mise bas pour atteindre un poids de 2864,0±270,4g au sevrage de la 2ème portée. Cette étude peut permettre une meilleure gestion de la ration et de la qualité d'aliments distribués aux lapines reproductrices de race locale en Algérie.

Mots clés: Algérie, cuniculture, Lapine locale allaitante, post partum, pic de lactation.

#### 58. The development of astrocytes in the sheep cerebellum

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Astroglial cells make up the largest glial population in the brain and play a various vital function in the development of the CNS. Immunohistochemical study was performed in 19 ovine fetuses ranging from 2 to 5 months of gestation, one lambs in the first week after birth and three adult sheep were studied. Using the GFAP marker demonstrated several variations in the degree distribution of GFAP+ astrocytes between the different zones in the sheep cerebellum during brain development. The current study indicates that the first appearance of astrocytes occur about the 8<sup>th</sup> week of gestation from restricted zones in the cerebellum. The Bergmann cells were present about the 15<sup>th</sup> week of gestation. Our finding suggest that the maturation of astrocytes begins in the caudal parts of the cerebellum from their initial ventral regions to dorsal regions radially within the white matter followed by the more rostral parts of the cerebellum and that the astrocytes proliferate in the vermis before it does in the cerebellar hemispheres.

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### 59. Typologie des préférences des éleveurs de zébu Azawak pour le choix de reproducteurs dans les zones d'Abalak, Filingué et Niamey (Niger)

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Au Niger, le zébu Azawak, adapté aux conditions environnementales sahéliennes, a bénéficié d'un intérêt grandissant de la part de l'Etat nigérien et d'une grande diversité d'éleveurs. L'amélioration génétique par sélection ou par diffusion de bétail sélectionné nécessite une connaissance des objectifs des éleveurs et des critères d'appréciation en usage. Des méthodes économétriques, dites de préférences déclarées, permettent d'analyser de telles décisions non-observables sur le marché. Etablissant différents attributs des animaux, dont leur prix, et niveaux de ceux-ci par des méthodes participatives (empilement proportionnel en groupes de discussion), des animaux virtuels sont composés et proposés aux éleveurs, sous la forme de dessins associant différents niveaux des qualités retenues. Les choix effectués par les éleveurs entre ces animaux sont analysés afin de dériver l'importance relative des critères. Conduite auprès de 218 éleveurs des zones d'Abalak (pastorale), Filingué (agricole) et Niamey (périurbain), cette enquête établit une typologie du comportement d'appréciation des géniteurs Azawak. Toutes zones confondues, les critères les plus importants sont le format du corps (24%) et la robe (21%). Une classification hiérarchique des éleveurs selon les critères mobilisés a permis de distinguer trois groupes de préférences. 55% des répondants apprécient le reproducteur d'abord pour la robe et la taille des testicules. 34% valorisent la longueur de la gueue et le format. Le dernier groupe, 11% des éleveurs, juge sur base du format et du prix. Le groupe de préférence est indépendant de la zone d'appartenance des éleveurs (p=0,4). La compréhension des critères de sélection en usage permettra leur prise en compte dans les programmes de sélection et diffusion.

Mots clés: Typologie d'élevage Azawak, bovin, critères de sélection, reproduction, Niger.

### 60. Critères d'appréciation et consentement à payer pour des reproducteurs améliorés de zébu Azawak au Niger

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Au Niger, l'élevage bovin est caractérisé par un cheptel important et varié, adapté à son environnement semi-aride mais de faible productivité. Face à l'augmentation de la demande nigérienne en protéines animales, l'amélioration génétique des races bovines locales rustiques est nécessaire. Bénéficiant d'un plan de sélection mené dans la station de Toukounous depuis plus de 50 ans sur les caractères laitiers et viandeux, des géniteurs zébus Azawak sont à présent disponibles pour participer à cette amélioration, par diffusion de leur génétique. Dans cette perspective, cette étude propose d'estimer les valeurs attribuées par les éleveurs de zébu Azawak à différents critères d'appréciation des reproducteurs. Ces valeurs sont calculées sous forme de consentements à payer, par une analyse conjointe (enquête de préférences déclarées) menée auprès de 150 éleveurs au sein de 3 systèmes d'élevage différents. Les critères considérés sont l'exigence en alimentation, la docilité, l'orientation viandeuse ou laitière, les performances de reproduction, la couleur de la robe et la longueur de la queue. Les résultats ont montré que la reproduction et l'alimentation sont les attributs les plus importants dans le choix d'un reproducteur, présentant des poids relatifs de 20% et 19% respectivement. Les résultats ont montré une disposition à payer de 33871 FCFA (52€) pour l'amélioration de la reproduction, 33629 FCFA (51€) pour l'adaptation des exigences alimentaires aux systèmes d'élevage, 25564 FCFA (39€) pour la robe fauve avec extrémités noires. Par contre, l'orientation viandeuse ou laitière du reproducteur est moins importante pour les éleveurs. Distinguer l'importance relative et l'équivalent financier de l'utilité apportée par un attribut du bovin dans les différents systèmes de production sera utile pour améliorer la diffusion et la sélection du zébu Azawak au Niger.

### 61. Measurement of the estrogenic activity of migration products from plastic contact materials

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Baby bottles in plastic are widely used to feed newborns and infants. The polymers used for the manufacture of baby bottles are mainly polypropylene (PP), silicone, polyamide (PA) and polyethersulphone (PES), since the ban of the use of bisphenol A (BPA), in polycarbonate by European commission, in March 2011. For the plastic substitutes, chemical substances, such as monomers or additives (antioxidants, plasticizers, clarifiers, etc.), may migrate from the plastic baby bottle into milk. Like BPA, these monomers and additives may exhibit at low concentrations estrogenic activity, which can produce adverse effects, especially in newborn and infants. A recent study of Simoneau et al. (2012) gives an overview of the identity and amounts of chemical substances which migrate from plastic baby bottle. The objective of this study is to measure the estrogenic activity of these substances. The study focuses first on screening of human estrogen receptor agonistic and antagonistic activities of pure compounds using a cell based transactivational assay named "Chemically Activated LUciferase gene eXpression" (CALUX). Preliminary test with 3 Bisphenols (BPs) have been performed: BPA, Bisphenol S (BPS) and Bisphenol F (BPF). The dose–response curves obtained for BPA, BPS and BPF were compared with that of  $17\beta$ -estradiol (E2). An agonistic effect was observed with the 3 BPS and BPF exhibited an oestrogen-like response lower than that of BPA.

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### 62. Evasion of cytotoxic T cell response by *Alcelaphine herpesvirus 1* genome maintenance protein.

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Alcelaphine herpesvirus 1 (AlHV-1) is a gammaherpesvirus carried by wildebeest asymptomatically. After transmission to a variety of ruminant susceptible species, AIHV-1 is responsible for the development of an acute and fatal disease named malignant catarrhal fever (MCF). MCF is characterized by the proliferation and infiltration in many organs of CD8<sup>+</sup> T cells supporting latent viral infection. Recent studies have demonstrated that latency is essential in MCF. Among rare transcripts detected during MCF, the open reading frame (ORF) 73 is highly expressed. The ORF73 encodes AIHV-LANA (for latency-associated nuclear antigen), an orthologous protein of the genome maintenance protein conserved through the Gammaherpesvirinae subfamily. AIHV-LANA is expressed in tissues of animals developing MCF. From that observation, infected animals should therefore develop an adaptive immune response. Though such a response could exist, it is not protective since infected animals develop MCF. Orthologous proteins to AIHV-LANA are responsible for viral episome maintenance in infected cells and were proved to have cis-acting immune evasion properties. Thus, it is possible that AlHV-LANA has similar properties. In this study, we tested the capability of the AIHV-LANA protein to escape the cytotoxic T cell response by inhibiting its own presentation by the major histocompatibility complex class-I (MHC-I). These cis-acting immune evasion properties of AIHV-LANA were tested in vitro in an isolated gene context. The results revealed that AIHV-LANA is able to inhibit the MHC-I-restricted presentation to the cell surface of an ovalbumin peptide fused to this protein. We also demonstrate that the GE-rich domain of the central acidic repeat region of AlHV-LANA is involved in the cis-acting immune evasion mechanism. In conclusion, our results suggest that AIHV-LANA has intrinsic immune evasion properties that could be important in the pathogenesis of MCF.

### 63. Growth optimization of *Bifidobacterium crudilactis* and *Bifidobacterium mongoliense, two new potential probiotic strains*

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Two newly patented Bifidobacterium species, B. crudilactis and B. mongoliense have been identified in raw milk and raw milk cheese. Considering their potential as probiotic strains due to their immunomodulatory effect, two strains have been studied in order to determine their optimum growth conditions for industrial use. First, the culture medium was optimized. Growth kinetics were established for both strains for a period of 72 h, under anaerobic conditions, at 37°C and pH of 6.5. Next, cultures were investigated in 2L batch reactor with pH control at 33°C under anaerobic conditions. Finally, freeze-drying of B. mongoliense in skim milk was tested. The best growth for both strains was observed at 33°C in a media supplemented in cysteine, caseine peptone, soybean peptone and yeast extract. Both strains presented tolerance to oxygen, which could represent a huge advantage for use in industrial applications. At pH 5.5 and in 2L batch reactor, B. mongoliense cell concentration reached 1.63 10 CFU/ml with a lactate and acetate production of 5.40 g/l and 9.20 g/l respectively. Regarding B. crudilactis, cell concentration reached 6.97 10 CFU/ml with a lactate and acetate production of 7.01 g/l and 8.03 g/l respectively. The highest viability rate obtained after drying was 93%. However a huge loss of viability was observed after 2 weeks of conservation at 4°C. It can be concluded that for both strains, the best growth was observed at pH 5.5 and 33°C after optimization of the media. Next step will be to improve the stability of the dry formulation by using cryoprotectants.

### 64. Environment factors affecting racing performances of Thoroughbred horses in Algeria

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The results of the flat races organized in Algeria from 1995 to 2007 by the Algerian Horse Racing Society were used to estimate non-genetic parameters of racing performances of Thoroughbred horses. Performances were assessed through two earnings traits (the logarithm of annual virtual earnings: LAEV and the logarithm of average annual virtual earnings per start: LAEV/S) and a normalized ranking (PERF). The phenotypic correlations between these traits were calculated, in order to deduce what is common and what is specific to each measure. The environment factors that were investigated are age (3 to 8 years and older), sex (male or female), year of race (1995 to 2007) and the interactions between these factors. The General Linear Model (GLM) procedure from SAS software was used to identify and quantify the non-genetic factors affecting racing performances. The results showed significantly high positive correlations (p<0.001) between the three traits, hence considered as accounting for similar aptitudes. The effects of age (with a plateau between 4 and 5 years) and year (with an increasing trend for more recent years) turned out to be significant (p<0.001) for the three traits, the sex effect was only significant for the PERF trait (with better performances for males than females) and an interaction between the age and year of the performance was the only significant interaction (p<0.05) for the LAEV trait. The significant effects of these non-genetic factors indicate the need to adjust the earnings and ranks in the context of a program for genetic improvement of Thoroughbred horses in Algeria.

**Key words:** non-genetic factors, earnings, rankings, flat racing, Thoroughbred horses, Algeria.

### 65. Evaluation morphométrique des chevaux pur-sang Arabe en Algérie: mensurations corporelles et proposition d'équations barymétriques

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Cette étude vise à la caractérisation morphobiométrique des chevaux de course pur-sang arabe et à l'estimation d'équations barymétriques adaptées à cette race. La caractérisation a concerné 98 chevaux, dont 44 femelles et 54 mâles, tous âgés de trois ans et plus, auprès de 77 propriétaires-éleveurs dans 3 hippodromes d'Algérie (Zemmouri, Tiaret et Caroubier). Dix-neuf mensurations étaient relevées ainsi que le poids vif (PV). Le poids moyen est de 456,2 +/- 43,0 kg, variant de 335 kg à 545 kg. La sélection des variables à inclure dans les équations barymétriques a été réalisée à l'aide de la procédure *stepwise* du SAS. Quatre mensurations parmi les 19 réalisées ont été retenues pour la proposition d'équations d'estimation du poids vif des chevaux : le périmètre thoracique (PT), la hauteur à la croupe (HC), la longueur de l'encolure (LE) et le tour de l'encolure (TE). Ainsi, les équations proposées pour les mâles et pour les femelles sont respectivement de : PV= 7,024\*PT - 787,119 (R²=0,99); PV=6,207\*PT + 0,633\*HC + 0,668\*TE - 0,878\*LE - 746,370 (R²=0,96). Les résultats de cette étude devraient permettre aux propriétaires-éleveurs et entraineurs de suivre aisément le poids de leurs chevaux. Ce suivi est nécessaire pour adapter l'activité et l'alimentation des chevaux et favoriser leur performance en course.

Mots-clés: pur-sang arabe, mensurations corporelles, poids vif, équations barymétriques, Algérie.

#### 66. Genetic parameters of racing performance traits of Arabian horses in Algeria

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The data used in the present study were recorded at the Algerian Horse Racing Society for 36492 racing performance of 913 Arabian horses from 1995 to 2007. The aim of the study was to identify the genetic parameters underlying three traits: two earnings traits, namely the logarithm of average annual virtual earnings (LAEV) and the logarithm of average annual virtual earnings per start (LAEV/S), and a ranking trait, the normalized ranking (PERF). To identify the fixed effects to be included in the genetic mixed model, a preliminary analysis was conducted using the General Linear Models (GLM) procedure from SAS software. The effects of age, sex, year and the interaction between year of the race and age and between sex and age were included in the model for the three traits. Afterwards, two random effects, a direct genetic effect of the animal and a permanent environmental effect were included in the mixed model. The variance components and genetic parameters were estimated using the restricted maximum likelihood (REML) procedure with the MTDFREML program. The analyses with this repeatable animal model led to the following estimation of the genetic parameters: for LAEV, heritability was 0.225 (±0.041), while estimate of repeatability was 0.330 (±0.040). For LAEV/S, heritability was 0.164 (±0.027), while estimate of repeatability was 0.215 (±0.022). The heritability for the normalized ranking was higher, 0.369 (±0.054), indicating that this trait might provide faster progress for breeding programs of Arabian horses in Algeria. The repeatability estimate for the normalized ranking was 0.587 (±0.045). The genetic correlation between LAEV and LAEV/S was 0.99, revealing a almost complete genetic dependence between these two traits, 0.69 between PERF and LAEV and 0.79 between PERF and LAEV/S.

**Key words**: Arabian horses, genetic parameters, flat racing, Algeria.

### 67. Pedigree analysis in the Arabian horse in Algeria: estimation of inbreeding coefficient

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The studbook of the Arabian horse used in this study is recognized by international authorities such as the WAHO (World Arabian Horse Organization). The pedigree file of the horses includes 1812 animals from 166 stallions (with an average of  $10.11 \pm 17.33$  offspring) and 392 mares (with an average of 4.30 ± 3.76 offspring). The maximal pedigree depth was 18 generations. Inbreeding coefficients of horses present in our data were estimated using the software "Pedigree Viewer" and MTDFREML software on the basis of the provided pedigree. The estimation of the inbreeding coefficient for the 1812 horses showed that 1177 animals from all those present in the pedigree were consanguineous, with an inbreeding coefficients varying from 0,00002 to 0,265, with an overall mean of 0,0275  $\pm$  0,001. The average value of the inbreeding coefficient in the population of Arabian horses in Algeria is thus relatively high. It is to be noticed that this average coefficient of inbreeding is less than the threshold established as problematic in the inbreeding literature (6%); however, 13.5% of the total population shows inbreeding coefficients above this threshold. It is therefore important to educate owners-breeders to the problems that consanguinity can generate, and to avoid as much as possible practices that increase inbreeding, such as a too intensive use of a major ancestor, the use of a too small number of breeding animals and the use of crosses between related individuals. All these measures are necessary to prevent rapid inbreeding increase, which would result in a significant loss of genetic diversity, with a medium-term potentially negative effect on racing performances and reproduction.

**Key words**: Arabian horses, coefficient of inbreeding, pedigree, Algeria.

### 68. Morphological and Molecular Characterization of Lymnaeid Snails and Their Potential Role in the Transmission of *Fasciola* spp. in Vietnam

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Freshwater snails of the family Lymnaeidae play an important role in the transmission of faciolosis worldwide. In Vietnam, only two lymnaeid species, *Lymnaea swinhoei* and *L. viridis*, were morphologically identified so far and both of them are reported as intermediate hosts of *Fasciola* spp. However, recent studies have raised controversy about their role in the transmission of *Fasciola* spp. because of confusion in the identification of the snail hosts. The aims of this study area, therefore, to clarify the identities of lymnaeid snails in Vietnam by a combination of morphological and molecular approaches, and to survey for their role of transmission of *Fasciola* spp. Based on morphological characteristics, two species were recognized as *L. viridis*, *L. swinhoei* and a third one was considered as a *Lymnaea* sp.. The molecular analyses using the second internal transcribed spacer of the nuclear ribosomal DNA clearly showed that lymnaeids in Vietnam include in fact three species: *Austropeplea viridis* (morphologically identified as *L. viridis*), *Radix auricularia* (morphologically identified as *L. swinhoei*) and *R. rubiginosa* (morphologically identified as

**Keyword:** Radix rubiqinosa, Radix auricularia, Austropeplea viridis, Fasciola spp., Vietnam

### 69. An attempt to eradicate PRRS in Wallonia (Belgium), a low density area of swine production

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The South part of Belgium (Wallonia) is a low density area of swine production where PRRS eradication should be possible. For some farmers, its real economical impact is still unknown. In a way to demonstrate the possibility to become PRRS-free or to maintain a free status, continuous education and communication meetings with producers, technicians and veterinarians were organised. Eight independent farmers located in Wallonia were followed between October 2009 and October 2011. These 8 farms were farrowing-to-finish units with on average 210 sows [60 to 450]. Serological tests (Elisa and RT-PCR) were performed every 4-6 months. To reduce costs, PCR-tests and sequencing were realized on pools of 2 to 3 sera. ELISA-tests with s/p ratio < 0.4 were considered negative. The quality of the biosecurity on these farm was assessed through a scientific based questionnaire and accompanying scoring system (Biocheck UGent). When necessary, vaccinations of sows and/or 3 week-old piglets were realized with modified live virus in a way to reduce virus circulation. In 2010 and 2011, Biocheck scores for external and internal biosecurity were respectively 62 and 42% vs. 68 and 55%. Two farms were and stayed PRRSv-free until now. One farm became PRRSv-free on Augustus 2011. Since May 2011, one farm begins to produce negative finished pigs while sows continue to be vaccinated. Until now (November 2011), in four out of eight farms, active viral circulation was demonstrated in sows and/or growing pigs. In these farms, some mistakes (non respect of vaccination protocol and/or biosecurity measures such as introduction of PRRS-positive semen) were identified that could explain the active viral circulation. Otherwise, to become and remain PRRS-free is possible in Wallonia.

#### 70. Hepatitis E virus infection in wild boars and humans in Belgium

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Hepatitis E virus (HEV) possesses four genotypes. In Europe, genotype (G) 3 mainly circulates and its route of transmission is highly suspected to be zoonotic. The aims of this study were to obtain data on apparent viroprevalence and seroprevalence in wild boar (WB) and to compare the different strains identified in WB and human in Belgium. For the detection of the viral infection, a nested RT-PCR, an ELISA and a Western blot were used. A sample of 383 WB sera and 69 sera and 61 livers from young WB was obtained during the hunting season in 2010. The human samples concerned all the sera samples sent by physicians for HEV diagnosis in Belgium. An apparent seroprevalence of 33% (±4.6; 125/383) was obtained in WB. Five out of 61 livers and 4/69 sera of young WB were detected viropositive. The sequences obtained belonged to G3f. In humans, 25/340 sera in 2010 and 32/437 in 2011 were IgM positive and, from these 25 and 32 sera, 10 and 24, respectively, were viropositive. From these, 4 belonged to G1, 7 to G3 and 1 to G4. The high HEV seroprevalence in WB raises zoonotic concern about HEV transmission. The HEV sequences obtained from WB were all of G3f like most of the human HEV sequences. Therefore, these data are in agreement with the situation observed in other European countries and the links between HEV infection in pigs, WB and humans need to be further analysed to support the hypothesis of a zoonotic transmission in Belgium.Research supported by the Belgium Federal Public Service, Health, Food Chain Safety and Environment and by the Public Service Wallonia. We thank Dr R. Cariolet (Anses-Ploufragan) and Prof W. van der Poel (Central Veterinary Institute-Lelystad).

#### 71. Gestion des ressources génétiques ovines dans les élevages périurbains de Ouagadougou, Burkina Faso

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En Afrique, la demande urbaine en produits animaux entraîne le développement d'élevages périurbains, bénéficiant de leur accès aux marchés, à l'information et aux services vétérinaires. Au fondement de tout système de production animale, les ressources génétiques sont cruciales au développement durable du secteur. Pour l'accompagner, la connaissance des élevages et des animaux qui y sont élevés est nécessaire. Dans cette optique, une enquête auprès de 50 élevages ovins périurbains de Ouagadougou, Burkina Faso, a abordé les caractéristiques technico-économiques des élevages et leur gestion des ressources génétiques animales. Les conditions d'élevage sont diversifiées : 94% des élevages disposent d'abris, couverts ou non ; 85,7% utilisent les pâturages communautaires ; 12,77% pratiquent la stabulation permanente ; 42% distribuent des aliments concentrés commerciaux. Les résidus de récoltes sont souvent utilisés (66%). Les contraintes citées sont l'alimentation (100%), l'accès à l'eau (42%) et les problèmes sanitaires (57%). Le mouton Djallonké de type Mossi représente 62,9% de l'effectif total. Le mouton Peulh en constitue 6,9% et les croisements entre ces races 29,2%. L'objectif principal des éleveurs est la commercialisation d'adultes pour l'abattage. Des motivations d'ordre socio-culturel (don, sacrifice) sont également citées. La fumure organique est valorisée. La majorité des éleveurs pratiquent la sélection (92%) et /ou des croisements (38%), pour améliorer le poids (93,5%), la croissance (86,9%) et la rusticité (52,2%). Les critères de sélection cités sont la taille (72%), la conformation (42%), la robe (28%) et la rusticité (26%). L'organisation et le contrôle des croisements permettraient de meilleurs résultats et une gestion durable des ressources génétiques.

Mots clés : périurbain, ressources génétiques animales, ovin, Ouagadougou.

### 72. Gestion des ressources génétiques ovines dans des élevages ruraux dans la région du Plateau Central du Burkina Faso

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La demande en viande ovine en Afrique de l'Ouest pourrait bénéficier aux éleveurs ruraux du Burkina Faso, détenant la majorité du cheptel national. Les races locales, adaptées à leur environnement, constitueront la base du progrès à rechercher. Caractérisant 63 élevages ovins de la région du Plateau Central, cette enquête porte une attention particulière à leur gestion des ressources génétiques. Si tous les élevages reposent sur l'exploitation des pâturages naturels, 77,8% y combinent d'autres aliments. L'eau provient de puits et forages communautaires en saison sèche, de mares en saison pluvieuse. Parqués en saison pluvieuse, les moutons sont laissés sur parcours en saison sèche, avec un berger (31,8%) ou en divagation (68,2%). Les contraintes citées sont l'alimentation (84%), les maladies (34%) et le vol de bélier (32%). Le taux de prélèvement annuel pour commercialisation est variable, modéré et cible les mâles (ventes équivalant à 25,4±10,2%de l'effectif), signant un rôle d'épargne de l'élevage. Bien que le mouton Djallonké constitue la quasi-totalité des troupeaux, 58,7% des éleveurs ont déjà utilisé un bélier Peulh. Les critères de choix des reproducteurs mâles sont la taille et le poids (77,8%), la robe (38,1%) et la conformation (22,2%).La monte n'est pas contrôlée. Les objectifs sont l'amélioration du poids (84,1%), de la croissance (79,4%) mais aussi le maintien de la rusticité (73%) et de la robe (19,4%). Développant une certaine orientation commerciale, la volonté des éleveurs va vers des animaux de meilleurs formats, tout en gérant avec prudence la rusticité des troupeaux. Dans ce contexte, un programme de sélection sur base de la race Djallonké serait envisageable.

Mots clés : rural, ressources génétiques animales, ovin, Burkina Faso.

### 73. Evaluation des performances zootechniques et estimation d'une formule barymétrique adaptée à la race Zébu Azawak au Nord du Mali

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Le Nord Mali est caractérisé par un environnement aride et semi-aride et des terres fragiles, dont une forme traditionnelle d'exploitation est l'élevage mobile, généralement transhumant. L'élevage bovin y est dominé par le zébu, mieux adapté à la marche que les taurins. Menée au sein d'élevages de zébus Azawak, à orientation mixte, cette étude analyse les performances de croissance et de reproduction. Afin de faciliter le suivi des performances, une formule barymétrique est également développée. Le suivi a concerné 1109 animaux dans quatre communes du cercle de Ménaka, entre octobre 2007 et février 2008. Les variables de poids, périmètre thoracique et hauteur au garrot ont été analysées par des modèles linéaires généralisés à l'aide du logiciel R. Les résultats ont montré qu'à 30 jours, les mâles ont pesé en moyenne  $39,33 \pm 10,26$  kg et les femelles  $32,25 \pm 4,50$  kg. Le poids adulte des taureaux et des vaches (3 ans et plus) était respectivement de 370,99 ± 88,30 kg et 327,16 ± 46,53 kg. L'âge au premier vêlage a été de 50,07 ± 11,19 mois avec un intervalle entre vêlages de 16,42 ± 4,59 mois. Les corrélations du poids avec le périmètre thoracique et la hauteur au garrot étaient élevées, étant respectivement de 0,94 et 0,93. Des équations barymétriques ont été établies à travers une régression polynomiale du poids (y) sur le périmètre thoracique (x) pour chaque catégorie animale (veaux, génisses/taurillons, vaches/ taureaux). Cette dernière pourrait servir de table de conversion du périmètre thoracique en poids pour des besoins pratiques de manipulation des animaux.

**Mots clés** : Zébu Azawak, performance, croissance, reproduction, équations barymétriques, Ménaka, Mali.

### 74. Extraintestinal lesions associated with *Mycobacterium avium paratuberculosis* in wild cervids

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Paratuberculosis is a chronic enteritis of ruminants caused by *Mycobacterium avium subsp. paratuberculosis* (Map). Major lesions are mainly observed in the lower part of the small intestine and associated mesenteric lymph nodes. Disseminated infections are rarely reported in wild cervids. In this study, we report two cases with extraintestinal lesions associated with Map in adult red deer (*Cervus elaphus*). The first one was found dead in 2011 and the second was culled for sanitary reasons in 2012. After necropsy, samples of feces, ileocaecal junction, spleen, lung and lymph nodes (mesenteric, mediastinal and bronchial) were processed for bacterioscopy, histopathology, Map PCR and culture. Gross lesions were observed in intestinal tract, mesenteric lymph nodes, lung (focal consolidation, case #1) and bronchial/mediastinal lymph nodes (enlargement, case #2). Microscopic changes were characterized by granulomatous enteritis, pneumonia and associated lymphadenitis, with abundant acid-fast bacilli in macrophages and giant cells (multibacillary form). The presence of Map was shown by direct PCR performed on bronchial, mediastinal, mesenteric lymph nodes and spleen. Map cultures are currently in progress. Taken together, these observations suggest that systemic dissemination of Map may occur in paratuberculosis.

### 75. Effect of biocides on murine norovirus and feline calicivirus, surrogates of human norovirus

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Human noroviruses (HuNoV) are one of the major agents of human gastroenteritis and the main transmission occurs by faecal-oral route. The purpose of this work is to test biocide products on surrogate viruses of HuNoV in order to get informations on the viral infectivity and on the integrity of viral genomes. Two caliciviruses, murine norovirus (MNN) and feline calicivirus (FCV), have been chosen as HuNoV surrogates because presenting comparable structure and physico-chemical properties. Three commercial biocide products have been chosen (Kenocid 2100®, Virocid®, Alcocid®). The biocide product was tested according to Afnor norm EN 14476. The reduction of viral titer has been calculated and RNA extraction followed by a 1 step RT-qPCR was performed. Three biocides products tested are able to get a 3 log reduction on the viral titer of surrogates MNV and FCV and are so considered as effective. Efficacy against HuNoV can be extrapolated. The absence of effect of Alcocid® on genomic copies number indicates this biocide product doesn't interfere directly on viral genome but it acts maybe only on the viral structure, the capsid for example. In contrary with the 2 other biocide products (Kenocid 2100® and Virocid®), the increase of Ct values indirectly means an effect on the number of genomic copies. The effect of these two products could be on viral capsid but also directly on viral genome.

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#### **Addendum**

#### 76. Evaluation of some production parameters in Kabyle local rabbit population

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In Algeria, the rabbit production is mainly based on local population to ensure a cheap supply of urban markets proteins. This sector may represent an important source of protein which is in large deficit in the country. The rabbit production can be justified by its advantages such as short life cycle and high prolificacy. The objective of the present study is to evaluate production performances of Kabyle local rabbit population. Litter size, growth rate, body slaughter weight, feed conversion ratio and mortality rate are considered. The experiment was carried out on 30 animals (6 males and 24 females) for reproduction in the Ath Waghlis region (north-east Algeria). Rabbits have been raised in pens with 1 male and 4 females per pen. The commercial feed was supplied ad libitum to animals. Results reported shown that total number of rabbits born, number born alive, number to weaned, slaughter weight at 84 days of age, feed conversion ratio (from weaning to slaughter) and survival rate (from birth to slaughter) were 7.1, 6.1, 5.3 rabbits, 1.90kg, 3.16 and 73% respectively. The performances of the Kabyle breed could be improved by purebred selection as well as by crossing with exotic breeds.

**Key words**: Algeria, feed conversion ratio, growth rate, Kabyle local rabbit, reproduction traits, slaughter weight.

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