

Day 0: Sunday 25/08/2013				
15:00-17:00	Standing Committee AITVM (only for members)			
16:00-18:00	Registration Indaba Reception			
19:00	Dinner in Epsom Room			
	Day 1: Mond	lay 26/08/	13	
07:30-08:30	Registration desk open			
	Plenary session first part: Chair: Moritz v	an Vuuren (D\	/TD)	
08:30–09:00	Official opening ceremony (Prof Koos Coetzer, President of the AITVM & Prof Gerry Swan, Dean of the Faculty of Veterinary Science, University of Pretoria)			
09:00–09:45	Key-note presentation on diagnosis, surveillance and control at the interface, Eric Fèvre, United Kingdom			
09:45–10:30	Key-note presentation on drivers of emerging and re-emerging diseases: Understanding epidemiological processes for better control of emerging animal diseases: stakes and challenges for surveillance in Africa, Renaud Lancelot, France			
10:30-11:00	Coffee Break, exhibition, posters			
	Plenary session second part: Chair: Eric Thys (ITM)			
11:00–11:45	Key-note presentation on food safety and food security: The road that should be more travelled, Gavin Thomson, South-Africa			
11:45–12:30	Key-note presentation on training opportunities in the context of the interface: Enhancing health systems in Africa through training in one health, Serge Niangoran Bakou, Senegal			
12:30-14:00	Lunch break (Epsom Terrace Restaurant)			
Reminder:pres	sentation (maximum 15 minutes + 5 minut	es discussion)		
	Parallel session 1: diagnosis, surveillance and control at the interface (a) Chair: Eric Fèvre, United Kingdom	Parallel sessio diseases (a) Chair: Renauc	n 2: drivers of emerging and re-emerging	
14:00–14:40	Global elimination of rabies in humans: actions targeted at the animal reservoir, M Martinez	14:00–14:20	The prevalence of zoonotic tuberculosis in cattle at the livestock/human interface in the Mnisi community, South Africa, J Musoke	
		14:20–14:40	The prevalence of bovine tuberculosis in Swaziland, MEM Dlamini	
14:40–15:00	Knowledge and perceptions of dog ownership and rabies control among the Mnisi community, Mpumalanga, South Africa, S Thys	14:40–15:00	A review on bovine tuberculosis at the wildlife/livestock/human interface in sub-Saharan Africa, M De Garine-Wichatitsky	
15:00–15:20	Portable platforms for the detection of African swine fever virus tested in field conditions in East Africa, N LeBlanc	15:00–15:20	<i>Brucella abortus</i> is circulating in both, man and animals of Bangladesh, AKMA Rahman	
15:20–15:40	Understanding the dynamics and spread of African swine fever virus - a study on the role of the bushpig, <i>Potamochoerus</i> <i>larvatus</i> , at the wildlife-livestock interface, K Ståhl	15:20–15:40	Brucellosis and bovine tuberculosis at an animal-human interface in Zimbabwe, EMC Etter	
15.40-16.10	Coffee Break, exhibition, posters			

	Parallel session 1: diagnosis (b) Chair: Marta Martinez, France		Parallel session 2: drivers (b) Chair: Sofie Dhollander, Italy
16:10–16:30	Using participatory epidemiology to prioritize swine diseases at commune level in the Red River Delta region, Vietnam, TTH Pham	16:10–16:30	Brucellosis in small ruminants– seroprevalence and household practices in peri-urban farming around the capital Dushanbe, Tajikistan, U Magnusson
16:30–16:50	Spatial and seasonal patterns of foot- and-mouth disease outbreaks in domestic cattle in Zimbabwe between 1930 and 2006: a role of the wildlife/ livestock interface in FMD emergence in cattle in Zimbabwe? L Guerrini	16:30–16:50	Zoonoses in humans and domestic animals in Cambodia, a cross disciplinary approach, K Osbjer
16:50–17:10	Contact rates with buffalo explain foot-and mouth disease dynamics in cattle at the periphery of Transfrontier Conservation Areas in southern Africa, A Caron	16:50–17:10	Feeding behaviour of <i>Culicoides</i> spp. (Diptera: Ceratopogonidae) on cattle and sheep in northeast Germany, PH Clausen
17:10–17:30	-	17:10–17:30	Current knowledge of cryptosporidium at the wildlife-livestock human interface of the Kruger National Park, N Abu Samra
17:45	Photo of the group		
18:00	Farewell ceremony for Robert Paling follo	wed by a cock	tail evening
	Day 2: Tuesda	y 27/08/2	013
	Parallel session 1: diagnosis (c) Chair: Hellen van der Maazen, the Netherlands		Parallel session 2: drivers (c) Chair: Max Baumann, Germany
08:30–08:50	Bovine tuberculosis in the highlands of Cameroon: Risk factors analysis and implications for public health, J Awah-Ndukum	08:30–08:50	Alpha viruses as a cause of neurological disease in farm- and wild animals in southern Africa, S van Niekerk
08:50–09:10	Zoonotic tuberculosis at human- livestock interface in South-East Ethiopia, B Gumi	08:50–09:10	Bridge host, definition and methods: avian influenza virus transmission at the wildlife/domestic bird interface, A Caron
09:10–09:30	Bovine tuberculosis in the border area of the Natural Park of W in Niger: Impacts and risk factors for the disease transmission in cattle and humans in rural, peri-urban and urban areas, AR Boukary	09:10-09:30	Understanding the ecological drivers of avian influenza virus infection in wildfowl: a continental-scale study across Africa, A Caron
09:30–09:50	Measuring the prevalence and potential economic impact of <i>Mycobacterium</i> <i>bovis</i> and <i>Brucella abortus</i> in community-owned livestock bordering Hluhluwe-iMfolozi Park, South Africa, C Geoghegan	09:30–09:50	Landscape attributes driving avian influenza virus circulation in Lake Alaotra region, Madagascar, L Guerrini
09:50–10:10	Parasitic Zoonoses in Georgia, N Kokaia	09:50–10:10	The risk of introduction of Rift Valley fever virus into the southern Mediterranean area through undocumented movements of infected ruminants–assessment by Expert Knowledge Elicitation, S Dhollander
10:30-11:00	Coffee Break, exhibition, posters		
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11:00 -11:20	Wildlife and livestock use of extensive farm resources in South Central Spain: implications for disease transmission, J Vicente	11:30 -11:50	A metapopulation mechanistic model for Rift Valley fever virus persistence in the Comoros archipelago, L Cavalerie
11:20–11:40	Participatory and active surveillance for disease syndromes in communal cattle at the livestock/wildlife interface, South Africa, DL Knobel	11:50–12:10	Modelling spatial spread of Rift Valley fever virus in a temperate and mountainous area of Madagascar, G Nicolas

11:40–12:00	Application of mobile technologies in resource-limited environments for national One Health Disease Surveillance Systems, A Oukharov	12:10–12:30	Evidence of circulation of Rift Valley fever virus in the Comoros Archipelago and Madagascar in a post-epidemic period, M Maquart
12:00–12:20	Improvement of the current SAT3 liquid phase blocking ELISA to enhance the control of FMD in SADC, I Anahory	-	-
12:30-14:00	Lunch break (Epsom Terrace Restaurant)		
	Parallel session 2: drivers (e) Chair: Michel De Garine-Wichatitsky, Zin	nbabwe	
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14:40-15:00	Emerging zoonotic arboviruses causing ne	eurological dise	ease in animals in South Africa, M Venter
15:00-15:20	Genomics of South African West Nile viru	ses, C Kortenh	oeven
15:20-15:40	Ndumu virus in domestic pigs: a livestock	revolution tim	e bomb for public health, C Masembe
15:40-16:10	Coffee Break, exhibition, posters		
	Parallel session 3: food safety and food security (a) Chair: Hassen Chaka, Ethiopia		Parallel session 4: animal health and production (a) Chair: Norbert Mbahin, Kenya
16:10–16:50	Reconciling international trade imperatives with novel wildlife conservation approaches, P Bastiaensen	16:10–16:30	Transovarial passage and transmission of lumpy skin disease virus by Amblyomma hebraeum, Rhipicephalus appendiculatus and Rhipicephalus decoloratus ticks, EH Venter
		16:30–16:50	Altitudinal and spatial distribution of <i>Rhipicephalus (Boophilus) microplus</i> (Canestrini, 1888), <i>Amblyomma</i> spp. and <i>Ixodes boliviensis</i> Neumann, 1904 (Acari: Ixodidae) in Ecuador, R Rodríguez- Hidalgo
16:50–17:10	Thela entweni, ungakhi phakathi! Linking pathogens and people with farming and food practices to determine public health risks and design novel intervention strategies in remote farming communities of South Africa, C Geoghegan	16:50–17:10	Husbandry, health and welfare in cattle breeding feedlots in Indonesia—a unique production system, M Laurence
17:30-19:00	General Assembly AITVM (only for repres	entatives)	
19:00	Gala Dinner (Chiefs Boma Restaurant)		
	Day 3: Wedness	day 28/08/	2013
	Parallel session 3: Food safety (b) Chair: Anita Michel, South Africa		Parallel session 4: Animal health and production (b) Chair: Jean-Paul Dehoux, Belgium
08:30–08:50	Measuring the effects of time and temperature on the longevity of <i>Mycobacterium bovis</i> in unpasteurised, souring milk from Kwazulu-Natal, South Africa, C Geoghegan	08:30 –08:50	Efficacy and effectiveness of Waterbuck repellent blend (<i>Kobus ellipsiprymnus</i>): tsetse-repellent technology developed for the control of bovine trypanosomiasis in sub-Sahara Africa, N Mbahin
08:50–09:10	The use of polymerase chain reaction (PCR) to confirm presence of selected pathogenic bacteria along the milk value chain in the Tanga region, F Shija	08:50–09:10	Evolution of the trypanosome transmission cycle: the effect on pathogenicity and transmissibility of Trypanosoma congolense, S Chitanga
09:10–09:30	The importance of the zoonotic bacterium <i>Brucella abortus</i> in the milk chain in Gulu and Soroti regions of Uganda, KT Rock	09:10–09:30	Salmonellosis in pig farms in Reunion Island: Prevalence assessment and identification of risk factors, C Tessier
09:30–09:50	Fit for Human Consumption? A qualitative survey at a Ugandan pig abattoir, K Roesel	09:30–09:50	Bacteriophages against <i>B. anthracis</i> - causative agent of severe infectious diseases in humans and animals, M Kutateladze

09:50–10:10	Longitudinal investigation of <i>Salmonella</i> spp. from farm to fork in the pig industry in Reunion Island , C Tessier	09:50–10:10	Evaluation of Enzyme-Linked Immunosorbent Assays and Haemagglutination Inhibition tests for the diagnosis of Newcastle disease virus infection in village chickens using a Bayesian approach, H Chaka
10:10–10:30	-	10:10–10:30	How can joint Master programmes and their alumni contribute to the `One Health' concept? Experiences from the Master of Veterinary Public Health (MVPH) for Southeast Asia and the Master of Transboundary Animal Disease Management (MTADM) for Eastern Africa, MPO Baumann
10:10-11:30	Exhibition, posters		
11:30-13:00	Early Lunch break		
13:00-18:00	Afternoon free and tours for delegates w	ho booked tou	rs or would like to book a tour
	Day 4: Thursda	ay 29/08/2	013
	Session 5: Training opportunities in the context of the interface (plenary) Chair: Serge Niangoran Bakou, Senegal		
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09:20–09:40	Capacity building in EcoHealth: Experiences and evaluation of training using a "learning by doing" approach, within academic and non-academic contexts, J Gilbert		
09:40–10:00	Of Specialists and Generalists: Transdisciplinarity and 'One Health' training in South Africa, C Geoghegan		
10:00-10:20	Study abroad: an enrichment for many veterinary students of Utrecht University, WGGM van der Maazen		
10:20–10:40	Research platform "Production and Conservation in Partnership", promoting collaborative applied research and postgraduate training to study wild/domestic interfaces in southern Africa, M de Garine-Wichatitsky		
10:45-11:15	Coffee Break, exhibition, posters		
	Plenary session Chair: Robert Paling, The Netherlands		
11:15 -12:30	Presentation of Dik Zwart Awards for best	t poster and or	al presentations
	Announcement of successful bid for the 2016 conference		
	Official closure (Prof Koos Coetzer, Presid	ent of the AIT∖	(M)
12:30-14:00	Lunch break		
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Plenary presentations

Understanding epidemiological processes for better control of emerging animal diseases: stakes and challenges for surveillance in Africa

R Lancelot

CIRAD, UMR CMAEE / INRA, UMR CMAEE 1309, 34398 Montpellier, France

Africa is facing major challenges such as a dramatic increase in the human population, together with rapid and profound environmental changes, including climate and a significant increase in agricultural encroachment in fragile areas: forests, river deltas, etc. These changes are recognized as the drivers of animal or human disease emergence. Recent phylogenetic studies for example suggested African swine fever (ASF) and Rift Valley fever (RVF) have both emerged in eastern Africa during the 18th and 19th centuries, when exotic livestock were introduced in new agro-ecosystems. These viruses have subsequently spread with livestock movements, or exports of animal products. In addition, climate changes result in more frequent El-Niño events, causing heavy autumn rainfall in eastern and southern Africa, and consecutively RVF epizootics and epidemics.

However, many epidemiological questions remain unanswered, because of insufficient disease surveillance and collection of biological samples in outbreaks. Even when such samples are collected, tools - and most importantly human skills - are lacking to generate appropriate genomic data and analyse them. Also, with the availability of new diagnostic tools such as next-generation sequencing, new designs for disease / vector surveillance surveys will be needed.

Indeed, neither passive (event-based) surveillance, nor other methods such as sentinel animals or cross-sectional serological surveys, provides enough information for a correct assessment of epidemiological processes. A set of complimentary methods is needed to reach this goal, combining participatory epidemiology with surveys actively searching for pathogens or their serological traces in outbreaks.

Regarding the drivers of disease emergence, livestock movements – especially unofficial ones, are poorly documented and analysed. For environmental variables, the situation has recently improved with the availability of remotely sensed data collected over an extended period. Here again, the main bottleneck is the lack of skills to analyse these data.

Promotion and reinforcement of sustainable regional and sub-regional animal health networks is an efficient way to improve this situation. These networks must encompass veterinary services, research institutions, veterinary schools and universities, private stakeholders, and regional organisations. Beyond formal meetings and communication actions, more research programmes are needed, in close association with disease surveillance and control programmes.

The livestock-human-wildlife interface: Challenges in animal health and production in urban/peri-urban and extensive farming/conservation systems. The road that should be more travelled

GR Thomson¹, M Penrith¹, MW Atkinson², SJ Atkinson³, SA Osofsky³

¹TAD Scientific & DVTD, University of Pretoria; ²AHEAD-WCS, Gaborone; ³AHEAD-WCS, New York; gavin@tadscientific.co.za

The complexity of the livestock-human-wildlife interface is arguably most starkly illustrated by the long-standing conflict between livestock production and wildlife conservation in the extensive rangeland systems of southern and East Africa. Paradoxically, a presenting 'symptom' of this conflict - constraints to market access for commodities and products derived from animals - is not obviously associated with the underlying cause; somewhat analogous to the phenomenon of referred pain.

This conundrum has resulted from the way control of transboundary animal diseases (TADs), particularly those that involve wildlife, has evolved over many decades, largely dictated by international trade practices and standards that determine 'safe' access to markets. These practices and standards currently pay little heed to environmental circumstance and the consequential requirement for flexibility. Safe access to markets is determined by: (1) food safety and (2) negation of the risk of transmission of TADs such as foot and mouth disease (FMD). These two issues are driven by differing sets of international standards based on divergent principles which results in a constraint to trade. Fortunately, there is a solution: Value chain management of sanitary and phyto-sanitary (SPS) risk. This approach, supported by a recently published guideline provided by the FAO (Food & Agriculture Organisation), is accommodated by the principle of 'equivalence' (Article 4 of the WTO's [World trade Organisation] Agreement on the Application of Sanitary and Phyto-sanitary Measures (SPS Agreement). So the alternative road is not, in principle, faced with a 'planning permission' problem, although there are practical issues associated with implementation.

It is argued in this paper that value chain-based systems are not only logical and potentially beneficial to international trade, they are also much less costly to implement and simpler to audit and certify than the current geographically-based system for

animal disease risk-management, i.e. the need for freedom from TADs of the locality of production. This requirement for TADsfreedom is particularly problematic in southern Africa where export performance of beef, for example, has declined sharply in the last few years. This is due to a combination between the increasing incidence and severity of FMD outbreaks in the region and escalating private trade standards related to traceability and animal welfare. The proposed new road, importantly, does not constitute weakening or 'watering down' of standards; it simply provides alternative, potentially more reliable and robust SPS management which, at the same time, would greatly reduce the negative socio-economic and environmental impacts of SPS risk management, particularly beneficial to wildlife conservation given the historical and ongoing impacts of veterinary cordon fencing on free-ranging wildlife.

The potential advantages of this alternative road to improved and integrated rural development in the extensive rangeland systems of southern and East Africa are truly enormous.

Enhancing health systems in Africa through training in One Health

Serge Niangoran BAKOU

Ecole Inter-Etats des Sciences et Médecine Vétérinaires (EISMV) de Dakar, Member of Consortium Afrique One and OH NextGen

The interdependence of humans, animals, and their environment has never been so important than today. The recent epidemics (H1N1, severe acute respiratory syndrome, salmonellosis, *Escherichia coli* 0157:H7) highlight the growing globalization of health risks as well as the importance of human-animal-ecosystem interface in the evolution and emergence of pathogens.

The emergence and re-emergence of the disease incidence is now the result of the imperfect ability of policy makers and stakeholders to assess the impact of current systems for monitoring and controlling animal diseases. In Africa, the economic reforms of the 80s (eg Structural Adjustment Programs) with the dismantling of veterinary and other services have led to an increase of public health problems. Unfortunately, the regions with most severe health care shortages are the same regions in which high burdens of disease prevail. For example, Africa has 24% of the global burden of disease, but only 3% of the global health care workforce, and only 1% of the world's health expenditure. This shortage is first driven by insufficient training capacity and second personnel flow due to higher salaries, better working conditions, and more advanced training opportunities in higher-income countries settings.

Current global health challenges have called for more holistic, collaborative, action-oriented approaches toward logical and practical solutions. "One health" is exactly one of these approaches. The One Health approach is based on the paradigm of transdisciplinarity and added value of intersectoral collaboration between health professionals, veterinarians, wildlife and environment scientists and social scientists. Transdisciplinarity is defined as a unique epistemological perspective to the collaborative effort and is distinct from other cooperating disciplines. It is based on the principle that combined investments have a greater output than separate investments in each sector. Greatest added values are expected in instances where human and animal health issues are closely interrelated as with zoonoses and food safety, and if each sector reaches excellence in its own field of expertise. The challenge is to make each sector aware of the competences of the other sectors and the potential benefits of intersectoral collaboration.

How can this intersectoral collaboration be implemented, when veterinarian and physicians are trained separately, rarely communicate and work together.

To truly address "One health" in enhancing health system, developing centres of excellence for education and training in specific areas through collaboration among multiple professions, veterinary medicine, human medicine, environmental, wildlife and public health must be made available in Africa. It is well known that veterinary training in most parts of sub-Saharan Africa has focused on producing veterinarians to serve the livestock sector although socio-economic changes and privatization of veterinary services have caused curriculum adjustments, as have globalization and the increased risk of the spread of transboundary diseases. This is the case with the Vet school of Dakar in Senegal, which has incorporated One health approaches in its undergraduate veterinary curriculum and Master degree in Veterinary Public Health.

Moreover, agencies such as the Wellcome Trust, WHO' Essence on Health Research, the Council on Health Research for Development and European Commission FP7 are paying increasing attention to building institutional and overall system capacity for research. For example, collaboration, management and communication needs have been assessed by Afrique One the research consortium funding by Wellcome Trust in order to implement the One Health concepts in Africa. Afrique One developed supplementary modules known as "Welcome Package" for enrolled and well established researchers from Masters to Postdoc levels. These modules introduce the trainees to the tools identified as the most critical to improve research environment, to raise the trust and to build individual capacity in African institutions.

Another example, is training of the "One Health" Next Scientific Generation in the Sahel and Maghreb (OH-NEXTGEN), a 4 years European Commission FP7 coordination action aiming at improving the control of neglected zoonoses in the Sahel and the Maghreb through the training of local medical and veterinary scientists using a One Health approach.

Day 1: Monday 26/08/2013

Parallel session 1: diagnosis, surveillance and control at the interface (a)

Global elimination of rabies in humans: actions targeted at the animal reservoir

P Bastiaensen, <u>M Martinez</u>, A Dehove & D Bourzat

World Organisation for Animal Health (OIE) 12, rue de Prony, 75017 Paris, France; m.martinez@oie.int

At a high level technical meeting in Mexico in 2011, rabies control was identified as a model for inter-sectoral collaboration to address health risks at the human-animal- ecosystem interface. The Global Conference for Rabies Control in 2011, organised by OIE, together with WHO and FAO, highlighted the importance of rabies control in the animal reservoir to achieve worldwide elimination of rabies in humans.

Despite being 100% preventable, rabies still causes between 50,000 and 70,000 human deaths annually, mainly in developing countries in Africa and Asia. The majority of these deaths are caused by rabid dogs, which are reservoirs of the infection. Dog vaccination and dog population management are the most cost-effective interventions to control rabies in the animal reservoir. Dog health, particularly in stray dog populations, is often neglected in rabies-endemic countries. Add to this a lack of awareness in the community and the veterinary services, inadequate distribution of resources, as well as technical deficiencies, and the result is a perpetuation of neglect and a continuation of preventable human deaths due to rabies.

Recent changes to the rabies chapter 8.10. of the *Terrestrial Animal Health Code* of the OIE, may have important consequences for public health, international trade and transport of dogs and other carnivores from and to rabies-infected countries. For the purpose of the *Terrestrial Code*, a country that does not fulfil the requirements of Article 8.10.2. is now automatically considered to be infected with rabies virus. Furthermore, and again for the purpose of the *Terrestrial Code*, rabies is now considered to be linked to only one member of the Lyssavirus genus, namely rabies virus (formerly referred to as classical rabies virus, genotype-1). In line with the ongoing process to integrate wildlife into the *Terrestrial Code* where relevant (for the status of domestic animals), several articles on the importation of a variety of domestic and wild animals (captive, feral or wild) have been amalgamated into two articles dealing with "domestic ruminants, equids, camelids and suids" (Article 10.6.) and "wildlife" (Article 10.8). The model veterinarians to vaccinate and for official veterinarians to certify has been made clearer. New scientific information on serological testing of animals for rabies antibodies and on oral vaccination has been included in the rabies chapter 2.1.13 of the *Manual of Diagnostic Tests and Vaccines of Terrestrial Animals* of the OIE. The articles on rabies remain intrinsically linked to those on stray dog population control (Article 7.7.), which were recently adopted as part of the international animal welfare standards of the OIE.

In 2012, the OIE launched an EU-funded vaccine bank for rabies in Asia. With an overall capacity of 4 million doses of rabies vaccine for dogs, 750,000 doses have already been delivered to Lao PDR, Vietnam and the Philippines up to March 2013.

Finally, the OIE continues to work together with FAO, WHO and other international partners, such as the Global Alliance for Rabies Control, in the fight against rabies at the main source of human deaths.

Knowledge and perceptions of dog ownership and rabies control among the Mnisi community, Mpumalanga, South Africa

S Thys¹, DL Knobel², J Van Rooyen², G Simpson³, J Coetzer², T Marcotty⁴

¹Epidemiology and Tropical Diseases Control Unit, Department of Public Health, Institute of Tropical Medicine; ²Department of Veterinary Tropical Diseases, Faculty of Veterinary, University of Pretoria; ³Hluvukani Animal Health Clinic, Onderstepoort Veterinary Academic Hospital, Faculty of Veterinary Science, University of Pretoria; ⁴Department of Biomedical Sciences, Institute of Tropical Medicine; sthys@itg.be

Background: Canine rabies is endemic in many parts of the developing world, where domestic dogs are the primary maintenance host of the causative virus and responsible for the vast majority (>90%) of human exposures. Rabies is efficiently controlled through mass vaccination of dogs. Based on the concept of herd immunity, the efficiency of mass vaccination depends on the accessibility of dogs and the ease of restraint for vaccination, which, in turn, is determined by factors such as the proportion of unowned dogs in the population, the type of relationship people have with their dogs, and dog population dynamics. Understanding these aspects – all of which may vary geographically or be affected by socio-cultural determinants – is therefore helpful in guiding

long-term rabies control efforts in particular areas. Awareness in the community of the risk of rabies transmission from infected animals and the appropriate response to potential exposure to rabies are also essential to reduce human cases and deaths.

Objective: The general objective is to to improve educational messages used in rabies vaccination campaigns. The specific objective of this study was to assess the communities' knowledge and perceptions of dogs, rabies and the related risk and control behaviours in a recently-infected area of South Africa.

Method: Seventeen focus group discussions with men, women and children, stratified by dog ownership status were organised in three villages included in the Mnisi Community Programme, a study site located in Bushbuckridge local municipality in Mpumalanga Province in north-east South Africa.

Results: Preliminary results indicate that communities own dogs for guarding houses, cattle and fields. Hunting dogs are highly valued in the Mnisi community in spite of the illegality of hunting bushmeat. People are aware of the presence of rabies and its zoonotic nature. A dog's bite is always associated with rabies risk and children are the most aware about health seeking behaviours and the preventive value of dog vaccination. According to women groups, humans can also be infected if they eat animals that were bitten by a rabid dog or share their drinking water. On the other hand, none of the three groups have a clear idea of how dogs become infected. For some people, dogs are infected by other animals or when feeding on, for example, fish bones. "Street dogs" are regarded as the ones who suffer from diseases and who bite people. Vaccination is perceived as a means to prevent diseases from spreading to humans and other animals, to cure and/or to protect from diseases.

Conclusion: Lack of awareness and misinterpretation of promoting messages among adults seems to hinder participation in vaccination campaigns. Dog owners are aware of the usefulness of vaccination in general but may fail to have their dogs vaccinated as they believe dogs may become less aggressive afterwards to guard and hunt. Owners believe that training a dog well to not bite people will reduce the risk of rabies infection among the community. Different intersectoral strategies to improve awareness and action among adults are needed.

Portable platforms for the detection of African swine fever virus tested in field conditions in East Africa

N LeBlanc¹, E Okoth², JF Mayega³, C Masembe³, K Ståhl¹, M Hakhverdyan¹, R Bishop²

¹National Veterinary Institute (SVA), Uppsala, Sweden; ²International Livestock Research Institute, Nairobi, Kenya; ³Makerere University, Kampala, Uganda; neil.leblanc@sva.se

African swine fever virus (ASFV) is a serious transboundary animal disease in *Suidae*, causing high rates of morbidity and mortality in *Sus domesticus*. In southern and eastern Africa, the presence of a sylvatic cycle involving wild *Suidae* and soft ticks (*Ornithodoros* family) means that the risk of introduction of ASFV into domestic swine is always present. Low biosecurity, pig-to-pig transmission and contamination are considered the main factors in maintaining circulation of the virus in domestic pigs. In many parts of East Africa, smallholder pig farmers form a key part of the food supply. The ability to detect ASFV at pen-side can be a key aspect in the control of this disease. The objective of this study was to produce a portable molecular detection platform that is suitable for use in the field or modestly equipped laboratories.

The T-COR 4 portable real-time PCR thermocycler was used. Three methods of DNA extraction from whole blood were tested, including a magnetic bead extraction method (Nordiag AB), FTA elute indicating cards, and Tego sample cards. A real-time PCR assay based on the universal probe library (UPL; Roche) was used for detection (1). The field locations were in Gulu District, Uganda and in the Busia area on both the Kenyan and Ugandan sides of the border.

The UPL assay was tested with the Epizone ASFV ringtrial, a panel with all 22 genotypes, clinical samples from the European Union Reference Laboratory for African swine fever, blood samples from ASFV outbreaks and field samples collected in a pilot study. The UPL assay was prepared with a PCR kit which is highly stable at ambient temperature. The complete assay was premixed, so preparation on-site consisted of simply pipetting the mix into reaction tubes. The magnetic bead extraction method was robust and efficient. However, it involves a good deal of pipetting and reagents. The Tego extraction is extremely simple and requires little time (except drying) but the sensitivity was lower. The FTA method also worked well but there is significant drying time and a 30 minute heating step, so although the method is simple, it does take some time. The sensitivity was in between the two former methods. The UPL assay was robust and sensitive. It was simple to use because it can be pre-mixed before going in the field. The T-COR 4 instruments worked well with many runs in the field without fail. Re-charging the instruments from a vehicle outlet using a 12V universal adapter was effective in keeping the field lab functional while off the electrical grid for several days.

The results indicate that the platform can be used effectively at pen-side or in a field laboratory with performance at a level comparable to sophisticated molecular laboratories. The field trials revealed that these methods have potential for application in research, monitoring and outbreak scenarios.

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Understanding the dynamics and spread of African swine fever virus - a study on the role of the bushpig, *Potamochoerus larvatus*, at the wildlife-livestock interface

<u>K Ståhl</u>¹, P Ogweng², E Okoth³, T Aliro², D Muhangi², L Björnheden⁵, N LeBlanc¹, P Atimnedi⁴, S Boqvist⁵, R Bishop³, H Rasmussen⁶, C Masembe²

¹National Veterinary Institute (SVA), Uppsala, Sweden; ²Makerere University, Kampala, Uganda; ³International Livestock Research Institute, Nairobi, Kenya; ⁴Swedish University of Agricultural Sciences, ⁵Savannah Tracking Ltd, Nairobi, Kenya; karl.stahl@sva.se

African swine fever virus (ASFV) is a serious animal disease in members of the family *Suidae*, causing high mortality in domestic swine. In Africa, the presence of a sylvatic cycle involving wild pigs and soft-shelled ticks means that the risk of introduction of ASFV into domestic swine populations is always present. Although warthogs are considered the main wild vertebrate host of the virus in the endemic African setting, they are not the only wild African suids with a potential role in ASF epidemiology. The bushpig, *Potamochoerus larvatus*, is an elusive, nocturnal pig known to be susceptible to ASF. Naturally infected animals have been reported, and like warthogs infected bushpigs show no clinical signs. Bushpigs can transmit ASFV to domestic pigs, unlike the warthog, which only transmits through the tick. The interface between bushpig and domestic pig habitat is likely to be significant in many regions, as bushpigs. Such interaction between the genera in areas where ASFV is circulating is likely to result in virus transmission. An overlap between warthog and bushpig habitats in national parks is also likely, possibly also resulting in virus transmission. In the absence of a natural interface between the sylvatic and non-sylvatic cycles. With this in mind, the aim of this ongoing study is to investigate the role of the bushpig in the epidemiology of ASF at the wildlife-livestock interface.

Bushpigs are captured at the interface between farmland and major national parks in Uganda using game capture nets. Pigs are sedated with Zoletil forte[®] before blood sampling. One pig per location is equipped with a GPS/GSM tracking collar. Samples are screened for presence of virus and antibodies using commercial PCR and ELISA. To monitor bushpig movements, data from deployed tracking collars is collected at regular intervals and uploaded to a database at Savannah Tracking Ltd. All data can be visualized in real-time through Google earth, and downloaded for further analysis.

So far 10 bushpigs have been captured and sampled, and tracking collars have been deployed on three individual pigs. For the first time seropositivity to ASFV has been demonstrated in bushpigs, and weak positive PCR reactions have been detected. Analysis of movement data showed very limited movements during daytime, and peak distances travelled between sunset and midnight. Daily travel distances reached 12 km. During daytime pigs rested in swamps or thick bush, with nightly ventures into farming areas were domestic free-ranging pigs are kept.

These preliminary results confirm that bushpigs can be naturally infected by ASFV, and shows for the first time that detectable seroconversion may occur. Moreover, they demonstrate a spatial overlap between bushpig and domestic pig home range. In conclusion, our findings support a possible role of the bushpig in the epidemiology of ASF, but more work is needed as the project continues.

Parallel session 1: diagnosis, surveillance and control at the interface (b)

Using participatory epidemiology to prioritize swine diseases at commune level in the Red River Delta region, Vietnam

TTH Pham¹, TT Nguyen², VK Nguyen², TH Dao³, DT Vu³, E Baudon^{1,4}, B Cowling⁴, M Peyre¹

¹CIRAD-AGIRs Vietnam/France; ²National Institute of Veterinary Research, Vietnam; ³CIRRD, Hanoi Agriculture University, Vietnam; ⁴School of Public Health, Hong Kong University, Hong Kong; thithanhhoapham@ymail.com

Swine production in Vietnam is increasingly threatened by a range of swine infectious diseases such as PRRS, FMD, and pneumonia, with significant economic impact on the national economy and livelihoods of local producers. Early detection of diseases through efficient surveillance systems is critical. Previous studies have highlighted the limits of animal health surveillance in Vietnam,

which might be in part linked to the difference in disease surveillance priorities between local and national levels. The objective of our study was to assess the disease surveillance priority for swine producers in Vietnam using participatory epidemiology (PE) techniques, and to compare the results with the priorities defined by the national surveillance strategy.

The study area was selected based on the importance of swine production, diversity of swine farming systems, and occurrence of swine diseases such as PRRS, FMD and CSF. A total of four villages from one commune were included in the study. Key informant interviews involving officials, veterinarians and para-veterinarians were done step-by-step from province level to village level. Farmer focus group discussions were held within each village. A range of PE tools (e.g. proportional piling, matrix scoring, seasonal calendar, pair wise ranking, disease impact matrix scoring, and probing) were applied to characterize and quantify animal production, major diseases and their economic impacts on producers' livelihoods.

Swine and chicken were described as the most important livestock species in the study area both in term of numbers and contribution to farmers' income. Most of the pig farms in the commune (around 96%) were described as smallholder production (holding less than 20 sows and/or 200 fattening pigs) but in both systems, swine production was mainly for income. The impact of swine diseases was high, with a large range of diseases occurring all year round in both smallholder and semi-commercial farms (PRRS, FMD, pneumonia, diarrhoea). Blue ear (PRRS) was considered as the most important disease in all pig farming systems (relative score of 69) and had the highest relative incidence and impact on the livelihood of the producers. Diarrhoea and pneumonia were ranked in the 2nd and 3rd positions (relative scores of 42 and 38 respectively) with FMD classified in the 4th position (relative score of 30). According to the national surveillance strategy, the swine disease surveillance priorities in Vietnam are FMD and PRRS. Additional differences in surveillance priorities were also highlighted according to the administrative levels (e.g. at district level the priorities were PRRS, FMD as well as diarrhoea).

Further work is continuing to try to understand the socio-economic factors underlying the discrepancies in priorities. These differences might have major impact on the level of reporting of swine disease in Vietnam and therefore on the performances of surveillance systems.

Spatial and seasonal patterns of Foot-and-Mouth Disease outbreaks in domestic cattle in Zimbabwe between 1930 and 2006: a role of the wildlife/livestock interface in FMD emergence in cattle in Zimbabwe?

L Guerrini^{1,2}, A Caron^{1,2,3}, C Njagu⁴, M de Garine-Wichatitsky^{1,2}, A Murwira⁵, E Etter^{1,2}, DM Pfukenyi⁶

¹UR AGIRs Cirad Zimbabwe, Harare, Zimbabwe; ²UR AGIRs Cirad, Montpellier, France; ³Mammal Research Institute, University of Pretoria, Pretoria, South Africa; ⁴Department of Field Veterinary Services, Harare, Zimbabwe; ⁵Department of Geography en Environmental Science, University of Zimbabwe, Harare, Zimbabwe; ⁶Faculty of Veterinary Science, University of Zimbabwe, Harare, Zimbabwe; laure.guerrini@cirad.fr

Foot-and-Mouth Disease (FMD) is an important disease of livestock in southern Africa that requires important control and tradeassociated measures. Understanding the patterns of disease emergence and diffusion are important to adapt surveillance and control to local contexts.

We analysed a dataset of primary FMD outbreaks (n=73) in cattle in Zimbabwe from 1931 and 2006 and tried to answer the questions relative to the spatial, temporal and seasonal clustering of these outbreaks. In addition, we explored the relation between the occurrence of these outbreaks and the distance to national parks where resident buffalo populations are believed to be the maintenance population and the source of FMD for cattle. We also used a data set of FMD outbreaks in Zimbabwe between 2000 and 2006 (n=576) to explore the mechanisms of diffusion of the disease from the primary outbreak.

Results indicate a clear spatial clustering of primary outbreaks in the South-East Lowveld of Zimbabwe, a semi-arid region receiving less than 600mm of rainfall per year but hosting a large livestock population. No inter-annual temporal pattern could be identified from the first data set. However, the majority of the outbreaks occurred at the end of the cold-dry season (mainly June and July) and beginning of the hot-dry season (September). The distance of FMD outbreaks to national parks was shorter than average distance of random location to national parks at the country level. The descriptive study of the second data set on FMD outbreaks detected by the Department of Veterinary Services between 2000 and 2006 indicate how from primary outbreaks occurring in cattle close to (private or public) conservation areas the disease has spread in the cattle population.

The South-East Lowveld of Zimbabwe is a clear historical hotspot of FMD emergence in Zimbabwe. Close association between buffalo and cattle populations in this region could explain this pattern. However, other regions in Zimbabwe present the same properties without experiencing the same FMD pattern. The seasonal pattern of FMD primary outbreaks could be explained by the decrease in water availability in the course of the (cold then hot) dry season and the need for buffalo and cattle to access water from the same waterholes. Once FMD has jumped from buffalo to cattle, the disease spread easily within the cattle population. These different hypotheses are discussed in the light of recent studies on the topic in the area.

Contact rates with buffalo explain foot-and mouth disease dynamics in cattle at the periphery of Transfrontier Conservation Areas in southern Africa

E Miguel¹, V Grosbois¹, <u>A Caron</u>^{1,2,3}, T Boulinier⁴, H Fritz⁵, D Cornelis¹, C Foggin⁶, V Makaya⁷, P T Tshabalala⁷, M de Garine-Wichatitsky^{1,2}

¹Cirad, UR AGIRs, Montpellier; ²Cirad/RP-PCP, Harare, Zimbabwe; ³Mammal Research Institute, University of Pretoria, South Africa; ⁴CNRS - CEFE UMR 5175, Montpellier, France; ⁵CNRS - LBBE UMR 5558, UCB Lyon Villeurbanne, France; ⁶Governmental Veterinary Services: Wildlife Unit, Harare, Zimbabwe; ⁷Governmental Veterinary Services: Veterinary Diagnostic and Research Branch, Harare, Zimbabwe; degarine@cirad.fr

Wildlife is a maintenance host for several significant livestock diseases. Interspecific pathogen transmission often occurs at wildlife-domestic animal interfaces within complex socio-ecological systems but is yet poorly studied. We investigated the relationship between the dynamics of foot and mouth disease (FMD) in vaccinated or unvaccinated cattle populations and the frequency of contacts with African buffaloes at different buffalo-cattle interfaces.

A total of 36 GPS collars were deployed on African buffaloes (*Syncerus caffer*) and cattle (*Bos taurus, Bos indicus*) to assess contact patterns at the periphery of 3 protected areas in Zimbabwe. Simultaneously, a longitudinal survey of 300 cattle with five repeated sampling sessions on known individuals during 16 months was undertaken. Immunological assays (ELISAs), that allowed detection of antibodies following infection or vaccination, were used to assess serological transitions (*i.e.* incidence and reversion) in the surveyed cattle. Variation in rates of serological transitions across seasons, sites and as a function of the frequency of contact with buffaloes was analyzed using generalized linear mixed models.

The incidence in the cattle populations of FMD antibodies produced following infection varied among sites and as a function of contact rates with African buffaloes. The incidence was higher for sites which had substantial interactions between the two species. The serological incidence was also related to seasons, being higher during the dry season. The reversion rate pattern was the opposite of this incidence rate pattern. Vaccination seemed partly efficient at the individual level, but it did not prevent the diffusion of FMD viruses from the wild reservoir host to the domestic cattle population. Furthermore, antibodies were detected in areas where cattle had not been vaccinated, suggesting that the virus may have spread without being detected. Access to resources shared by carrier buffaloes and livestock, particularly water and grazing areas during the dry season, could explain the observed patterns of FMD transmission. We discuss how insights on ecological processes leading to wildlife-livestock contacts may provide some innovative solutions to improve FMD management, including surveillance, prevention or control of buffaloborne outbreaks, by adopting strategies targeting risky areas and periods.

Parallel session 2: drivers of emerging and re-emerging diseases (a)

The prevalence of zoonotic tuberculosis in cattle at the livestock/human interface in the Mnisi community, South Africa

J Musoke¹, T Marcotty^{1,2}, A Michel¹

¹Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa; ²Institute of Tropical Medicine, Belgium; jolly.musoke@hotmail.com

In African countries, including Sub-Saharan Africa, high numbers of informal animal slaughters are observed. In addition, many rural households consume animal products, such as milk, untreated. This is of great public health concern as often there is no adequate inspection of the meat and the animal products consumed, and few of these countries have policies implemented to control zoonotic diseases. The aim of this investigation is to determine the presence and, if applicable, the prevalence of bovine TB (BTB) in cattle within the Mnisi community, as well as to investigate possible risk factors for transmission of bovine and human TB between livestock and humans.

The comparative intradermal test was performed in Mnisi's livestock using a cross-sectional as well as a targeted survey at the 15 dip-tanks. The cross-sectional survey entailed random sampling at the 15 dip-tanks, whereas the targeted survey entailed selecting cattle from the HDSS-live database that were recorded with respiratory syndrome reported by the farmer (e.g. chronic cough). The target number of cattle to be tested using the cross-sectional survey is 1 200, whereas approximately 300 cattle are to be tested using the targeted approach. To date, 800 cattle were tested as part of the cross sectional survey, while, 37 cattle were identified and tested in the targeted approach. A total of 4/800 cattle (0.5%) tested positive for BTB, three of which also tested positive on the interferon gamma assay (Bovigam). None of the 37 cattle tested using the targeted approach were positive.

The low prevalence could be attributed to low test sensitivity or challenges and constraints encountered at the dip-tanks, which

will be discussed further. To achieve the aims and objectives of this investigation, the three interferon gamma test positive cattle were slaughtered and inspected for TB lesions. Lymph nodes from the head and thorax and any organs showing lesions suspect of BTB were collected and cultured for mycobacteria. A study in humans is also to be performed in order to screen for BTB in humans and possibly isolate and characterize the mycobacteria. The Mycobacterium isolates obtained will be genetically characterized using spacer oligonucleotide types (spoligotyping). The genetic profile obtained will be compared to genetic profiles of the livestock study group, the human study group, the milk study and reported cases in Kruger National Park to determine genetic relatedness. A short questionnaire will also be administered to identify possible risk factors of bovine and human TB at the livestock/human interface.

The prevalence of Bovine tuberculosis in Swaziland

MEM Dlamini^{1,2}, A Michel¹

¹Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa; ²Meat Hygiene Services (Veterinary Public Health), Department of Veterinary and Livestock Services, Ministry of Agriculture, Swaziland; mcebodlamini@ymail.com

Bovine Tuberculosis (BTB) is a chronic insidious debilitating contagious disease primarily affecting cattle and a wide range of domestic and wild animals with a worldwide distribution and transmitted mainly through the inhalation of aerosols. Human beings consuming products of infected cattle, such as unpasteurised milk and meat, are at risk of being infected by *Mycobacterium bovis*, the causative agent of BTB. A study has been conducted in which carcasses found to be infected with BTB on *post mortem* inspection at an export abattoir, and later confirmed positive for BTB by laboratory diagnosis, were traced back to their dip tanks of origin which were consequently selected for a BTB prevalence study. The distribution of the fifteen selected dip tanks of study was geographically representative of all provinces in the country. Ten percent of the animals in the selected dip tanks of study, whose populations range from 500-1800 cattle, owned by about 10- 120 farmers per dip tank, were randomly selected and tested for BTB using the comparative intra-dermal skin test; with each kraal in a dip tank included in the testing. Eight BTB test positive animals were sacrificed, a *post mortem* conducted and samples collected for the isolation of *M. bovis*. Furthermore, the zoonotic risk to farmers whose cattle are infected with BTB was assessed by means of a questionnaire survey.

The prevalence of BTB was found to be 6.75 % in the study population and gross lesions typical of *Mycobacterium bovis* infection were observed in the eight skin test positive cattle slaughtered and *M.bovis* was isolated from seven of them and from at least one herd. Of the farmers interviewed, 88.9% consumed unpasteurized milk obtained from their lactating beef cattle while 92.9% consumed meat that has not been inspected. Of the latter, 100% consumed high risk organs such as the head and visceral organs, without removing any lymph nodes. Furthermore, 94.8 % admitted to some form of undercooking of the meat, through cooking the meat over an open fire. Lastly, about 68.1% of respondents consume meat from animals that die on their own whenever it is available.

This study is the first to determine the BTB prevalence and zoonotic risk factors for humans in Swaziland. BTB was found to be an important cattle disease in the country with a significantly high prevalence. Consumption of unpasteurized milk and uninspected meat, including high risk organs, and the rampant practice of undercooking meat, expose consumers to infection with *M*.bovis from BTB infected animals. Farmers lack knowledge about BTB as a cattle disease and a serious zoonosis. At best, livestock owners' knowledge of the zoonotic aspects of BTB is insufficient and inadequate, with inferences of BTB drawn from human TB. There is a need to investigate the extent of *M. bovis* infections in the human population in Swaziland.

A review on bovine tuberculosis at the wildlife/livestock/human interface in sub-Saharan Africa

M De Garine-Wichatitsky^{1,2}, A Caron^{1,2,3}, R Kock⁴, R. Tschopp^{5,6,7}, M Munyeme⁸, M Hofmeyr⁹, A Michel¹⁰

¹Cirad, UPR AGIRs, Montpellier, France; ²Cirad/RP-PCP, Harare, Zimbabwe; ³Mammals Research Institute, University of Pretoria, Pretoria, South Africa; ⁴Royal Veterinary College, University of London, United Kingdom; ⁵Swiss Tropical and Public Health Institute, Basel, Switzerland; ⁶Armauer Hansen Research Institute, Addis Ababa, Ethiopia; ⁷MRC National Institute for Medical Research, London, United Kingdom; ⁸Department of Disease Control, University of Zambia, School of Veterinary Medicine, Lusaka, Zambia; ⁹South Africa National Parks, Kruger National Park, South Africa; ¹⁰Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa; michel.de_garine-wichatitsky@cirad.fr

This talk is based on the findings of a recent article reviewing the current epidemiological situation, risk of emergence and control options at the wildlife-livestock-human interface in sub-Saharan Africa (1). Infection of wild animals by bovine tuberculosis (bTB) is raising concern worldwide. In livestock, bTB has been confirmed in the majority of countries from all parts of the continent. Wildlife infection is confirmed in 7 countries from southern and eastern Africa, apparently spreading in the southern Africa region. *Mycobacterium bovis* has been isolated from 14 wild mammal species, although only 4 are suspected to play a role as

maintenance host. Zoonotic risks are a concern, but no direct spill-over from wildlife to human has been documented, and no case of bTB spill-back from wildlife to livestock has been confirmed.

Transmission of *M. bovis* at wildlife-livestock-human interfaces in sub-Saharan Africa is driven by a complex interplay between biological, ecological and anthropogenic processes. We discuss the main risk factors of bTB spill-over at the wildlife-livestock-human interface and identify several knowledge and technical gaps which should be filled in priority in order to improve the control of the disease in the African context. These gaps include the absence of understanding of the role of individual wildlife species/populations in the epidemiology of bTB in complex multi-host systems, the low number of longitudinal surveys and *M. bovis* isolates from various sympatric species, the absence of understanding of the ecological and anthropogenic drivers of contacts between wildlife and livestock, the environmental source of bTB infections and the persistence of infectious material in various African ecosystems, the need for accurate, affordable, and reliable diagnosis for African wild species and the development of effective vaccination strategies.

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Brucella abortus is circulating in both, man and animals of Bangladesh

AKMA Rahman^{1,2,3}, C Saegerman², B Dirk³, F Melzer⁴, H Neubauer⁴, D Fretin⁵, MU Ahmed¹, A Hossain⁶, E Abatih²

¹Department of Medicine, Bangladesh Agricultural University, Mymensingh, Bangladesh;²Research Unit of Epidemiology and Risk Analysis applied to the Veterinary Sciences (UREAR), Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Lie`ge, Lie`ge, Belgium;³Unit of Epidemiology, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerpen, Belgium;⁴Federal Research Institute for Animal Health, Reference Laboratory for Brucellosis and CEM, Naumburger Str. 96a, 07743 Jena, Germany;⁵Department of Bacteriology and Immunology, Veterinary and Agrochemical Research Centre, Brussels, Belgium;⁶Department of Microbiology, Mymensingh Medical College, Mymensingh, Bangladesh; arahman97@yahoo.com

In endemic areas with low prevalence e.g. Bangladesh, brucellosis is not only a food-borne disease but is also often acquired occupationally and veterinarians, abattoir workers, farmers and laboratory personnel are at high risk. Consequently, Brucellae isolated from humans mirror those species which are also endemic in livestock. However, the Brucella species which cause human or animal disease are still unknown for Bangladesh. Laboratory detection of Brucella and species identification is based on culture which is time consuming, resource-intensive and poses the risk of laboratory-acquired infections (1). In order to avoid these problems, polymerase chain reaction (PCR) techniques have become popular for both, rapid detection from clinical samples and subsequent species confirmation. Blood and serum are the clinical samples routinely taken for diagnosis but serum is the preferred for PCR (2-3) probably because serum contains fewer PCR inhibitors than blood. The Brucella real time PCR assays so far applied for human samples were designed to detect isolates at the genus level (4-5) but not at the species level. Thus, a study was made to identify the species of Brucella circulating in animals and humans of Bangladesh. Seventeen milk ring test (MRT) positive pooled milk samples (cattle, goat and gayal), 23 placentas (cattle, goat and sheep), 15 vaginal swabs from different animals (cattle, goat and sheep) and of two women, 5 semen samples of bulls and 19 sero-positive human sera were tested for Brucella species by using IS711 real-time PCR. (6). Only five milk (three cattle, one goat and one gayal), one semen but all 19 human sera were positive in the Brucella abortus specific real-time PCR. Interestingly, none of the samples was positive for Brucella melitensis in the specific real time PCR which is the species most often associated with human disease. Now it can be supposed that B. abortus is circulating in animals of Bangladesh. The presence of B. abortus DNA in milk and semen also supports this hypothesis. Serum based IS711 real time PCR is a promising alternative tool for the species level detection of Brucella in a resource poor setting like Bangladesh. Future studies have to investigate whether B. abortus is the only prevalent Brucella species in Bangladesh.

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Brucellosis and bovine tuberculosis at an animal-human interface in Zimbabwe

BM Gadaga¹, GMC Matope², B Mukamuri³, J Magwangudze⁴, D Pfukenyi², EMC Etter^{5,6}

¹Department of Veterinary Technical Services, Central Veterinary Laboratory, Harare, Zimbabwe; ²Veterinary Faculty, University of Zimbabwe; ³Centre for Applied Social Sciences, University of Zimbabwe; ⁴Veterinary Field Services, District of Chiredzi, Zimbabwe; ⁵UR AGIRs, CIRAD, Zimbabwe; ⁶UR AGIRs Cirad, Montpellier, France; eric.etter@cirad.fr

South East Lowveld (SEL) in Zimbabwe is part of agro-ecological region V which receives less than 450 mm annual rainfall and characterised by extensive farming. SEL encompasses the Gonarezhou National Park, which is part of the Great Limpopo Transfrontier Park. Fences of the park in Zimbabwe were partly removed and the frontier between wildlife and domestic cattle is extremely porous. In this area 70% of households own cattle while 88% and 100% owned sheep and goats, respectively. In 2008, the first isolation of bovine tuberculosis in buffaloes was reported in Gonarezhou (1). Brucellosis was also diagnosed in SEL but only in cattle (5-16%) (2). The importance of the interface between wildlife, domestic animals and human in this region revealed a high potential for transmission of zoonoses. This study tackles the zoonotic risk of brucellosis and bovine tuberculosis in SEL. In a first approach the study assessed the perception of different categories of the population concerning these two diseases. Then it determined the principal modes of transmission and the critical points of the commodity chain where these diseases could be introduced or expanded. Analysis of bulk milk samples as well as sera and lymph nodes collected in slaughterhouse showed that brucellosis is a real hazard for human population. Health centre in SEL used malaria rapid kits in case of acute febrile illness but there is no confirmatory test for the disease. There is no diagnostic follow-up of people having malaria negative test to rule out brucellosis. Concerning abortion, no systematic research is performed in human population and awareness of human health workers concerning symptoms of human brucellosis is negligible. In perspective with the awareness and food habits of animal health workers, human health workers, commodity chain workers and farmers, the study highlights the high probability for brucellosis to be an occupational disease as well as a food borne zoonosis most often under-diagnosed. Even though no cattle samples were found positive for bovine tuberculosis, due to the similar wavs of transmission, the potential transmission of bovine tuberculosis from wildlife to cattle would constitute an important threat for human population. In addition to these results, HIV prevalence in Zimbabwe is high with a prevalence of 14.9% amongst adult aged 15 to 49 in 2011 (3). Therefore the potential of brucellosis and bovine tuberculosis spread into human population is really to be considered by government and relevant measures linking animal and human health has to be taken.

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Parallel session 2: drivers of emerging and re-emerging diseases (b)

Brucellosis in small ruminants – seroprevalence and household practices in peri-urban farming around the capital Dushanbe, Tajikistan

I Ljung¹, C Grahn¹, N Sattorov², E Lindahl¹, <u>U Magnusson¹</u>

¹Division of Reproduction, Department of Clinical Sciences, Swedish University of Agricultural Sciences (SLU), SE- 750 07 Uppsala, Sweden; ²Laboratory of veterinary Microbiology, Tajik Agrarian University, Dushanbe, Tajikistan; Ulf.Magnusson@ slu.se

The bacterial zoonosis brucellosis is endemic among ruminants in the Central Asian Tajikistan where about 80% of the households keep livestock, most commonly shoats but also smaller numbers of cattle. This makes the risk for transmission of brucella from livestock or livestock products to humans considerable. The public health concern might be extra high in peri-urban farming where livestock are closer to large and dense human populations. The aims of the current study were to, 1) describe the farm structure in peri-urban located villages around the capital Dushanbe; 2) assess the seroprevalence of brucellosis in shouts from small households in peri-urban villages in four districts around Dushanbe; 3) identify practices in the households known to increase the risk for animal-to-animal or animal-to-human transmission.

Twenty-two villages were selected for the study partly randomly and partly by convenience in order to be evenly distributed in the 4 districts adjacent to Dushanbe. In these villages a total of 97 households were randomly chosen and subjected to systematic interviews by using a questionnaire. In the households sera were collected from 908 animals (329 sheep and 579 goats, respectively). Samples were tested for antibodies to *Brucella* spp. by i-ELISA. All positive or suspicious positive samples were tested with Brucella-Ab c-ELISA.

- Animal husbandry and management practises were at large homogenous in the study area with most farmers owning a small number of shoats (<20) and communal grazing occurred frequently and almost all households also own at least one cow.
- The seroprevalence in sheep was double that in goats, 14 and 7%, respectively. More than 50% of seropositive sheep and goats were between four and five years old. 68% of the 22 villages had at least one seropositive shoat. A large variation in seroprevalence between the districts was seen.
- 57% of the households had heard of the disease brucellosis previously and all these knew that the disease was zoonotic. In 18 % of the households was milk drunk without previous boiling.

These preliminary data show a very high seroprevalence for brucella among the shoat in periurban Dushanbe and suggest several possible risk factors for transmission of the infection between livestock as well as to humans.

Zoonoses in humans and domestic animals in Cambodia, a cross disciplinary approach

<u>K Osbjer</u>¹, C Kannarath², S San³, S Sokerya⁴, S Boqvist⁵, M Berg⁵, U Magnusson¹

¹Division of Reproduction, Department of Clinical Sciences, Swedish University of Agricultural Sciences (SLU), SE- 750 07 Uppsala, Sweden; ²National Institute for Public Health, Phnom Penh, Cambodia; ³National Veterinary Research Institute, Phnom Penh, Cambodia; ⁴Centre for Livestock and Agriculture Development, Phnom Penh, Cambodia; ⁵Department of Biomedical Sciences and Veterinary Public Health, SLU, Uppsala, Sweden; Kristina.osbjer@slu.se

Introduction: There is an increasing insight that animal-human interfaces are crucial for the transmission of zoonotic pathogens. By studying the interplay between peri-domestic wildlife, domestic animals and humans in the same rural area of Cambodia simultaneously, it will be possible to analyze contrasting and commonality risk factors for transmission of the two pathogens Influenza A and *Campylobacter* with the final aim to make future recommendations for more upstream preventive measures.

Methods: Three agro-ecological zones of Cambodia have been selected where information on village demographics, socioeconomic situation, human health, livestock health and practices related to disease transmission risks was captured in 330 questionnaires at village and household level. In the same households fecal samples were collected from humans and domestic or captured peri-domestic wildlife (including buffaloes, cattle, chickens, doves, ducks, geese, horses, pigs and quails) for *Campylobacter* analysis. For Influenza analysis tracheal and cloacal swabs were collected from birds of various species and nasal swabs from pigs.

Initial Results: To date, isolation and identification of *Campylobacter* spp. has been conducted on 250 animal samples. 35 samples (14%) were positive to *Camplobacter* spp. (*C. jejuni* and *C. coli*) with the highest prevalence in chickens below the age of one. Of the 449 animals screened for Influenza A on the for influenza samples recommended rRT-PCR method, 8 samples (1.6%) were found to be positive. The subtypes of these have not yet been determined.

Questionnaires analyzed from Mekong lowland swamp villages in Kampong Cham province (KC) and lakeshore villages in Battambang province (BB) show notable regional differences.

Divergences are suggested to be partly explained by the short distance from KC villages to the urban areas of Phnom Penh:

- Monthly average income per household is USD 10 more in KC than in BB and 23% of the adult population in the household generate off farm income in KC compared to 14% in BB
- In KC, 27 households enrolled in the study benefited from an ongoing development project compared to six households in BB
- A higher proportion of households kept pigs and ruminants in KC while poultry was more abundant in BB. Responsibility for different species was also more gender specific in BB
- No regional difference was observed in risk factors for zoonoses. However while 61% of the households knew of a zoonotic disease, only 3 % saw a risk for zoonotic disease transmission in their village despite risk behaviors such as "sick animals are slaughtered for consumption" (27% of the households) and "animals found dead are eaten" (29% of the households)

Feeding behaviour of *Culicoides* spp. (Diptera: Ceratopogonidae) on cattle and sheep in northeast Germany

T Ayllón¹, AM Nijhof¹, W Weiher¹, B Bauer¹, X Allène², P-H Clausen¹

¹Institute for Parasitology and Tropical Veterinary Medicine, Freie Universität Berlin, Königsweg 67, D-14163, Berlin, Germany; ²CIRAD, UMR Contrôle des maladies, F-34398, Montpellier, France; clausen.ph@vetmed.fu-berlin.de

Culicoides spp. play an important role as vectors of several vector-borne pathogens such as bluetongue virus (BTV) and Schmallenberg virus (SV). The unexpected outbreak of bluetongue in Europe in 2006, its fast dissemination and the emergence of Schmallenberg disease in Europe had an enormous impact on livestock production (1,2). Although the importance of autochthonous *Culicoides* species in the dissemination of BTV and SV is generally recognized, many aspects of the ecology of native *Culicoides* species are still not fully understood, and there is a lack of information concerning their dispersal, vectorial capacity, feeding and host-seeking behaviour. In order to better understand the biology of *Culicoides* species in Central Europe, we performed a field study in northeast Germany which aimed to clarify the daily activity patterns, preferential landing and feeding sites and host feeding preferences of *Culicoides* spe. using cattle and sheep as baits.

The study was performed in July 2012 and was divided in three parts (A, B and C). In study A, 3545 Culicoides belonging to 13 species were collected from the vicinity of cattle and sheep over a 72h period using drop traps and backpack aspirators. We observed a bimodal pattern of activity, with peaks at dawn and dusk, although the highest midge activity was found around sunset (24% of the catches). By direct aspiration of midges from the body surface of cows and sheep in study B, we aimed to determine the preferential landing and feeding sites of Culicoides species. We collected 2024 Culicoides from four different body regions, whereby a high number of midges were collected from the lower body regions of cattle and sheep. This has implications for the use of topical (pour-on) insecticides to prevent midge bites, as their availability in the skin of different body regions may not be homogenous (3). Host feeding preferences of *Culicoides* were tested in study C where we collected midges using 2 drop traps placed at an intermediate distance of 10m with a cow or three sheep placed as baits inside. The origin of the host bloodmeal was confirmed by a PCR targeting the cytochrome B gene. In study C, 3710 Culicoides were collected, 83% of them from cattle and 17% from sheep. Nearly half (46.7%) of the midges collected from cattle were engorged, significantly more than sheep where 7.4% of the collected midges were engorged, which suggests that cattle are more attractive for *Culicoides* midges in comparison to sheep. All Culicoides species collected were morphologically identified, according to standard identification keys. Culicoides from the Obsoletus complex (C. obsoletus and C. scoticus) were the most common Culicoides species collected, followed by C. punctatus. Other species identified were C. dewulfi, C. chiopterus, C. pulicaris, C. lupicaris, C. pallidicornis, C. subfascipennis, C. achrayi, C. griseidorsum, C. subfagineus and C. stigma, the last two species described for the first time in Germany. Significantly more engorged C. chiopterus midges were collected from sheep in study C, suggesting a potential host preference of this species for sheep.

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Current knowledge of cryptosporidium at the wildlife livestock human the interface of the Kruger National Park

N Abu Samra¹, F Jori², S Caccio³, J Frean⁴, B Poonsamy⁴, P Thompson¹

¹Epidemiology Section, Department of Production Animal Studies, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa; ²CIRAD, UR AGIRs, Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, 0002, South Africa; ³European Union Reference Laboratory for Parasites, Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy; ⁴National Institute for Communicable Diseases, South Africa; nada.nada@gmx.de

Cryptosporidium spp. is an oocyst-forming apicomplexan protozoan, which infects a large number of animals and humans (Putignani and Menichella 2010). Several species/genotypes have a relevant zoonotic potential and ruminants are considered as an important source of infection (Xiao and Feng 2008). In general, young individuals show higher rates of infection compared to adults (Santín *et al.* 2004). *Cryptosporidium* infection has been demonstrated in a wide number of wild animals, which might contribute to environmental contamination (Feng 2010). In sub-Saharan Africa, where HIV infections are the highest in the world, high rates of severe and even fatal *Cryptosporidium* infection have been reported (Mor and Tzipori 2008). However, nothing is known about the epidemiology of this disease in a wildlife livestock interface context representative of Southern Africa.

Samples from buffalo, impala and cattle where collected between 2008/2009 at the wildlife, livestock and human interface of the Kruger National Park (KNP). The modified Zieh- Neelsen (ZN) staining technique and an immunofluorescent antibody test kit detected *Cryptosporidium* in 5.5% (5/91) and 1.4% (2/140) of buffalo respectively and 4.2% (4/94) and 1.8% (3/161) in impala. Four of the 241 wildlife samples were PCR-positive (2.8% in each species) and sequencing revealed the presence of *C. ubiquitum* in two impala and one buffalo and *C. bovis* in one buffalo. Among cattle samples, 8% (4/51) were PCR-positive and were identified as *C. andersoni* (2/4) and *C. bovis* (2/4). Additional sampling took place in 2012, this time targeting young individuals such as calves (0-6 months), children (> 5years) and buffalo calves. All ZN positive and a random number of ZN negative samples from human and cattle were analysed by PCR and identified DNA was sequenced: 5% (7/140) of children samples were positively amplified at the GP60 gene and sequencing identified *C. hominis* (3/4) and *C. meleagridis* (1/4). Twelve out of 350 calves samples (3.4%) were confirmed positive by 18S rRNA-PCR and sequencing revealed two cattle specific *Cryptosporidium* species (*C. bovis* and *C. ryanae*). None of the buffalo calves sampled at the centre of the KNP were found positive with ZN.

The prevalence of *Cryptosporidium* detected in our samples was low compared to the ones reported in other sub-saharan African studies. The species/genotypes detected in humans were predominantly of anthroponotic nature, while wildlife species were shown to be infested with zoonotic species. However, the public health importance of this parasite should be investigated further; environmental samples such as water should be analysed for *Cryptosporidium* spp. in order to fully understand the exposure pathways of this parasite.

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Day 2: Tuesday 27/08/2013

Parallel session 1: diagnosis, surveillance and control at the interface (c)

Bovine tuberculosis in the highlands of Cameroon: Risk factors analysis and implications for public health

J Awah-Ndukum¹, AC Kudi³, G Bradley² PL⁴ Dickmu

¹School of Veterinary Medicine and Sciences, University of Ngaoundere, Cameroon; ²School of Biomedical and Biological Sciences, University of Plymouth, UK; ³Department of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria; ⁴Department of Mathematics / Computer Sciences, University of Dschang, Cameroon; awahndukum@yahoo.co.uk

Bovine tuberculosis (TB) is a neglected zoonosis of cattle that is prevalent but under-investigated in Cameroon. Based on epidemiological data of the disease, this study was designed to assess the risks and public health implications for zoonotic *M. bovis* infection in cattle and humans in the highlands of Cameroon. Evidence of bovine TB in cattle in the study region was confirmed by the following surveys: abattoir slaughter meat inspection TB lesion detection rates of 0.20% - 1.69% (over 60.94% of all pathologies that warranted partial or whole carcass condemnation were due to TB lesions); sero-prevalence rates of 37.17% and comparative cervical tuberculin test estimations of 4.67% - 7.15%, 12.02% - 15.67% and 20.56% - 24.98% at the ≥ 4 mm, ≥ 3 mm and ≥ 2 mm cut-off points, respectively. Genomic deletion analysis of cultured isolates showed evidence of *M. tuberculosis* from suspected cattle tissue and *M. bovis* from infected human sputa while spoligotyping identified five cattle *M. bovis* strains including four unique spoligotype patterns that had not been previously described. The study revealed that age, sex, breed and husbandry practices served as significant (P<0.05) risks to the prevalence and exposure of bovine TB in cattle. The feedback from cattle professionals suggested that there was a high possibility of cattle to cattle and cattle to human transmission of bovine TB through intimate and repeated cattle / cattle and cattle / human interactions, unawareness of control TB measures, consuming unpasteurised milk and eating raw meat. The findings have important public health implications requiring prompt and decisive actions from the Cameroonian authority towards controlling zoonotic bovine TB in both humans and animals.

Zoonotic tuberculosis at human-livestock interface in South-East Ethiopia

<u>B Gumi¹</u>, E Schelling², A Aseffa³, J Zinsstag²

¹Bule Hora University Faculty of Agriculture , PO Box 307 balako.gumi@yahoo.com, Bule Hora , Ethiopia; ²Swiss Tropical and Public Health Institute, PO Box CH-4002 Basel, Switzerland; ³Armauer Hansen Research Institute, PO Box 1005, Addis Ababa, Ethiopia

The occurrence of human tuberculosis (TB) cases and their main causative strains in remote pastoral zones of Ethiopia are hardly known. Since in these zones people live in close contact to their livestock, a proportion of human TB cases could be due to infection with cattle strains (*Mycobacterium bovis*). During 2008-2010 we have sampled suspected tuberculous patients (sputum and fine needle aspirates of swollen cervical lymph nodes) and cattle, sheep and goats at slaughter in two pastoral areas of South-East Ethiopia. Specimens were cultured and cultures differentiated with spoligotyping. Most human isolates (160/173) were *Mycobacterium tuberculosis*, but three were *M. bovis*. Twenty-four *M. bovis* strains were isolated from cattle and one *M. tuberculosis* from a camel. Given that *M. bovis* was isolated in people and one strain was identical to a strain from cattle, this study strongly suggests that tuberculosis is transmitted between livestock and humans.

Bovine tuberculosis in the border area of the Natural Park of W in Niger: Impacts and risk factors for the disease transmission in cattle and humans in rural, peri-urban and urban areas

AR Boukary^{1,2,4}, E Thys², E Abatih², L Rigouts³, C Saegerman⁴

¹Department Animal Health and Livestock promotion, ONG Karkara, Niamey, Niger BP 2045, Niamey, Niger;

²Unit of Biostatistics and Epidemiology, Department of Biomedical Sciences, Institute of Tropical Medicine (ITM), Nationalestraat 155, B-2000, Antwerp, Belgium; ³Unit of Mycobacteriology, Department of Biomedical Sciences, Institute of Tropical Medicine (ITM), Nationalestraat 155, B-2000, Antwerp, Belgium; ⁴Research Unit in Epidemiology and Risk Analysis applied to the Veterinary Sciences (UREAR-ULg), Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liege, Boulevard de Colonster, 20, B42, B-4000 Liege, Belgium; Razacboukary@yahoo.fr

Background: A retrospective study on data from the abattoir of Niamey has shown that 53.7% of carcasses condemned for TBlike lesions were from the rural district of Torodi while this region provided less than 25% of total animals slaughtered at the abattoir (Boukary *et al.*, 2011). This fact indicates that Torodi may constitute an endemic area of bovine Tuberculosis (BTB) and a source of contamination for the urban population of Niamey. Torodi is located at the edge of the W Natural Park shared by Niger, Benin and Burkina Faso. The proximity of this natural reserve and the grazing system inevitably leads to close contact between domestic animals and wildlife, which can be a source of transmission of *M. bovis* (Zieger *et al.*, 1998). A study was conducted to assess the true prevalence of BTB in Torodi, to type strains of *M. bovis* circulating in that area and to identify risk factors for disease transmission in rural, peri-urban and urban areas.

Methods and Principal Findings: A survey was carried out at the level of 51 households keeping livestock randomly selected from the district of Torodi. The Comparative Intradermal Tuberculin Test was conducted in 339 cattle and 27 animal samples with suspected TB gross pathological lesions were subjected to post mortem microbiological analysis and molecular typing of strains. Results showed that the overall BTB herd prevalence was 60%, whereas the individual true prevalence was estimated at 0.8% (CI: 95%, 0.0-5.0). *M. bovis* was identified in 8 animals showing five different spoligotypes, belonging to the 'African 1' type previously identified in Central and West Africa. In addition, a profile (SB1982) not previously reported, distinguished by the absence of spacers 3, 4, 9, 16, 22, 30 and 39–43 has been characterized in the study. The main risk factors identified are the lack of hygiene within households (74%), the presence of animals coughing in the herd (OR 4.7, 95% CI: 1.1-19.7) and the lack of the practice of quarantine (OR 4.2, 95% CI: 1.0-18.4).

Conclusion: The study confirmed that BTB is endemic in cattle in Torodi and further investigations of the disease must be conducted in wildlife. Collaboration between the veterinary and the medical sectors, in the diagnosis, monitoring and control of BTB is strongly encouraged.

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Measuring the prevalence and potential economic impact of *Mycobacterium bovis* and *Brucella abortus* in community-owned livestock bordering Hluhluwe-iMfolozi Park, South Africa

<u>C Geoghegan</u>¹, T Marcotty², M Robertson³, H van Heerden⁴, J Godfroid⁵, W Getz^{1,6}

¹Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, South Africa; ²Department of Biomedical Science, Institute of Tropical Medicine, Antwerp, Belgium; ³Department of Zoology and Entomology, University of Pretoria, South Africa; ⁴Department of Veterinary Tropical Diseases, University of Pretoria, South Africa; ⁵Department of Food Safety and Infection Biology, Norwegian School of Veterinary Science, Tromso, Norway; ⁶Department of Environmental Science, Policy and Management, University of California - Berkeley, USA; cgeoghegan@zoology.up.ac.za

Cattle are an integral part of local livelihoods for many rural communities in southern Africa; helping to support agro-pastoral activities that provide families with food resources, financial security and social status. The emergence of zoonotic pathogens like *Mycobacterium bovis* and *Brucella abortus* in rural livestock not only threaten the health and production value of livestock herds, but also compromise the health of families in direct contact with infected animals and dairy products. Furthermore, in areas with poor access to health services, the progressive morbidity and eventual mortality of infected animals threatens the economic and nutritional resilience of local households whom often have few alternative livelihood options.

This study describes the clinical results of a multi-disciplinary programme designed to detect and diagnose the emergence of two

zoonotic pathogens (*M. bovis* and *B. abortus*) in rural farming communities neighbouring Hluhluwe-iMfolozi Park in KwaZulu-Natal, South Africa. This area is of particular interest due to the remote location of subsistence farms and the historical but continuing prevalence of *M. bovis* in African buffalo (*Syncerus caffer*) and other wildlife species within the park conservation area.

Over the course of one year, 12,000 cattle were observed at 26 community diptanks located within 10km of the park boundary. Over 1,200 animals from 400 household herds were tested for *M. bovis* using the laboratory based gamma-interferon assay, with half undergoing additional field-testing using the comparative intradermal tuberculin skin test (CITT). The *B. abortus* status of the same 1,200 cattle was assessed by the local service laboratory using serology (Rose Bengal, confirmed using Complement Fixation and Serum Agglutination tests), prior to further independent analyses using an ELISA test.

Both *M. bovis* and *B. abortus* was detected in communal cattle herds around the park boundary, with spatial clustering commensurate with cattle movements driven by local communal farming practices, extreme topological features and cultural ceremonies. However, the higher prevalence of *B. abortus* than *M. bovis* in community cattle compared to the contrasting higher prevalence of *M. bovis* and absence of *B. abortus* in park buffalo populations, prompts discussion regarding the independence of infections at the interface between wildlife and livestock populations.

Finally, the potential direct and perceived economic impact of both diseases was estimated using cattle and livelihood evaluations provided by local farmers prior to the start of the project. These results demonstrate an acute concern regarding the impacts of degrading livestock health on family health, wealth and resilience. When combined with epidemiological analyses, this information highlights the need to improve the clinical surveillance of zoonoses in rural farming areas to ensure the sustainability of animal health and rural livelihoods in southern Africa.

Parasitic Zoonoses in Georgia

N Kokaia, N lashvili, M Murusidze, O Zenaishvili

S. Virsaladze Research Institute of Medical Parasitology and Tropical Medicine, Tbilisi, Georgia; irma.kokaia@mail.ru

Background: Parasitic zoonosis cause significant medical and veterinary problems in Georgia. The clinical manifestation of these diseases in humans is characterized by non-specific clinical signs, progression to chronic illness, and severe immune suppression which causes serious difficulties in timely diagnosis and treatment. In Georgia the main route of transmission of parasitic zoonotic diseases is undercooked meat of diseased animals.

Metods and results: We analysed 68 683 patients who visited the S. Virsaladze Research Institute of medical Parasitology and Tropical medicine from 2002 to 2011. Based on laboratory findings 32 699 (47.6%) patients were diagnosed with parasitic diseases. Among these patients 4 666 (14.2%) had zoonotic infections. Among patients with zoonosis 10.6% had echinococcosis, 7.9% had trichinellosis, 26.3% had brucellosis, 30.9% had visceral leishmaniasis, 8.5% had fascioliasis, 0.4% had toxoplasmosis, 0.9% had cutaneous leishmaniasis, and 14.5% had taeniasis. We analysed trends of new cases and found that from 2007 to 2012 the cumulative incidence for taeniasis was 32% and the number of new cases of trichinellosis decreased significantly by 78%. The number of new cases of brucellosis, echinococcosis and fascioliasis remained unchanged during the period 2007 to 2011. The sharp decrease of trichinellosis is associated with killing large numbers of pigs as a prophylactic measure after an outbreak of African swine fever in 2007. The increased numbers of new cases of certain zoonosis is associated with the improvement of laboratory diagnostic systems in the country. Around 45% of patients with zoonotic parasitic diseases reported having no direct contact with animals and were presumably infected through food purchased at uncontrolled stores.

Conclusion: Based on our study results parasitic zoonotic diseases present a serious health problem for Georgia. Improvement of the epidemiologic surveillance systems in the healthcare and veterinary sectors are currently in process and will decrease morbidity and mortality caused by these diseases among humans and animals in the near future.

Parallel session 1: diagnosis, surveillance and control at the interface (d)

Wildlife and livestock use of extensive farm resources in South Central Spain: implications for disease transmission

J Vicente, R Carrasco, JA Barasona, C Gortázar

University of Castilla-La Mancha Ciudad Real Spain; joaquin.vicente@uclm.es

The interactions of extensively-farmed livestock with wildlife contribute to disease spill back at the interface, impeding the total success of eradication programs. In South Central Spain farming typically occurs close to wild ungulate populations. We describe and quantify the use of extensive farm resources by livestock and wildlife, and the factors involved (farm and wildlife management, habitat). Camera traps (CT) were placed at a priori risk points (n=143 points, 2145 CT days) for interspecies interactions: water points, buildings, food points, and acorn fields in representative extensive cattle (n=6) and Iberian pig (n=5) farms. The use of extensive farm resources by wild ungulates was frequent (over 50% of daily presence of either wild boar or red deer) and positively depended on the local abundance of wild ungulates in game estates. No direct interactions were detected, and livestock and wild ungulates partitioned use temporally, so that the interaction was mainly indirect. Daily presence of wild boar was higher at water points. High red deer abundance resulted in a more even use of farm resources by deer. Cattle presence was positively associated with that of wild boar and red deer. Pigs positively associated with red deer but negatively with wild boar, probably because of competence-mediated behavioral avoidance, which may prevent contacts and disease transmission. These findings at specific risk points for contacts show that the use of extensive farm resources by wild ungulates is frequent and widespread, and that farm practices (aggregation at specific points, namely water holes and pastures), habitat, big game management, species-specific behavior and between-species interactions, determined differences in the use. This study provides understanding of wildlife/livestock pathways, which is discussed in order to implement scientifically-based husbandry to reduce contact and risks for disease transmission at the interface.

Participatory and active surveillance for disease syndromes in communal cattle at the livestock/wildlife interface, South Africa

DL Knobel, M Wassung, A Meyer, A Conan

Department of Veterinary Tropical Diseases, University of Pretoria, Onderstepoort, 0110; darryn.knobel@up.ac.za

Currently, 41% of all cattle in South Africa are run on communal grazing land, predominantly in the former homelands. Many of these areas occur at interfaces with wildlife. Productivity in the communal livestock sector is estimated to be markedly lower than in the commercial sector. One of the constraints to improved, sustainable production faced by communal farmers is the presence of infectious, nutritional, metabolic, toxic and other diseases in livestock. There is a lack of data on either the perceived burden of livestock disease among communal farmers, or on the true incidence of disease in their livestock. We used participatory epidemiology approaches to assess farmers' perceptions of important disease syndromes of cattle in a communal farming area at the livestock/wildlife interface in Mpumalanga, South Africa, and followed this with 12 months of active syndromic surveillance in a population of 4,000 cattle, to determine the farmer-reported incidence of disease syndromes.

The study area lies within the foot-and-mouth disease control zone in South Africa. Within the control zone, all cattle are registered at a particular diptank, and are brought together weekly for inspection and dipping. The population is also served by a satellite veterinary clinic of the University of Pretoria. We conducted one-on-one interviews with 129 livestock keepers and herdsmen from five diptanks, asking about disease conditions seen by the participants in their cattle. This was followed by pairwise ranking of disease syndromes by groups from each diptank of male and female livestock keepers separately. Farmer-reported syndromes were compared against clinic records for the preceding 12 months. Of the 129 respondents, 23 reported that they did not see any diseases in cattle. The remainder reported between 1 and 4 conditions (n=175). Helminths were the most frequently-mentioned condition (n=30), followed by mastitis (n=19), unassigned conditions (n=19), abscesses (n=17), lameness (n=15), and poor body condition (n=15). Helminths also attained the highest score in the analysis of the pairwise ranking exercises, again followed by mastitis. Helminths were the most frequently-recorded presumptive diagnosis for cattle seen by the clinic, accounting for 17% of visits over 12 months, followed by trauma (14%), abscesses (14%), lameness (7%) and diarrhoea (5%).

Assigning a diagnosis to a disease syndrome in the absence of diagnostic confirmation is subjective. To avoid this, we recorded the farmer-reported presence/absence of 17 clinical signs during the active surveillance. During the period 1^{st} July – 30^{th} October 2012, 48 disease episodes were reported, with a total of 94 clinical signs. The most frequently-reported clinical signs were wounds or abscesses (reported in 29% of episodes), followed by weakness (27%), loss of body condition (23%), skin conditions (23%), and lameness (21%).

In conclusion, helminths are perceived by farmers as the most important cause of disease in communal cattle. Other frequentlyreported conditions are mastitis, abscessation (often resulting from trauma caused by long-mouthed ticks), lameness and poor

body condition. These perceptions are supported to an extent by presumptive veterinary diagnoses and active surveillance for clinical signs, but further diagnostic work is needed to determine the true burden of disease.

Application of mobile technologies in resource-limited environments for national One Health Disease Surveillance Systems

T Wahl, A Burdakov, A Oukharov

Black & Veatch, Moscow, Russia; oukharov@bv.com

Background: Regions with limited finance, health workforces and infrastructure have a high risk of disease spread. Disjointed vertical monitoring programmes cannot provide a cohesive approach to surveillance on a national level nor meet requirements of information timeliness, data quality and integration of veterinary, human and wildlife data.

Objective: To facilitate improvement of national disease surveillance systems through an integrated electronic approach, which corresponds to national surveillance needs and integrated disease surveillance and response strategies while recognising specific existing environments and limitations.

Methods: The Electronic Integrated Disease Surveillance System (EIDSS) is an Open Source system developed to improve national disease surveillance. It provides a secure way to collect, share and analyse human, veterinary, vector and laboratory data in one integrated database using mobile phones, web access and desktop solutions Through customization the system is tailored to meet country-specific needs and surveillance priorities. The development is based on more than 100,000 man-hours of cutting-edge expertise from the U.S. Centers for Disease Control and Prevention and other institutions. Implementation of EIDSS normally takes 3 to 5 years. This timeframe includes piloting, evaluation of the system, adoption to health priorities, development of reports and case definitions, building the backbone of the system, operations and maintenance.

Findings: EIDSS is currently operated at more than 350 sites in Kazakhstan, Georgia, Azerbaijan, Ukraine and Armenia, as a part of the Cooperative Biological Engagement Program sponsored by the U.S. Defense Threat Reduction Agency. EIDSS was officially adopted in Azerbaijan and Georgia. Tens of thousands of cases are entered into the EIDSS systems across these countries.

Conclusions: Implementation of an integrated electronic approach to surveillance based on mobile technologies in resourcelimited environments improves timeliness and data collection quality through the use standardized formats. The approach provides the ability for One Health data analysis throughout the national systems.

Improvement of the current SAT3 liquid phase blocking ELISA to enhance the control of FMD in SADC

IV Anahory^{1,2}, FF Marre³, RM Dwarka³, P Mutowembwa³, JJ Esterhuizen³, B Botha³

¹Agricultural Research Institute, Directorate of Animal Science, Central Veterinary Laboratory, Av de Mozambique Km 1.5, PO Box 1922. Maputo, Mozambique, ²Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X 04, Onderstepoort, 0110, Pretoria, South Africa, ³Transboundary Animal Disease Programme (TADP), Onderstepoort Veterinary Institute, gricultural Research Council, Onderstepoort; ivanahory@gmail.com

Foot-and-mouth disease virus (FMDV) is the causative agent of the most serious infection in cloven-hoofed animals. The disease has a significant economic impact. The southern African Territories (SAT) types 1-3 are endemic to the region. Southern Africa has an abundant wildlife especially in National Parks and game reserves. Wildlife, particularly buffalo (*Sincerus caffer*), play an important role in virus maintenance and epidemiology of the disease. In communities within the proximity of National parks and game reserves, the wildlife-livestock interface present unique challenges towards disease eradication and control.

A Liquid phase blocking ELISA (LPBE) for detection and measurement of antibodies against FMD virus was improved. The sensitivity of the LPBE is dependent on the antigen used in the test and the ability of antibodies in the sera to cross-react with the antigen. We have prepared and standardized purified and non-purified virus as antigen as well as capture and detector antibodies for this purpose.

Despite the considerable intratipc antigenic variation that exists in FMD isolates, the LPBE that was adapted in this study proved to be antigenically more diverse, because it incorporated the appropriate strains (more than one antigen) to detect antibodies to the various antigenic subtypes of SAT3.

Five hundred and fifteen sera from a SAT-2 outbreak that occurred in Mpumalanga during 2011-2012, 286 sera from vaccinated cattle next to the Kruger National Park and 1 398 sera from a FMD survey conducted in the Northern Cape (negative sera) were

tested with the improved ELISA. A statistical analysis was performed to compare the results obtained with the new developed ELISA with the current in house ELISA.

This assay plays a key role in the control of FMD and is sensitive, specific and suitable for identification and typing of all SAT3 serotypes across the range of genetic variation within the SAT3 serotype of FMDV.

Parallel session 2: drivers of emerging and re-emerging diseases (c)

Alpha viruses as a cause of neurological disease in farm-and wild animals in southern Africa

S van Niekerk¹, S Human¹, J Williams², J Steyl², R Swanepoel¹, M Venter^{1,3}

¹Faculty of Health Sciences, University of Pretoria, South Africa; ²Faculty of Veterinary Science, University of Pretoria, South Africa; ³National Institute for Communicable Diseases, Sandringham, South Africa; Stephanie.vanniekerk@up.ac.za

Zoonotic arboviruses such as Alphaviruses (*Togaviridae*) contribute to significant medical and veterinary morbidity and mortality worldwide. Sindbis (SINV) and Middelburg (MIDV) viruses are serologically widespread in South Africa (SA); SINV causing annual cases of fever and rash in humans. MIDV was considered avirulent until isolated from a horse in Zimbabwe displaying clinical signs resembling severe African horse sickness. The aim of this study was to investigate the molecular epidemiology of SINV and MIDV to define the characteristics of the strains involved.

Veterinarians were invited to send EDTA/clotted blood, cerebrospinal fluid (CSF) and/or post mortem central nervous system (CNS) tissue from farm-and wild animals displaying unexplained neurological disease and fever to the Zoonoses Research Unit (ZRU), University of Pretoria. Specimens from 2007-2013 were screened with an alphavirus family-specific real-time RT-PCR as well as other zoonotic arboviruses for differential diagnosis. All positive cases were confirmed by sequencing and phylogenetic analysis.

Of the 549 horse specimens received from 2007 to June 2013, a total of 43 (7.8%) alphavirus positive cases were identified. Of these 43 positive cases, eight (19%) belonged to the SINV group and 35 (81%) belonged to MIDV group. Of the 136 other farmand wild animals tested, nine positive alphavirus cases were identified in the years 2010, 2011 and 2013 of which two (22%) belonged to the SINV group and seven (78%) belonged to MIDV group.

In general the SINV infections in horses were more associated with febrile disease unless it occurred with another virus such as West Nile virus. The MIDV infections were generally more severe with increased mortality.

Other farm- and wildlife animals affected include five rhinoceroses, two warthogs, one bovine and one giraffe. All nine cases resulted in severe neurological disease and death. For all except two of the rhinoceroses, alphavirus was detected with RT-PCR in the brain and spinal cord. The other two rhinoceroses had only spleen available for testing.

Alphaviruses were shown to play a role in causing neurological disease and death in horses and different animal species, which does indicate the importance of these viruses. Surveillance programmes should be established to identify any further outbreaks in animals and humans in southern Africa.

Bridge host, definition and methods: avian influenza virus transmission at the wildlife/ domestic bird interface

<u>A Caron^{1,2,3}</u>, J Cappelle^{1,4}, GS Cumming⁵, M de Garine-Wichatitsky^{1,2}, N Gaidet¹

¹UR AGIRs, Cirad, Montpellier, France; ²UR AGIRs, Cirad-RP-PCP, Harare, Zimbabwe; ³Mammal Research Institute, University of Pretoria, Pretoria, South Africa; ⁴Epidemiology and Public Health, Institut Pasteur du Cambodge, Phnom Penh, Cambodia; ⁵PercyFitz Patrick Institute, DST/NRF Centre of Excellence, University of Cape Town, Rondebosh, Cape Town 7701, South Africa; Alexandre.caron@cirad.fr

Understanding the concept of bridge species in epidemiology is intuitive: a host that transmits a pathogen from a maintenance source to a receptive population otherwise not connected. The bridge species function is particularly important to understand emerging infectious disease dynamics at wildlife/domestic/human interfaces where pathogens can spill-over from one host to the next. However, to our knowledge, the literature lacks a proper definition of the concept of bridge species and its epidemiological function. We propose that a bridge species can only be defined in the context of a reservoir-target species system as previously described in the literature. A bridge species for a pathogen in a specific system is: 1) able to get infected, replicate and excrete

the pathogen; 2) in direct contact or share habitat with the reservoir population and the target population. Achieving this demonstration necessitates a conceptual framework and specific methodologies that we review and illustrate using our field experience on the transmission of Avian Influenza virus at wild bird/poultry interfaces in Africa. Notably, we present a summary of potential bridge species sampled in Africa (n = 8 500 wild birds) either with RT-PCR or serological testing. Various methods to assess host competence to a specific pathogen and inter-species infectious contacts are presented and integrated protocols using these techniques are suggested. In Zimbabwe, a full implementation of bridge species identification method has led to identifying barn swallows (*Hirundo rustica*) and red-billed quelea (*Quelea quelea*) as bridge species in this particular ecosystem. Their identification allows the design of prioritized control and management strategies aiming at limiting the susceptibility of the target species or stopping/decreasing wild/domestic contacts when the risk of transmission from the bridge species to the target species is the highest.

Understanding the ecological drivers of avian influenza virus infection in wildfowl: a continental-scale study across Africa

N Gaidet¹, <u>A Caron</u>¹, J Cappelle¹, GS Cumming², G Balança¹, S Hammoumi1, G Cattoli³, C Abolnik⁴, R Servan de Almeida¹, P Gil¹, SR Fereidouni⁵, V Grosbois¹, A Tran¹, J Mundava⁶, B Fofana⁷, AB Ould El Mamy⁸, M Ndlovu², JY Mondain-Monval⁹, P Triplet^{10,11}, W Hagemeijer¹², WB Karesh¹³, SH Newman¹⁴, T Dodman¹²

¹CIRAD, 34398 Montpellier, France; ²Percy FitzPatrick Institute, University of Cape Town, Cape Town 7701, South Africa; ³Istituto Zooprofilattico Sper.le delle Venezie, Legnaro, Italy; ⁴RC-Onderstepoort Veterinary Institute, Onderstepoort, 0110 South Africa; ⁵Friedrich-Loeffler-Institut, Insel Riems, Germany; ⁶National University of Science and Technology, Bulawayo, Zimbabwe; ⁷Direction Nationale des Eaux et Forêts du Mali, Bamako, Mali; ⁸Centre National d'Elevage et de Recherches Vétérinaires, Nouakchott, Islamic Republic of Mauritania; ⁹Office National de la Chasse et de la Faune Sauvage, 13200 Arles, France; ¹⁰SMBS, 80100 Abbeville, France; ¹¹OMPO, 75017 Paris, France; ¹²Wetlands International, 6700 AL Wageningen, The Netherlands; ¹³EcoHealth Alliance, New York, NY 10001 USA; ¹⁴FAO, Infectious Disease Group, Rome, Italy; alexandre. caron@cirad.fr

Despite the investment of considerable effort in avian influenza viruses (AIV) detection in wild birds in recent years, empirical investigations of ecological drivers of AIV prevalence in wild birds are still scarce. Here we used a continental-scale data set to test the relative role of a range of ecological drivers on patterns of AIV prevalence in wildfowl, including species traits, migration patterns, climate and seasonal fluctuations in the abundance and composition of the wild bird community. Our analysis is of unusually broad scope in this field, including data from c. 8 500 birds sampled for AIV infection from 15 African countries during 2006-2009.

Our results validate several of the ecological drivers of AIV infection in wildfowl (host density, timing of congregation and migration), that, though often hypothesized, had not been tested empirically before. Interestingly, in marked contrast to temperate regions, our findings suggest a predominant role in Afro-tropical regions for direct transmission (*ie.* via individual contact) rather than environmental transmission (*ie.* via a persistent viral reservoir). Higher prevalences were found in *Anas* species than non-*Anas* species even when we account for differences in their foraging behaviour (primarily dabbling or not) or their geographic origin (Eurasian or Afro-tropical), suggesting the existence of intrinsic differences between wildfowl taxonomic groups in receptivity to infection. Birds were found infected as often in oropharyngeal as in cloacal samples, but rarely for both types of samples concurrently, indicating that both respiratory and digestive tracts may be important for AIV replication.

Finally our results support the hypothesis of an endemic cycle of AIV in Afro-tropical ecosystems rather than a seasonal introduction and spill over from Eurasian migratory water birds wintering in Africa. These results have important implications for understanding the general influence of animal movements and seasonal fluctuations in animal abundance on infectious disease dynamics. Our study provides an extensive, mechanism-focused contribution to the exciting and fast-growing field of eco-epidemiology.

Landscape attributes driving avian influenza virus circulation in Lake Alaotra region, Madagascar

L Guerrini¹, M Paul², L Leger³, HR Andriamanivo⁴, OF Maminiaina⁴, M Jourdan³, S Molia³, R Rakotondravao⁴, V Chevalier³

¹CIRAD/RP-PCP, CIRAD - UR AGIRs Department Environment and Societies, TREP Building, University of Zimbabwe, PO Box 1378, Harare, Zimbabwe; ²INRA, UMR 1225, ENVT, 23 chemin des Capelles, BP 87614, 31076 Toulouse Cedex 03, France; ³CIRAD, UPR AGIRs, TA C-22/E, Campus International de Baillarguet, 34398 Montpellier, France; ⁴FOFIFA-DRZV, BP 04, Antananarivo, Madagascar; laure.guerrini@cirad.fr

Landscape attributes are known to influence spatial distribution in disease risk. While the spatial pattern of H5N1 virus has been studied throughout southeast Asia, little is known of the spatial risk factors for circulation of avian influenza viruses (AIV)

in African countries. In the present paper, we combined serological data collected from poultry with landscape characterization from remote sensing images in order to investigate the environmental factors that contribute to the spatial pattern of avian influenza in Lake Alaotra, Madagascar.

Serological data from cross-sectional surveys carried out on poultry in 2008 and 2009 were examined. A Landsat 7 satellite image was analyzed through supervised classification of land cover. Two environmental variables were extracted for risk factor analysis, namely predominant landscape in a 1-km buffer around farmhouses and distance to the closest water body. The association between AIV seroprevalence and these two variables was quantified using logistic regression models. The analysis was adjusted for other farm characteristics (poultry species, flock size, and sampling date).

A total of 1,038 individual bird blood samples distributed among 241 flocks were analyzed. Three predominant landscapes were identified around the poultry farms: grassland savannah, rice paddy fields and wetlands. We observed that palmiped flocks were significantly more at risk than chickens. AIV seroprevalence also varied with flock size and sampling date. A significant negative relationship was found between AIV seroprevalence and distance to the closest body of water. Lastly, we found that flocks with predominant wetlands or rice landscapes in their neighbourhood were exposed to greater risk than those surrounded by dry savannah.

We showed that AIV circulated more intensely in the areas of Lake Alaotra which had predominant wetlands or rice landscapes. Results from the present study suggest that if the H5N1 virus was introduced in Madagascar through movements of wild birds or poultry products, the environmental conditions that prevail in Lake Alaotra region may allow the virus to spread and persist. This study provides useful information for tailoring risk-based surveillance of avian influenza in the Madagascan highlands or similar agro-ecosystems.

The risk of introduction of Rift Valley Fever virus into the southern Mediterranean area through undocumented movements of infected ruminants – assessment by Expert Knowledge Elicitation

<u>S Dhollander¹</u>, JA Stegeman², A O'Hagan³

¹European Food Safety Authority, Via Carlo Magno 1/A, IT-43126 Parma, Italy; ²University of Utrecht, Dept. Farm Animal Health, PO Box 80.163, NL-3508TD Utrecht, The Netherlands; ³Department of Probability and Statistics, University of Sheffield, Hicks Building, Sheffield S3 7RH, The United Kingdom; sofie.dhollander@efsa.europ.eu

Since Rift Valley Fever virus (RVFV) was first identified in 1931, its range has expanded from East-Africa across the sub-Saharan region to North Africa, the Arabian Peninsula and Mayotte in the Comoros Archipelago. Rift Valley Fever (RVF) is a mosquitoborne, viral disease, mainly affecting ruminants and humans. The virus can cause abortion in pregnant susceptible ruminants, high mortality rates in newborns and can have a high impact on public health and the economy in the infected regions.

To assess the risk of introduction of RVFV through undocumented movements of infected animals into designated countries of the southern Mediterranean area, also referred to in this presentation as the Region Concerned (RC), a quantitative model was developed and parameterized using expert opinion, elicited using the Sheffield methodology, available online at: http://tonyohagan.co.uk/shelf.

The major conclusions of the assessment were that in 2013 (a year in which there appears to be a strong possibility of an outbreak occurring in the RVFV-infected areas trading with the RC), it is likely that a few hundred RVFV-infected animals are moved into the RC, but this number has substantial uncertainty. The number of infected ruminants to be moved into the RC from the 'East Source' (East Africa and the Arabian Peninsula) would be higher compared to animals moved from the 'West Source' (West and Central Africa). This is mainly due to the higher number of movements of animals expected from the East Source, and the shorter duration of the journey, resulting in a higher probability of remaining infected when entering the RC. Although expert opinion is per definition subjective, and the accuracy and uncertainty of the estimates are largely influenced by a number of factors, such as the group dynamics, expert selection, and validation of the results, the EKE workshops provided an overview of the current knowledge of the parameters needed to carry out a quantitative risk assessment for introduction of RVFV into the RC.

Parallel session 2: drivers of emerging and re-emerging diseases (d)

A metapopulation mechanistic model for Rift Valley fever virus persistence in the Comoros archipelago

L Cavalerie^{1,2,3}, MVP Charron⁴, P Ezanno⁴, E Cardinale^{1,2,3}

¹CRVOI: Research and Intelligence Center on Emerging Diseases in the Indian Ocean, Reunion Island, France; ²Emerging and Exotic Animal Disease Control Joint Research Unit, UMR 15, CIRAD: Center for Agricultural Research for Development, F-34398 Montpellier, France; ³French National Institute for Agriculture (INRA), UMR 1309: Emerging and Exotic Animal Disease Control Joint Research Unit, F-34398 Montpellier, France; ⁴INRA, UMR 1300: Biology, Epidemiology and Risk Analysis for Animal Health Joint Research Unit, Oniris, F-44307 Nantes, France; lisa.cavalerie@cirad.fr

Rift Valley fever (RVF) is a zoonosis of major concern in most of Africa. After its first identification in Kenya in 1930(1), it has caused abortion storms among livestock of many countries including Tanzania, South Africa and Mauritania, as well as epidemics in Egypt (200 000 human cases in 1977) and Kenya (155 deaths in 2006). Being arthropod-borne as well as directly transmitted through infected body fluids (abortions, raw milk, etc.), its epidemiologic cycle adapts itself from country to country and through time following the evolution of environmental and socio-economic parameters. Most outbreaks have been explained by major ecological changes: dam construction in Egypt, irrigation in Madagascar and abnormally high rainfall in Kenya.

In 2007, RVF virus was isolated in Mayotte, a French overseas territory (2) in the Mozambique channel. It was found to be genetically closely linked to the 2006-2007 isolates from Kenya (3). The presence of RVF virus has been retrospectively detected in cattle as far back as 2004. While no more cases were detected in the horn of Africa, RVF virus has been circulating in the Comoros archipelago ever since, with a report of only a few human cases (the latest having been reported in October 2012).

Is the tropical environment and/or the tradition of trading in live animals responsible for the persistence of RVF in that region? To answer this question, we decided to build a mechanistic model for RVF virus dynamics. First, we adapted existing models (4) to take into account the Comoros environment at an island level. This new stochastic model includes the available biological information on transmission (transovarian transmission in mosquitoes and vector-borne and direct transmission among livestock) and mosquito dynamics to consider seasonality. We performed a sensitivity analysis on this model to test the relative influence of the biological parameters on RVF persistence, 5 years after the introduction of an infectious animal and in various vector abundant contexts. This single island model was then extended to a metapopulation structure in order to represent the live animal trade in the archipelago and assess its effect on persistence. The aim of the model is also to serve as a tool to assist with the evaluation of the efficiency of control measures.

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Modelling spatial spread of RVFV in a temperate and mountainous area of Madagascar

<u>G Nicolas</u>^{1,2}, V Chevalier¹, L Tantely³, D Fontenille⁴, B Durand²

¹CIRAD, Department of Environment and Society, UPR AGIRs, Montpellier, France; ²ANSES, French Agency for Food, Environmental and Occupational Health & Safety, Laboratory of Animal Health (EPI Unit), Maisons-Alfort, France; ³Pasteur Institute of Madagascar, Antananarivo, Madagascar; ⁴IRD, Montpellier, France; gaelle.nicolas@cirad.fr

Rift Valley fever virus (RVFV) is a mosquito-borne infection of livestock and humans that causes a potentially severe disease. In 2008-2009, a RVF outbreak occurred in a temperate and mountainous area located on the highlands of Madagascar. A three-year cattle follow-up (2009-2011) was conducted in a pilot area. A seroprevalence rate of 28% was estimated in 2009 and a seroconversion rate of 7% in 2009-2010 and of 23% in 2010-2011 (unpublished work). In 2011 the study suggested RVFV circulation during the year. In this area, cattle exchange was shown to be linked to local spread of the virus. However, mechanisms allowing the virus to circulate during four successive years in this area where vectors are absent in winter (cold and dry season) and have a relatively low population density during the warm and wet season remain unknown. Therefore, we propose a model coupling cattle exchange practices and vector-borne transmission to explain RVFV spread and persistence in this temperate and mountainous area.

The epidemiological system is represented by a metapopulation of cattle, coupled with a metapopulation of mosquitos, with both metapopulations being spatially explicit. Each cattle population corresponds to the set of animals that live in a specific village (n=48). Four health states are considered: susceptible (S), incubating (E: Exposed), infected and infectious (I), and recovered (R), with recovered animals considered immune. Each mosquito population corresponds to a rice field (n=562), the larval habitat for the potential vector species of the area (*Culex* and *Anopheles* spp.). In the absence of *Aedes* spp., vertical transmission is not considered. Mosquito successive health states are S (susceptible), E (extrinsic incubation period) and I (infected and infectious). Each cattle population may take blood meals on cattle from the populations located within a 1km radius. Similarly, each mosquito population may take blood meals on cattle from the populations located within a 1 km radius. Villages are linked by cattle exchange movements. RVFV may be introduced in this epidemiological system by cattle introduction from markets located outside the modelled area. The model is parameterized to reproduce the local conditions of the Madagascan highlands, using observational data collected in the pilot area: location of villages and of rice fields, number of cattle per village, cattle birth and death rates, cattle exchange practices and related networks, monthly abundance of mosquitos, and monthly variations of the parity rate. Non-observable parameters such as transmission parameters were calibrated using seroprevalence and seroconversion data collected between 2009 and 2011.

The model was used to analyse three potential scenarios that could explain the recurrent circulation of RVFV in the pilot area: (i) a disappearance of RVFV during winter but recurrent introductions from other regions of Madagascar, (ii) RVFV overwintering thanks to a direct transmission between cattle, vectors being absent during the winter, (iii) a low level vector-based circulation during winter thanks to a residual vector population, without direct transmission between cattle. The results of the model are discussed.

Evidence of circulation of Rift Valley fever virus in the Comoros Archipelago and Madagascar in a post-epidemic period

<u>M Maquart</u>^{1,2}, MM Olive^{1,2,3}, M Roger^{1,2}, S Girard^{1,2}, C Foray^{1,2}, S Temmam², M Rakotoharinome⁴, JM Heraud³, K Dellagi², E Cardinale^{1,2}, H Pascalis²

¹CIRAD, UMR Contrôle des Maladies, CYROI, CRVOI, 2, Rue Maxime Rivière, 97490 Sainte Clotilde, La Réunion, France; ²Centre de Recherche et de Veille sur les maladies émergentes de l'Océan Indien, 97490 Sainte Clotilde, France; ³Institut Pasteur de Madagascar, BP 1274, Antananarivo 101, Madagascar; ⁴Direction des Services Vétérinaires, Antananarivo, Madagascar; mariannemaquart@yahoo.fr

Rift Valley fever virus (RVFV) has been responsible for two human and animal outbreaks in Madagascar (1990-1991 and 2008-2009). In 2008, human Rift Valley fever (RVF) cases were noticed in two islands of the Comoros archipelago, Mayotte and Grande Comore. No cases were reported in this area subsequently, but evidence of seroconversion in the animal population has been described in Madagascar and observed in the Comoros archipelago (unpublished data) showing possible virus circulation in this post-epidemic phase. To detect the genome of the virus, we designed a novel powerful semi-nested RT-PCR detection system able to detect low RVF viral loads. A total of 2,756 samples were collected from farm animal species in Madagascar, the Republic of Comoros and Mayotte, between May 2010 and August 2011. Out of these, thirty-eight samples (1.38%) were found positive for RVFV. Interestingly, the 38 samples detected were not associated with clinical symptoms except for one goat abortion in Mayotte. Isolation of the virus was performed by intracerebral inoculation on newborn mice with 3 successive passages and its presence was confirmed by indirect Immunofluorescence assays. Sequencing of the three segments (S, M, L) of the virus is still underway but preliminary results revealed that the RVFV strains isolated in the Comoros archipelago are closely related to the clade of strains circulating during the two epidemic outbreaks in Kenya (2006-2007) and Madagascar (2008-2009). Moreover, recent published data showed that these two epidemic events were phylogenetically related. These results highlight an active circulation of the RVFV during a post-epidemic period in Madagascar and the Comoros archipelago and raise questions about the origin of these strains, as it could be reemergence of an older circulating strain or silent maintenance in domestic fauna, awaiting better environmental conditions to emerge.

Parallel session 2: drivers of emerging and re-emerging diseases (e)

My Animals, My Health, My People: Linking human, livestock and zoonotic disease awareness with local farming and food practices to improve rural public health in KwaZulu-Natal, South Africa

C Geoghegan¹, M Robertson², W Getz^{1,3}

¹Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, South Africa; ²Department of Zoology and Entomology, University of Pretoria, South Africa ³Department of Environmental Science, Policy and Management, University of California - Berkeley, USA; cgeoghegan@zoology.up.ac.za

Many households in remote communities neighbouring conservation areas in South Africa rely heavily on traditional agropastoral activities for their nutritional, financial and social security. However, in areas with poor access to basic infrastructure, transport, communication and medical or veterinary services, families are often isolated and vulnerable to emerging and zoonotic disease derived through their close association with animals. Improving the detection and reporting of health problems in rural communities is essential for local development, public health and agricultural production. But to fully understand the potential for zoonoses to emerge and spread requires better recognition of the associations between local human, animal and environmental health in relation to daily household activities, decision making and farming practices.

This study describes the outcome of a One Health programme designed to link clinical, environmental and social information to assess zoonotic disease risks and local perceptions in 26 communities within 10 km of Hluhluwe-iMfolozi Park in KwaZulu-Natal. We discuss the results of 1 300 household interviews conducted over a period of four years, describing the consumption patterns of high risk animal-derived homemade foods (including milk, traditional soured milk products, livestock and bushmeat); reported contact between people, livestock and wildlife species while performing farming, household and water-collecting activities; and the social influence over livestock movements that can enhance or reduce disease risk. Furthermore, we discuss the local awareness of potential human and animal health risks associated with food and farming activities, comparing this data to medical and veterinary problems reported during the study, and analyses of economic and geographical factors influencing access to, and use of government, private and traditional health services in the area.

Finally, we argue that understanding local household and farming behaviours, as well as disease awareness and perceptions of risk can fundamentally enhance the focus, local relevance and subsequent compliance of health interventions in rural communities; ideally being designed within a One Health philosophy, to address health issues holistically across disciplines for maximum economic, social and health benefits.

Toilet practices among pig owners in Eastern Zambia: A socio-anthropological study

S Thys¹, EK Mwape², S Gabriël³, P Lefèvre¹, P Dorny³, A Phiri², IK Phiri², and T Marcotty³

¹Epidemiology and tropical diseases control Unit, Department of Public Health, Institute of Tropical Medicine; ²School of Veterinary Medicine, University of Zambia; ³Unit of Veterinary Helminthology, Department of Biomedical Sciences, Institute of Tropical Medicine; sthys@itg.be

Background: *Taenia solium* cysticercosis is a neglected parasitic zoonosis in many developing countries including Zambia. Sociocultural determinants related to its control remain unclear. Studies in Africa have shown that the underuse of sanitary facilities and the widespread occurrence of free roaming pigs were the major risk factors for porcine cysticercosis. Latrine use could have positive sanitary implications.

Objective: Assess the communities' practices, knowledge and perceptions regarding latrines in a *T. solium* endemic rural area in Eastern Zambia inhabited by the Nsenga tribe and identify possible barriers to their construction and use.

Methods: Twenty-one focus group discussions on pig husbandry practices and latrine use, with men, women and children were organised separately in 7 villages of Petauke district.

Results: Latrines are not constructed in each household because of the convenient use of existing latrines in the neighborhood and because men do not see them as a priority. Latrines were perceived to contribute to good hygiene mainly because they prevent pigs from eating human faeces. Men expressed reluctance to abandon open-air defecation practice mainly because of toilet-associated taboos with in-laws and grown-up children of the opposite sex. Latrine use does generally increase when people are freed from such taboos, encounter diarrhea-related illnesses or when visitors are around.

Conclusions: Socio-cultural barriers and perceptions related to privacy, dignity and comfort hinder latrine use and, consequently, construction. Because the Nsenga observe traditionally matrilineal descent, a newly married couple has to live in the wife's

relative's household and the custom implies that when a man gets married, he has to build his own toilet for his family because of the taboo's involving parents and sons-in-law sharing the same toilet. Since only men are responsible to build latrines and mostly men prefer open defecation, sanitation programmes should focus on men and address related sanitary taboos to be effective.

Emerging zoonotic arboviruses causing neurological disease in animals in South Africa

<u>M Venter</u>¹, J Williams², J Steyl², S van Niekerk¹, C van Eeden¹, S Human¹, R Swanepoel¹

¹Medical Virology, Zoonosis research unit, University of Pretoria, South Africa; ²Department of Paraclinical Sciences, Section of Pathology, Faculty of Veterinary Science, University of Pretoria; marietjiev@nicd.ac.za

Background: Zoonotic arboviruses in the families *Flaviviridae, Togaviridae* and *Bunyaviridae* may be the cause of severe neurological disease in humans and animals, but despite many being endemic are rarely reported from Africa. Recent identification of West Nile virus, Wesselsbronvirus and Shunivirus in several fatal cases of neurological disease in horses in South Africa, prompted us to investigate neurological cases submitted over five years as part of a sentinel surveillance programme in horses and several other species to detect potential zoonotic arboviruses in Africa. The objective was to detect emerging or previously missed zoonotic viruses associated with neurological disease in animals and define their epidemiology and host range in Africa.

Blood, brain and spinal cord specimens were submitted from 538 horses, as well as post mortem specimens of several wildlife species and livestock with nervous signs before death. Specimens were screened by family RT-PCRs for Flavi- and Alphaviruses as well as specific PCRs for WNV, Wesselsbronvirus, Shunivirus, equine encephalosis virus, Sindbisvirus, Middelburg virus, equine herpes virus and African horse sickness virus (AHSV). Rabies virus was excluded by the Onderstepoort Veterinary Institute. Electron microscopy and random amplifiacation and sequencing were used to classify unknown isolates. Histopathological investigations were carried out on fatal cases.

During the 5 years under investigation, WNV occurred in 7-21% of horses (fatality rate of 42%), in an outbreak in cattle and a fatal case in a giraffe. Wesselsbronvirus was detected in two horses, one of which was fatal. Shunivirus was identified in 0-10% of horses from year to year, 53% of which were fatal, while Middelburg virus was identified in 2-16% of cases with 39% being fatal. Sindbisvirus and equine encephalosis virus as well as AHSV were also detected in a few neurological cases in horses without co-infections. Shuni and Middelburg viruses were also detected in the brains of several rhinoceros, warthogs, buffalo and crocodiles. Sindbisvirus was detected in the brains of a rhino and giraffe while and equine encephalosis virus was also identified in the brain of 1 rhinoceros. Horse cases were detected across the country while wildlife cases occurred in reserves in the Limpopo, Northern Kwazulu Natal, North-West and Gauteng Provinces.

West Nile virus, Shunivirus, Middelburg virus, Wesselsbronvirus and Sindbis virus are potential zoonotic arboviruses associated with neurological disease in several animal species in Africa and should be investigated in human disease, geographic range, epidemiology, pathogenesis and ecology.

Genomics of South African West Nile viruses

<u>C Kortenhoeven¹, ADS Bastos¹, C Abolnik²</u>

¹Department of Zoology and Entomology, Faculty of Natural and Agricultural Science, University of Pretoria, Hatfield, 0028, South Africa; ²Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa; cornellkortenhoeven@gmail.com

Since its introduction into the Western Hemisphere during the 1990's, West Nile virus (WNV) has been a recognised cause for concern worldwide. WNV, a member of the genus *Flavivirus*, is the aetiological agent of West Nile fever; and in more severe cases WNV neuroinvasive disease (1). The WNV positive-sense single-stranded RNA genome is ~11 Kb in length and encodes a single open reading frame (ORF) that is post-translationally cleaved into three structural proteins and seven non-structural proteins (2). WNV is a zoonotic arthropod-borne virus transmitted primarily by *Culex sp.* mosquitoes in enzootic/epizootic cycles where migratory birds act as the reservoir host (2). Even so, the incidental infection of vertebrate hosts such as humans and equines frequently occur (3).

The global increase in frequency and severity of WNV infection is consistent with the epidemiological pattern of an emerging pathogen. Genetic change in a pathogen is, amongst many factors, considered a fundamental driver of disease emergence (4). The interactions between population variants, termed quasispecies dynamics, dictate the influence of genetic change on the fitness of a consensus- or master genotype of a virus (5). Large population sizes coupled with high mutation rates during viral replication result in increased genetic diversity within a quasispecies (6).

The evolutionary change that shapes emergence is seldom observed in the consensus genotype of an arbovirus such as WNV (6).

Population variants are subject to the adaptive constraints associated with host cycling, resulting in a reduced rate of consensus level evolution (7). However, minority variants that contribute to quasispecies dynamics without reflecting in the consensus genotype of a virus are more than often overlooked (6).

The advent of ultra-deep sequencing technologies enables the reconstruction of quasispecies structure and dynamics from whole genome sequencing data (6). In this study, the quasispecies dynamics of four previously uncharacterised South African WNV isolates were determined. Each isolate was subject to changes in propagation system, alternating between passage in baby hamster kidney (BHK-21) cells and suckling mice. Viruses were genetically characterised on a genome-wide scale and quasispecies were reconstructed from short-read Illumina® sequence data. It was found that the quasispecies structure of viruses exhibiting identical consensus genotypes differed significantly. The changes in frequency of population variants indicated a correlation with the changes in propagation system. A large number of minority variants were present consistently throughout the reconstructed quasispecies of each virus without reflecting in the consensus genome. To our knowledge, this study represents the first successful quasispecies reconstruction of lineage 2 West Nile Virus isolates from ultra-deep sequencing data.

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Ndumu virus in domestic pigs: a livestock revolution time bomb for public health

<u>C Masembe</u>¹, G Michuki², M Onyango², C Rumberia², M Norling^{2,4}, RP Bishop², A Djikeng³, SJ Kemp², A Orth², RA Skilton³, K Ståhl^{1,4} and A Fischer^{2,5}

¹Makerere University, Department of Biological Sciences, Kampala, Uganda; ²International Livestock Research Institute (ILRI), Nairobi, Kenya; ³Biosciences Eastern and Central Africa (BecA)–ILRI Hub Nairobi, Kenya; ⁴Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden; ⁵International Centre of Insect Physiology and Ecology, icipe, Nairobi, Kenya; cmasembe@zoology.mak.ac.ug

The rising demand for livestock products in Africa has resulted in an increased use of intensive pig production systems across the continent. Pigs are frequently preferred to other livestock species due to their relatively rapid growth rate and large litter sizes. In Uganda, the pig production industry is rapidly developing, as it has the potential to provide financial returns over a relatively short time (walking banks). However, intensification of pig production leads to increased contact between humans and domestic animals with possible public health consequences. Zoonoses such as Cysticercosis, swine Influenza virus, Nipah virus, Menangle virus, porcine hepatitis E, *Staphylococcus aureus* and *Streptococcus suis* have previously been reported in pigs and there is clear potential for domestic swine to act as a reservoir for many emerging and re-emerging infectious diseases. A complete picture of other potential zoonoses carried by domestic pigs that could potentially cross over to humans due to close confinement of pigs and humans, especially in smallholder pig farmers in Africa, is still lacking. Early identification of the pathogen spectrum and diagnosis of potential zoonoses in domestic animals in close contact with humans is therefore important for public health surveillance and development of early warning systems.

During a routine general surveillance for African swine fever, domestic pigs from Uganda were screened for the presence of RNA and DNA viruses using a high-throughput pyrosequencing approach. Serum samples from 16 domestic pigs were collected from five regions in Uganda and pooled accordingly. Genomic DNA and RNA were extracted and sequenced on the 454 GS-FLX platform. Among the sequences assigned to a taxon, 53% mapped to the domestic pig (*Sus scrofa*). African swine fever virus, Torque teno viruses (TTVs), and porcine endogenous retroviruses were identified as well. Interestingly, two pools (B and C) of RNA origin had sequences that showed 98% sequence identity to Ndumu virus (NDUV). There was no indication of contamination with insect DNA since none of the reads had identity to the class Insecta. This is the first report of the domestic pig as a vertebrate host for Ndumu virus. NDUV had been previously isolated only from culicine mosquitoes. NDUV therefore represents a potential zoonotic pathogen, particularly given the increasing risk of human-livestock-mosquito contact.

Parallel session 3 food safety and food security (a)

Reconciling international trade imperatives with novel wildlife conservation approaches

P Bastiaensen, B Chardonnet¹, D Bourzat

World Organisation for Animal Health (OIE) 12, rue de Prony, 75017 Paris, France ; ¹African Protected Areas & Wildlife; p.bastiaensen@oie.int

The authors describe the various processes that have enabled the *World Organisation for Animal Health* (OIE) to reconcile its primary mandate of safeguarding animal health worldwide and its WTO mandate of regulating international trade of animals and animal products, with ever-increasing challenges resulting from novel approaches to wildlife conservation that are incompatible with many traditional trade standards and certification processes. In order not to encourage unjustified trade barriers linked to the management of wildlife populations, whilst always ensuring that marketed animals are healthy, and animal products are safe to consume, new standards and approaches have been developed to meet the specificities of regions such as eastern and southern Africa. These have not only successfully protected animal biodiversity at large, but turned their resources into an economic sector of tremendous importance (through tourism, game farming and derivatives).

The innovations in terms of conservation are mostly related to the creation of trans-frontier conservation areas, in which not only national parks are merged (and game fences abolished), but also communal areas in which human settlements exist and crop and livestock production is taking place, and to the rapidly expanding devolution of the natural resources user rights (including wildlife) to local communities in line with current approaches to decentralization and empowerment of local governance systems.

The innovations in terms of trade-facilitation are related to geographic concepts such as zoning and containment zoning in case of outbreaks, and non-geographic concepts such as compartmentalisation, differentiation between infection status in domestic animal species and wildlife species, and the concept of inherently safe commodities, irrespective of the disease status of country or zone.

The recent adoption in Botswana of the Phakalane Declaration (November 2012) is generally considered a milestone in creating a broader mutual understanding between two traditionally antagonistic technical and scientific communities. The declaration provides a list of common principles to which veterinary services and wildlife services in the 15 SADC Member States have agreed.

The authors also briefly refer to ongoing programmes to improve university curricula to address new market-driven and conservation-driven requirements through the proposed day-one-competencies of graduate veterinarians; and the various networks that have been established in Africa to foster better communication between veterinary educational establishments and with veterinary statutory bodies or veterinary councils.

Thela entweni, ungakhi phakathi! Linking pathogens and people with farming and food practices to determine public health risks and design novel intervention strategies in remote farming communities of South Africa

C Geoghegan^{1,2}, S Mdletshe², M Robertson³, S Lu⁴, W Getz ^{1,5}

¹Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, South Africa; ²Pathogens, Parks and People, One Health Project, Hluhluwe-iMfolozi Park; ³Department of Zoology and Entomology, University of Pretoria, South Africa; ⁴School of Public Health, University of California – Berkeley, USA; ⁵Department of Environmental Science, Policy and Management, University of California - Berkeley, USA; cgeoghegan@zoology.up.ac.za

In many rural farming villages, the use of homemade animal-derived foods contributes significantly to the diet of poor households, especially those with few alternative sources of income and food. The consumption of milk and traditional dairy products provides access to an often scarce source of micronutrients that are essential for family health, child growth and cognitive development; and are often recommended to support the nutrition of patients on chronic medication for conditions including TB and HIV/ AIDS. However, many of these communities have poor access to basic utilities, including electricity and piped water, and are often outside the reach of under-resourced veterinary and medical health services. Consequently, the cumulative presence of zoonotic, environmental and food-derived pathogens in remote areas threatens to replace the dietary benefits of animal-derived foods with increased risks of disease for both human and animal populations.

This study describes the clinical and social results derived from a 10-month programme designed to assess the pathogen contamination of milk, traditional dairy and water sources in 65 rural farming households neighbouring conservation areas in KwaZulu-Natal. We discuss clinical results in relation to observed household, food and farming practices that introduce pathogens into the food system during animal production, milking practices and via poor household hygiene. We describe the potential

influence of educational backgrounds on local household awareness of disease, and assess the role of economic stability on household ability to practice improved hygiene standards given local infrastructure restrictions. Finally, we discuss the outcome of a One Health inspired public health programme designed to provide locally-relevant public health interventions to over 1,000 farmers and community members enrolled in the project. Using video and oral productions delivered at community venues and individual households, we illustrate the need to understand local livelihood and living conditions, and incorporate traditional knowledge into public health programmes for greater local understanding and compliance. In turn, this provides authorities with a unique way to build dialogue with remote communities while tackling public and animal health in unison for mutual community and service benefit.

Parallel session 4: animal health and production (a)

Transovarial passage and transmission of lumpy skin disease virus by *Amblyomma hebraeum*, *Rhipicephalus appendiculatus* and *Rhipicephalus decoloratus* ticks

EH Venter¹, JC Lubinga¹, JAW Coetzer¹, WH Stoltsz¹, ESM Tuppurainen²

¹Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, South Africa; ²The Pirbright Institute, Pirbright, Surrey, UK; estelle.venter@up.ac.za

Lumpy skin disease is an economically important disease of cattle occurring in Africa and parts of the Middle-East. It is an acute, sub-acute or inapparent disease and is caused by the lumpy skin disease virus (LSDV), a member of the genus *Capripoxvirus* in the family *Poxviridae*. The disease is characterised by high fever, enlarged lymph nodes and the formation of multiple firm, circumscribed nodules in the skin of affected animals, and necrotic plaques in the mucous membranes, mainly of the upper respiratory tract and oral cavity, as well as generalized lymphadenopathy. It is an economically important disease due to the permanent damage to hides, the reduction in productivity and trade restrictions imposed on affected areas.

It is currently believed that the most important mode of transmission of LSDV is likely to be through mechanical transmission of the virus by blood-feeding vectors. Mechanical transmission, mainly by biting flies such as *Stomoxys calcitrans, Aedes* and *Culex* spp. of mosquitoes has been demonstrated. Recent studies have shown that ticks can play a role in the transmission of the virus. Mechanical (intrastadial) and transstadial transmission by *Amblyomma hebraeum* and *Rhipicephalus appendiculatus* have been reported^{1,2}.

In this study the passage of LSDV from infected female ticks through the eggs to the next generation of larvae has been demonstrated in *A. hebraeum*, *R. appendiculatus* and *R. decoloratus*. Transmission to recipient animals by *A. hebraeum* and *R. appendiculatus* larvae was also shown. The finding of transovarial passage of LSDV in female ticks shows the potential for *A. hebraeum*, *R. appendiculatus* and *R. decoloratus* to be reservoir hosts for LSDV.

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Altitudinal and spatial distribution of *Rhipicephalus (Boophilus) microplus* (Canestrini, 1888), *Amblyomma* spp. and *Ixodes boliviensis* Neumann, 1904 (Acari: Ixodidae) in Ecuador

S Enríquez¹, <u>R Rodríguez-Hidalgo²⁻³</u>, F Vaca-Moyano¹, L Ron¹, J Ron¹, M Coral¹, E Villacrés¹, P Lahuatte¹, V Guasumba¹, W Benítez-Ortiz¹⁻³

¹Centro Internacional de Zoonosis, Universidad Central del Ecuador, Av. América s/n. Quito-Ecuador.; ²Nucleo de Investigadores, Universidad Central del Ecuador, Av. América s/n. Quito-Ecuador; rrodriguez@uce.edu.ec; ³Facultad de Medicina Veterinaria y Zootecnia, Universidad Central del Ecuador, Av. América s/n. Quito-Ecuador; rrodriguez@uce.edu.ec

Ticks are blood-sucking ectoparasites that utilise all land vertebrates. In Ecuador, ticks affecting cattle are widely distributed throughout the country, especially in the tropics. They cause damage by feeding on the host, resulting in tick-borne diseases, which are exacerbated where inappropriately used chemicals leads to high levels of resistance. Consequently, control is difficult in highly endemic areas, thereby leading to economic losses that have not been quantified

The lack of information about tick species, their distribution and the degree of drug resistance, has prompted the International Centre for Zoonosis at Central University of Ecuador to study the spatial and altitudinal distribution of ticks in the entire country. Farms were randomly selected based on the Ecuadorian Agricultural Census of 2000. Three animals parasitized by ticks were sampled from each farm. The ticks were collected directly from the animals, identified using dichotomous keys, and then stored in absolute ethanol for further molecular studies. Farm coordinates were allocated using a Garmin GPSMap 60sc and subsequently analyzed with the use of Manifold v.8.0 software.

Preliminary results have been obtained from 1618 animals on 623 farms in 12 of the 24 provinces of Ecuador. To date, four species of ticks have been identified: (1) *Rhipicephalus (Boophilus) microplus* (Canestrini, 1 888), identified from approximately 70% of farms and over 2 600 metres above sea level (masl); (2) *Amblyomma cajennense* (Fabricius, 1 787), reported from approximately 35% of farms and at altitudes between 0 to 2 457 masl; (3) *Amblyomma maculatum* (Koch, 1 844), identified in two provinces of the coast region in very arid conditions, at altitudes below 100 masl and (4) *Ixodes aff. boliviensis* (Neumann, 1 844), discovered at 2 745 masl in 1 animal.

Importantly, the provinces of Chimborazo and Pastaza were those that showed fewer cases of animals affected by ticks. Livestock production in the former is informal and the terrain is mountainous, while Pastaza province has a traditional system of animal husbandry, using "Gramalote" (*Axonopus scoparius*), an annual grass that hinders tick development. According to the questionnaire, the animal infested with *R. (B.) microplus* and found in this province was introduced from the coast region where this parasite is endemic.

Our survey demonstrated a wide spatial and altitudinal distribution of ticks in Ecuador. The most widely dispersed species were *R. (B.) microplus* and *A. cajennense*. These findings indicate that tick infestations probably exert significant deleterious effects on livestock production in Ecuador. Interestingly, the presence of *R. (B.) microplus* and *A. cajennense* at altitudes above 2 400 m was demonstrated for the first time. This finding warrants further study and consideration in future monitoring programs. Finally, this research will assess the burden of ticks on Ecuadorian livestock production and public health.

Husbandry, health and welfare in cattle breeding feedlots in Indonesia – a unique production system

M Laurence¹, R Ainsworth², TF Jubb³

¹College of Veterinary medicine, Murdoch University, Perth Western Australia; ²Australian Livestock Services Pty Limited, NT, Australia; ³Livestock Health Systems, Bendigo, Victoria, Australia; m.laurence@murdoch.edu.au

Australian cattle have been shipped live to overseas destinations since 1893, mainly from Darwin, to the Dutch East Indies (Indonesia) and Singapore (5). By 2010, approximately 900 000 cattle *per annum* were being exported, with approximately 77% destined for Indonesia (2). These mainly *Bos indicus* Brahman cattle were fattened in Indonesian feedlots, slaughtered at local abattoirs and the meat sold in wet markets. Australian beef constituted the majority of the beef consumed by Indonesians. On 30 May 2011 the Australian Broadcasting Corporation aired *A Bloody Business* that documented incidents of extreme animal cruelty in Indonesian abattoirs (1). The ensuing public outrage led to an immediate cessation of live export of cattle to Indonesia. The trade resumed some months later, accompanied by stricter regulation of the supply chain, which had political ramifications and reduced the number of cattle exported to 376 148 in 2011/12 (4).

The Indonesian government now insists on a maximum import weight of 350kg, restricts the number of import permits issued to feedlot owners and in a move towards self-sufficiency of beef supply, stipulates that in order for an import permit to be granted, a feedlot owner must have a breeding herd as part of their enterprise. These breeding herds that are managed in intensive conditions are known as *breedlots*. There are approximately 20 intensive breeding sites situated mainly in East Java and Southern Sumatra. The number of breeders ranges from 300-9000. The inexperience of the feedlot operators combined with the uniqueness of the production system has led to serious health and welfare problems, the most severe of which is a calf mortality rate of over 50% at certain times of year (3). The main diseases of calves include enzootic calf pneumonia, neonatal calf diarrhoea and joint ill. The main causes are failure of passive transfer of immunity, overcrowded calf rearing sheds, inadequate shelter for calves, wet and muddy bedding, poor standards of hygiene in creep areas, inappropriate use of medications, lack of effective vaccines and an inability of feedlot managers to recognise early signs of disease and instigate correct treatment protocols.

Australian consultants are developing resources to aid in the mitigation of these problems. These include DVDs and manuals that provide practical, cost effective and clear instructions on how to address the high mortality rates and improve husbandry. Training of feedlot staff will accompany these resources and will include topics such as appropriate management of the breeding environment, colostrum management, measurement of passive transfer and disease identification and treatment. Animal welfare in intensive breeding enterprises remains a concern and the success of the proposed solutions will be measured over the next couple of years.

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Day 3: Wednesday 28/08/2013

Parallel session 3: Food safety and food security (b)

Measuring the effects of time and temperature on the longevity of Mycobacterium bovis in unpasteurised, souring milk from Kwazulu-Natal, South Africa

Claire Geoghegan¹, Tiny Hlokwe², Keneilwe Raseleka², Tanguy Marcotty³, Wayne Getz^{1,4}, Anita Michel^{2,5}

¹Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, South Africa; ²ARC Onderstepoort Veterinary Institute, South Africa; ³Department of Biomedical Science, Institute of Tropical Medicine, Antwerp, Belgium; ⁴Department of Environmental Science, Policy and Management, University of California - Berkeley, USA; ⁵Department of Veterinary Tropical Diseases, University of Pretoria, South Africa; cgeoghegan@zoology.up.ac.za

Unpasteurised fresh and souring dairy products form an essential component of household diets in many rural communities of southern Africa. The presence of zoonotic pathogens like *Mycobacterium bovis* (the causative agent of bovine tuberculosis, BTB), which may be transmitted from infected cattle via milk consumption, constitutes a public health threat, especially in remote areas with poor disease surveillance and highly compromised human health due to HIV/AIDS and human TB.

Using unpasteurised fresh milk mixed with 10% soured milk obtained from rural farming communities with historical BTB risk in KwaZulu-Natal, South Africa, this study evaluates the longevity of *M. bovis* at three concentrations (10², 10⁴, 10⁷ cfu/ml). Souring milk spiked with *M. bovis* was incubated at temperatures mirroring ambient indoor and outdoor milk storage conditions (20 or 33°C, respectively) and sampled for *M. bovis* culture at day 1, 3, 5, 8, 15, 22, 29, 36 and 43. At each sampling an aliquot of milk was decontaminated and cultured for *M. bovis* on six different Lowenstein-Jensen media slopes supplemented with pyruvate (total number of slopes: 342). Slopes were monitored for 10 weeks. Bacterial growth was assessed by microscopy using the Ziehl–Nielsen (ZN) staining method. Mycobacterium isolates were confirmed by RD-4 specific PCR.

M. bovis was detected by PCR analyses at the highest concentration (10^7) for 15 days at 20°C, but was absent from all concentrations at 33°C by three days after inoculation. Logistic regression models were used to assess the effect of ambient storage temperature on *M. bovis* longevity, while the effects of bacterial concentration and time since inoculation were used to determine the half-life of *M. bovis* in raw souring milk. At 20°C, approximately 20% of mycobacteria were alive after one day of storage (95% CI: 7-61%), giving an estimated half-life of *M. bovis* in raw souring milk of 10.5 hours (95% C: 6-33 hours).

This study demonstrates that storing souring milk at ambient temperature significantly reduces the viability of *M. bovis* and that higher ambient temperatures have a detrimental effect on the survival of *M. bovis* in souring milk. However, *M. bovis* survives in fresh souring aMasi for periods of time great enough to represent a risk of exposure to people consuming traditional soured dairy products. When combined with information of the frequency of dairy consumption in rural households, these results demonstrate a need to reduce potential zoonotic transmission of *M. bovis*. They also improve public health programmes by including locally-relevant and achievable recommendations to minimise zoonotic disease risks in rural farming communities.

The use of polymerase chain reaction (PCR) to confirm presence of selected pathogenic bacteria along the milk value chain in the Tanga region

<u>F Shija</u>¹, G Misinzo¹, H Nonga¹, LR Kurwijila¹, K Rosel², D Grace³

¹Sokoine University of Agriculture, Faculty of Veterinary Medicine, P O Box 3015 Morogoro; ²International Livestock Research Institute, c/o Biodiversity International, Plot 106, Katalima Road, P O Box 24384 Kampala, Uganda; ³International Livestock Research Institute, Box 30709, Nairobi, Kenya; taz3435@gmail.com

Background: Despite the fact that food of animal source are a minor constituent in most diets, they are responsible for incidents of food-borne illness; dairy products being implicated (De Buyser et al. 2001). Up to 90% of all dairy related diseases are due to

pathogenic bacteria found in milk (Ryser 1998). Emergence of new milk-borne bacterial pathogens with more serious challenges such as *E. coli* 0157:H7 has been reported (Sivapalasingams 2004).

Objectives: The main objective was to determine milk-borne zoonotic risks associated with milk handling and storage along the dairy value chain.

Specific objectives included:

- 1. To assess possible sources of microbial contamination of milk from farm to consumer
- 2. To identify practices that lead to microbial contamination along the milk value chain
- 3. To enumerate total number of bacteria and coliforms present in milk
- 4. To assess presence of E. coli and B. abortus along the milk value chain

Materials and methods: Milk samples and questionnaires from all the role players along the dairy value chains of Handeni and Lushoto districts in Tanga region were collected. The questionnaires were analyzed using Stata/IC 11 while standard microbiological cultures and polymerase chain reaction were used to identify and confirm microbial contamination in milk. Results: A total of 184 (118 farmers, 4 collection centres, 16 restaurants, 35 vendors, 11 consumers) milk samples were collected at a total of 81 (65 farmers, 11 restaurants, 15 vendors) sites. A checklist of questions for four collection centres was also obtained.

From the sociological data, different practices that could lead to microbial contamination of milk during milking, storage and delivery were identified. Preliminary analysis showed that only 43% of farmers cleaned the animal shed before milking, only 71% washed hands before milking, and only 71% of farmers washed the cows' teats before milking. Detailed results of the study including laboratory results and sociological results are presented.

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The importance of the zoonotic bacterium *Brucella abortus* in the milk chain in Gulu and Soroti regions of Uganda

KT Rock¹, DR Mugizi², K Ståhl³, U Magnusson⁴, S Boqvist¹

¹Dept of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences (SLU), SE- 750 07 Uppsala, Sweden; ²Dept of Veterinary Surgery and Reproduction, School of Veterinary Medicine, Makerere University, PO Box 7062, Kampala, Uganda; ³Dept. of Disease Control and Epidemiology, the National Veterinary Institute of Sweden (SVA), 751 89 Uppsala; ⁴Dept. of Clinical Sciences, SLU, SE- 750 07 Uppsala, Sweden; Kim.Rock@slu.se

Introduction: Livestock is essential to improving food security although diseases such as bovine brucellosis threaten efficient and safe food production. Increased post-conflict urbanization in Uganda has resulted in an increase in the number of livestock introduced to urban and peri-urban areas. The zoonotic bacterium *Brucella abortus* is of special concern in Uganda as people may be infected through consumption of unpasteurized milk and milk products and through contact with infected animals. This study aims to improve food safety and security in the peri-urban regions of Gulu and Soroti, Uganda by describing the milk value chain and the role of milk in infection.

Methods: Milk samples (N=330) were collected from milk deliverers at milk collection centres in Gulu, boiling points in Soroti, and from informal milk sellers in both regions. Milk sample prevalence of antibodies to *B. abortus* was determined by Elisa (I-Elisa, SVAnova) at Makerere University, Kampala, Uganda. Questionnaires (N=273) were administered to illicit information on risk factors and milk deliverer knowledge and practices concerning *B. abortus* in milk. Information was also collected in order to produce a map of the milk value chain at the level of collection centres and informal milk sellers.

Results: In Gulu, 11% of 142 bulk milk sample were sero-positive for *B. abortus* antibodies; in Soroti 35% of 188 milk samples were sero-positive. The regions differed in how cattle were housed, Gulu intensive and Soroti grazed (P<0.001). The Gulu region showed a higher number of female milk deliverers (P<0.001) than Soroti. Soroti region had more deliverers who answered that they drink fresh milk directly (P<0.001). It is known that at least one non-governmental organization (NGO) has focused on women smallholders of dairy cattle in Gulu and their influence is thought to be shown in our results in gender of milk deliverers. The fact that more Soroti deliverers report drinking fresh milk is an indication of less public health communication and less NGO presence.

Future plans: Results and suggestions will be shared with regional stakeholders in cooperation with local District Veterinary Officer (DVO) offices and Makerere University. Excess milk samples have been stored for future use. In this way, this project can provide future benefit to the communities from which samples were collected.

Fit for Human Consumption? A qualitative survey at a Ugandan pig abattoir

K Roesel

International Livestock Research Institute; k.roesel@cgiar.org

Since 1986, the numbers of pigs in Uganda have at least quadrupled and today's more than two million pigs are in the hands of 1.1 million households. Up to 70% of all pork is estimated to be consumed in urban and periurban areas mainly through informal road-side butcheries and 'pork joints'. In August 2012, a descriptive study was conducted at a large pig slaughter house in Kampala using participatory epidemiology methods. Focus group discussions with members of the slaughter house management and abattoir workers, as well as in-depth interviews with meat inspectors, were used to assess various parameters. These included the annual supply and influencing factors, description of the slaughter process, product flow and by-product management, identification of stakeholders' knowledge, attitudes and practices on meat hygiene, and potential risk factors that could compromise the health of abattoir workers as well as end consumers.

The supply of live pigs to the slaughter house has increased over the past 10 years, and numbers rise rapidly when schools fees are due, prior to festivals or in the event of a disease outbreak. Most of the pigs are supplied from the Central region followed by the Eastern, Northern and Western regions through a well-established network of agents and traders buying from smallholder farmers. Preliminary results showed several factors that could increase the risk of occupational or foodborne diseases, such as crowding of animals, insufficient separation between clean and dirty areas, and the lack of electricity. The movement over long distances to a central slaughter house could have implications for public health in a country with abundant wildlife and with frequent occurrence of haemorrhagic fevers such as Ebola which can also be carried by pigs. Symptoms frequently observed in live pigs were diarrhoea, nasal discharge or skin problems including signs compatible with African swine fever. Lesions that were commonly observed in slaughtered pigs included cysts and white spots in the liver, lung congestion and ectoparasites such as fleas and mites, but the policy on condemnation of carcasses was ambiguous.

The perception about who is responsible for meat hygiene is biased among members of management and staff. Further and ongoing research include the analysis of serum, tissue and faecal samples as well as an assessment of preferences and attitudes on the consumers' end of the pig value chain in Uganda. This will eventually feed into a comprehensive risk assessment and hence identify critical control points for interventions such as training of slaughterhouse personnel or a management system that could guarantee an independent and improved meat inspection process.

Longitudinal investigation of *Salmonella* spp. from farm to fork in the pig industry in Reunion Island

<u>C Tessier^{1,2}</u>, L Atiana¹, E Cardinale¹, M Denis³

¹CIRAD, BIOS, UMR CMAEE - CRVOI, Saint Denis, Réunion; ²Coopérative des Producteurs de Porcs de la Réunion, Saint Pierre, Réunion; ³ANSES, HQPAP, Ploufragan, France; ctessier@cppr.fr

Salmonella is, after *Campylobacter*, the most reported zoonotic pathogen in Europe. Pork is frequently incriminated in salmonellosis. However, asymptomatic carrying of *Salmonella* by pigs makes its detection difficult. Despite its tropical situation, the pig industry of Reunion Island is also concerned about this public health burden.

Our epidemiological study aims at identifying the pathways of *Salmonella* contamination along the pork chain of the swine industry of Reunion Island.

Three *Salmonella* positive farms were monitored from farrowing to the pork cuts. Three sows and five piglets by sow were selected from one batch per farm. At farm level, *Salmonella* excretion was assessed from individual samples of faeces, after farrowing and throughout each growing stage. At slaughterhouse level, gauze swabs from pigs entering, from carcasses after polishing, splitting and chilling, caecal contents and pork cuts were sampled. Environmental samples were also collected at both farm and slaughterhouse levels; they were collected at each visit in farms and all along the slaughtering and cutting process at the beginning of the day, before any other slaughtering.

The microbiological cultures were carried out according to an adapted method from ISO 6579 Annex D. Amongst the 900 analysed samples, one isolate per positive sample was genotyped by PFGE using *Xba*I enzyme and one isolate by pulsotype was serotyped.

Genotyping was performed on 229 isolates, divided into 42 *Xba*l pulsotypes and 12 serovars. *Salmonella* monovariant 4,[5],12:i, *Salmonella* Rissen, *Salmonella* Typhimurium and *Salmonella* Livingstone were predominant. Three different infection profiles were observed. At the nursing stage, piglets were *Salmonella* free. Infection occured either post-weaning (%) or during the fattening period (%). Truck and lairage are cross-contamination stages (100% are positive before pig loading). Converserly, the slaughterhouse is a decreasing contamination stage (equipment and pork cuts are *Salmonella* free). Farm surroundings play a major role in the reinfection of pigs (70% are contaminated) like other animal productions. This investigation provides for the first time an accurate picture of *Salmonella* spreading along the pork chain.

Parallel session 4: Animal health and production (b)

Efficacy and effectiveness of Waterbuck repellent blend (*Kobus ellipsiprymnus*): tsetserepellent technology developed for the control of bovine trypanosomiasis in sub-Sahara Africa

N Mbahin¹, H Affognon¹, J Andoke¹, M Makayoto² and SK Rajinder¹

¹Animal Health Division, African Insect Science for Food and Health, International Centre of Insect Physiology and Ecology (icipe), Nairobi, 00100; ²Engineering Department, Kenya Industrial Research and Development Institute (KIRDI), Nairobi, 00100; capild@yahoo.fr

Nature has her own defences against pests and diseases. Tsetse flies avoid many wildlife including the waterbuck (*Kobus ellipsiprymnus*). "The repellents have been identified from odours of animals avoided by tsetse, like the waterbuck, a big antelope species that is common in tsetse-infested areas of eastern Africa but which is rarely fed on by the flies. *icipe* – International Centre of Insect Physiology and Ecology scientists undertook studies to determine why these animals are not attacked by tsetse and have isolated powerful fly repellents from waterbucks which reduce tsetse bites by >90%. But in a bid to help farmers solved this menace, the International Centre for Insect Physiology and Ecology (*icipe*) has developed a repellent collar that is tied around the animal's neck and in the process repels tsetse flies. These repellent collars slowly dispense the chemicals in them, thereby protecting the animals and their herders from the flies. *icipe* scientists have also been able to produce repellents through molecular optimization of natural repellents found in the urine of cattle. These repellents have been used to develop repellent collars for protection of cattle.

Primary result from a large scale validation field trial with Pastoralists and Agro-pastoralists in Kenya and Uganda show that the disease levels in protected cattle had been reduced by more than 90 per cent and that repellent collars performed better than other conventional methods (chemotherapy, targets, pour-on). The annual incidence rate of trypanosomiasis was significantly higher in control (188 cases for 100 animals-years) than that of treatments (66 cases for 100 animals-years) and (P<0.001).

According to a livestock farmer, with the introduction of the repellent collars, weight of animals had significantly increased making them fetch more money as well as enabling those that use bulls ploughing utilize their land effectively. They are saying milk yields had doubled up, a sign that the repellent collars were very effective and their cattle were now more settled when grazing and they were grazing much closer to the park fence than before without being disturbed by tsetse flies.

Evolution of the trypanosome transmission cycle: the effect on pathogenicity and transmissibility of *Trypanosoma congolense*

<u>S Chitanga^{1,2}</u>, T Marcotty^{3,4}, V Delespaux⁴

¹School of Life Sciences, University of KwaZulu-Natal, Durban 4000, South Africa; ²Department of Biomedical Sciences, School of Medicine, University of Zambia, Zambia; ³Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, South Africa; ⁴Institute of Tropical Medicine, Antwerp, Belgium; schitanga@gmail.com

Trypanosomosis is naturally an infection of wildlife but has crossed over to domestic animals due to encroachment of people into areas normally inhabited by game animals. In livestock, the infection causes a disease of economic importance and can be expressed in different clinical forms ranging from acute and severe to chronic forms. Typically, three situations are observed in animals: (i) relative endemic stability in livestock in areas where wildlife was eliminated, (ii) endemic stability in game reserves (trypanotolerant hosts) and finally (iii) severe outbreaks in livestock living in the vicinity of game reserves.

The virulence of 23 *T. congolense* strains (six domestic, five mixed & 12 sylvatic) was tested in OF1 mice using the method described by Masumu *et al.* (2006). Survival of mice was summarized in a Kaplan-Meier survival curve and further analyzed in a parametric survival model in Stata 10. Median survival time was used to categorize the strains as either of low virulence or highly virulent.

Transmissibility of 22 isolates (12 sylvatic & 10 domestic) was determined by allowing teneral flies to take a blood meal infected with each of the trypanosome isolates. The engorged flies were then maintained and dissected after 21 days. The percentage of metacyclic infections (transmissibility) was calculated as the proportion of flies that had infections in both midgut and mouthparts. Mixed logistic regressions were applied on tsetse procyclic and metacyclic infection binary data, using individual strains as random effects. Pathogenicity, cycle type and the interaction of the two were the binary explanatory variables.

Survival of mice varied considerably between *T. congolense* isolates. Mice infected with strains from a domestic cycle survived significantly longer than those infected with strains from a sylvatic cycle (P<0.001). Mice infected with strains from an interface

cycle also survived longer than those infected with strains from a sylvatic cycle (P=0.03). All strains isolated from a domestic cycle were of low virulence while 20% (1/5) of the mixed cycle strains and 83.3% (10/12) of sylvatic strains were highly virulent.

Both pathogenicity and cycle of origin had a significant effect on transmissibility. Highly pathogenic isolates easily established a midgut infection whilst being from a domestic transmission cycle also allowed easier establishment of midgut infection. However, the isolates most common in the field (low virulence domestic cycle and highly virulent sylvatic isolates) had an equivalent transmissibility (OR=0.81; 95% CI; 0.51-1.28; p=0.37).

Domestication of the transmission cycle seems to select for isolates of low virulence, thus allowing for attainment of endemicity, but with a higher transmissibility than sylvatic isolates of equivalent pathogenicity.

Salmonella in pig farms in Reunion Island: Prevalence assessment and identification of risk factors

C Tessier^{1,2}, L Atiana¹, M Denis³, E Cardinale^{1,4}

¹CIRAD, BIOS, UMR CMAEE, Saint Denis, Réunion; ²Coopérative des Producteurs de Porcs de la Réunion, Saint Pierre, Réunion; ³ANSES, HQPAP, Ploufragan, France; ⁴CRVOI, Saint Denis, Réunion; ctessier@cppr.fr

Salmonella is the second biggest cause of food-borne diseases in France and pork and pork products are regularly incriminated. In Reunion Island, Salmonella is also a public health burden. The pig industry will be concerned, sooner or later, by a new European regulation regarding Salmonella; and that is why it is necessary before its implementation, to better understand the routes of infection on the pig farms to be able to propose adequate and specific control measures. Our epidemiological study aimed to determine the prevalence of Salmonella infection in fattening pigs and to identify the main risk factors for infection.

Fifty farms (farrow-to-finish and grower) were randomly selected and visited 4 times: pre-slaughtering of the previous batch; after cleaning and disinfection; beginning and end of the fattening period for the studied batch. Pools of fresh faeces, gauze socks and gauze swabs were sampled to assess the bacteriological status of these pig farms. Samples from rodents and cockroaches were also collected. A questionnaire was submitted to the farmers and analysed for further identification of risk factors.

To date, we observed a massive contamination $(75\%\pm7\%)$ of the pig farms, that may be related to poor disinfection procedures (68% of the farms were positive at this stage). *Salmonella* 4,[5],12,:i:- and Livingstone were the most prominent serovars. Moreover, 20% of the rats and 27% of cockroaches were also positive for *Salmonella*.

The present ongoing statistical analysis will highlight the main risk factors and the measures to be immediately taken to lower the high prevalence. Antibiotic susceptibility profiles and molecular typing (PFGE) could, by estimating the diversity of *Salmonella strains*, lead to a better understanding of the epidemiology of this zoonotic pathogen on pig farms on Reunion Island.

Bacteriophages against *B. anthracis* – causative agent of severe infectious diseases in humans and animals

S Rigvava, M Natidze, L Gubeladze, L Kvachadze, R Adamia, M Kutateladze

G. Eliava Institute of Bacteriophages, Microbiology and Virology, Tbilisi, Georgia; kutateladze@pha.ge

Anthrax is a life-threatening disease in humans and animals. In most industrialized countries, livestock are routinely vaccinated and cases of anthrax are rare. In developing countries, however, where animal vaccination is not regularly practiced, the disease in animals is a problem. This is especially true in tropical and sub-tropical environments. Bacteriophages are bacterial viruses that reveal specific lytic activity against host bacterial strains. The Eliava Institute, Tbilisi, Georgia is one of the most experienced institutes for phage isolation, research, and application. Phage preparations are used for detection, therapy and prophylaxis of various bacterial infections in humans and animals. Several bacteriophages active against *Bacillus anthracis* are included in the bacterial and phage collection of the Eliava Institute. The aim of our research was isolation and selection of new specific phages with lytic activity and to elaborate the phage typing scheme for these bacterial pathogens. Several lytic phages were isolated from two geographically distinct loci: one from West Georgia (12 phages from the Kutaisi region) and the second from Eastern Georgia (6 phages from the Gardabani region). The phages were isolated and then propagated on vaccine strains of *B. anthracis* phage Gamma – the phages have hexagonal heads and long, non-contractile tails, consequently morphologically they all belong to *Siphoviridae* family. All phages are very specific and do not lyse any other Bacillus species other than strains of *Bacillus anthracis*. Most of the phages isolated differ from each other by DNA restriction and structural proteins. The phages were tested against a virulent strain collection (A total of 37 strains both from the Eliava Institute and the National Centre of Disease Control

and Public Health, Tbilisi Georgia). All phages revealed different lytic activity against virulent strains; the phages differ from each other with parameters of phage reproduction on host bacterial cells. Further research will be focused on complete genome sequencing of genetically different phages, which could lead to a better understanding of the biodiversity of *B. antracis* phages, as well and elaborate and improved diagnostics capability of phage typing of *B. anthracis*.

Evaluation of Enzyme-Linked Immunosorbent Assays and Haemagglutination Inhibition tests for the diagnosis of Newcastle disease virus infection in village chickens using a Bayesian approach

H Chaka^{1,2}, V Grosbois³, F Goutard³, PN Thompson¹

¹Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, Pretoria, South Africa, ²National Animal Health Diagnostic and Investigation Center, PO Box 04, Sebeta, Ethiopia; ³CIRAD, Unite AGIRs, 34398 Montpellier Cedex5, France; hasscha@yahoo.com

Newcastle disease in village chicken is endemic in Ethiopia with significant economic importance. The sensitivity (*Se*) and specificity (*Sp*) of the blocking enzyme-linked immunosorbent assay (bELISA, Svanova Biotech), indirect ELISA (iELISA, Laboratoire Service International) and haemagglutination inhibition (HI) test for Newcastle disease virus (NDV) antibody detection were evaluated in a Bayesian framework in the absence of a gold standard test, on sera collected from unvaccinated chickens kept under the village production system in household flocks and at markets in Eastern Shewa zone, Ethiopia. The bELISA had both the highest *Se* (96.3%; 95% posterior credible interval (PCI): 88.1; 99.8%), and the highest *Sp* (98.9%; 95% PCI: 97.8; 99.9%), while the HI had a *Se* of 81.6% (95% PCI: 71.8, 91.9%), and a *Sp* of 96.1% (95% PCI: 95.1; 96.6%). The iELISA also had high *Se* (95.2%; 95% CPI: 88.5; 99.0%) but had very poor *Sp* (8.9%; 95% PCI: 6.4, 11.8%). The conditional correlation coefficient between the tests for both sensitivity and specificity clustered around zero indicating the likely conditional independence, but posterior distribution skewed more towards positive values for truly positive animals. The use of bELISA in screening and surveillance for NDV antibodies is indicated given its high *Se* and *Sp*, in addition to its ease of automation to handle large numbers of samples compared to HI. The latter can be used as confirmatory test where an ELISA test with moderate or low specificity is used. Evaluation of commercial ELISA kits is indicated before their wider use in similar situations like ours.

How can joint Master programmes and its alumni contribute to the `One Health' concept: experiences from the Master in Veterinary Public Health (MVPH) for Southeast Asia and the Master in Transboundary Animal Disease Management (MTADM) for Eastern Africa

MPO Baumann¹, K Kreausukon², K Belihu³, JD Kabasa⁴, F Ejobi⁴, K Elmalik⁵, AA Elfadil⁶, L Srikitjakarn², KH Zessin⁷

¹Faculty of Veterinary Medicine, Freie Universität Berlin, Germany; ²Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand; ³ FAO Ethiopia, Addis Ababa, Ethiopia; ⁴ College of Veterinary Medicine, Animal Resources & BioSecurity, Makerere University, Kampala, Uganda, ⁵Faculty of Veterinary Medicine, University of Khartoum, Sudan; ⁶College of Veterinary Medicine, Sudan University of Science and Technology, Khartoum, Sudan; ⁷Oldenburger Str. 2 D-26676 Barßel, Germany; Maximilian.Baumann@fu-berlin.de

The Faculty of Veterinary Medicine of the Freie Universität is developing and delivering joint master programmes together with partner universities in Asia and Africa since more than ten years. The Joint Master in Veterinary Public Health (MVPH) for South(-east) Asia is going into the 6th edition this year and the Joint Master in Transboundary Animal Disease Management (MTADM) for Eastern Africa has been delivered from 2010 to 2011.

Not only by its focus on food safety along the livestock value chain from producer to consumer but also due to the number of graduates being now in positions where new concepts are asked for an assessment of the MVPH programme's contributions to `One Health' allows a quantitative approach. However, the empirical database for the Joint African Programme is limited as operational funds were provided by the ACP EU Edulink Programme just for a single run so far; as the MTADM programme was concentrating more on animal diseases of transboundary nature here greater attention was paid to wildlife –ecosystem interface issues.

`One Health' relevant aspects of the respective curricula are outlined and examples for master thesis field studies at the livestockhuman- wildlife interface are given and its impacts assessed. Alumni contributions being it individual or in an organized way, i.e. the Veterinary Public Health Association for Asia Pacific, as well as challenges are demonstrated and discussed.

Day 4: Thursday 29/08/2013

Session 5: Training opportunities in the context of the interface

Training of the "One Health" next scientific generation in the Sahel and Maghreb

T Marcotty¹, S Bakou², A Rhalem³ and E Schelling⁴

¹Department of Biomedical Science, Institute of Tropical Medicine, Antwerp, Belgium; ²Ecole Inter-Etats des Sciences et de Médecine Vétérinaires, Dakar, Senegal; ³Laboratoire de Parasitologie, Institut Agronomique et Vétérinaire Hassan II, Rabat, Morocco; ⁴Epidemiology and Public Health, Swiss Tropical and Public Health Institute, Basel, Switzerland; tmarcotty@itg.be

Training of the "One Health" Next Scientific Generation in the Sahel and Maghreb (OH-NEXTGEN) is a 4 year European Commission FP7-funded programme aiming at improving the control of neglected zoonoses in the Sahel and the Maghreb through the training of local scientists from the medical and veterinary sectors using a One Health approach.

The One Health approach is based on transdisciplinarity and added value of intersectoral collaboration between health professionals, veterinarians, wildlife and environment scientists and social scientists. Transdisciplinarity is defined as epistemological perspectives unique to the collaborative effort and distinct from those of any of the cooperating disciplines. In other words, combined investments have a greater output than separate investments in each sector. Most important, added value is expected where human and animal health are closely interrelated such as with zoonoses and food safety. The challenge, but also the key to fostered cooperation, is to make each sector aware of the competences of the other sector and the potential benefits of intersectoral collaboration. This can be done by country- and region-specific case studies.

Consequently, the objective of OH-NEXTGEN is to develop a sustainable course and to provide Maghrebian and Sahelian postgraduate health practitioners with competences to research and effectively manage the neglected zoonoses prevailing in the region, namely bovine tuberculosis, brucellosis, echinococcosis, leishmaniosis and rabies. The course, which will be mostly web-based, will be available in 2014 in French and in 2015 in English. The modular course will be embedded in local academic institutions and OH-NEXTEGEN will train tutors for facilitation.

The following modules are being developed:

- One Health in the Sahel and Maghreb (3 ECTS)
- Concepts of Epidemiology in One Health (3 ECTS)
- One Health Survey Methodology (6 ECTS)
- Risk Analysis in a One-Health Framework (9 ECTS)
- Geographical Information System in One Health (6 ECTS)
- Health and Livestock Economics (6 ECTS)
- Specific Diseases and Integrated Control (3 ECTS)
- One Health Policy Development (6 ECTS)
- Health Education and Communication in a One Health framework (3 ECTS)
- Socio-cultural and Ethical Aspects in One Health (3 ECTS)

The course could eventually be extended to other zoonoses (e.g. emerging or re-emerging zoonoses) and tailored to other geographical regions.

Capacity building in EcoHealth: Experiences and evaluation of training using a "learning by doing" approach, within academic and non-academic contexts

J Gilbert, R Asse, K Tohtubtiang, F Unger, P Mehta

International Livestock Research Institute, Nairobi, Kenya, J.Gilbert@cgiar.org

EcoHealth and OneHealth are complementary approaches,- sharing the central vision of better solutions to health problems via collaboration across multiple sectors and disciplines. EcoHealth initiatives are active in Southeast Asia, West Africa and Latin America, - mostly supported by the Canadian International Development Research Centre. (www.idrc.ca)

The presentation will outline the background, context and results of training conducted within the IDRC-funded *Ecosystem Approaches to the Better Management of Zoonotic Emerging Infectious Diseases in SE Asia* (EcoZD) project that was implemented by the International Livestock Research Institute (ILRI) in six countries from 2008 – 2013.

The central theme (tagline) of the EcoZD project was '*learning by doing*'. Training inputs enabled the multi-disciplinary research teams conceptualise and develop research proposals; then conduct, analyse and write-up the research, – applying EcoHealth concepts. A combination of formal workshops and ad hoc mentoring by ILRI researchers was utilised.

The EcoZD project also established two EcoHealth Resource Centres at Chiang Mai (Thailand) and Gadja Mada (Indonesia) universities, with the objectives of providing sustainable capacity building in the Southeast Asia region, - including training, research and advocacy for Eco/One-Health.

'Lessons learnt' and substantial challenges will be shared, - for example standardising Eco/One-Health as a paradigm (and the difference between them), essential core capacities, and the operational challenges of administration research projects across different sectors and ministries.

In addition to supporting research activities ILRI also conducted on-going monitoring and evaluations to record how teams put into practice novel integrated health approaches, using inquiry/interviews and 'outcome mapping'. Researchers views were sought on whether Eco/One-Health approaches could be adopted beyond the EcoZD project. Key findings of these evaluations will be presented.

Of Specialists and Generalists: Transdisciplinarity and 'One Health' training in South Africa

<u>C Geoghegan^{1,2}</u>, M Robertson³, W Getz^{1,4}

¹Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, South Africa; ²Pathogens, Parks and People, One Health Project, Hluhluwe-iMfolozi Park, ³Department of Zoology and Entomology, University of Pretoria, South Africa ⁴Department of Environmental Science, Policy and Management, University of California - Berkeley, USA; cgeoghegan@zoology.up.ac.za

As our understanding of the intrinsic links between the health of people, animals and the environment improves, so must our integration of multiple disciplines into scientific programmes and health education. While the need for medical, veterinary and environmental specialists continues, so does that for a new breed of scientist, trained to incorporate diverse methodologies and develop innovative approaches to health problems in the 21st Century.

This 'One Health' philosophy mirrors the interconnectedness of daily life and environmental systems, and offers a wide range of tools designed to complement the specialist knowledge on which we currently rely.

Providing interdisciplinary training environments for specialists and students is critical to improving our ability to tackle emerging global health problems. Enabling students to 'experience' the links between disciplines is widely encouraged and seen as the primary method through which we may learn to think across traditional and perceived disciplinary and institutional barriers.

This paper describes the outcome of a programme designed to enable students and specialists to collaborate on 'One Health' projects assessing wildlife, livestock, and environmental and human health in rural communities and conservation areas in KwaZulu-Natal. Over a period of five years, over 60 individuals from 10 countries and 15 specialist disciplines have collectively sampled and analysed clinical, social and environmental data with the aim of improving public health, agricultural development and conservation programmes.

We discuss the initial philosophical and institutional challenges to implementing this programme, and note the benefits of interdisciplinary involvement for both the project objectives and individual participants involved in the scheme. We describe the evolution of the project in relation to the broadening interests of project participants, and simultaneous inclusion of a wide range of local, government and tribal authorities that responded positively to a more interdisciplinary approach to local health and environmental challenges.

Finally, we consider the future of 'One Health' programmes in South Africa, and the ongoing trend for educational and funding organisations to encourage interdisciplinary training programmes as part of health and environmental projects at the global scale.

Study abroad: an enrichment for many veterinary students of Utrecht University

WGGM van der Maazen, RW Paling

Office for International Cooperation, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands; H.vanderMaazen@UU.NL

Internationalisation is high on the agenda of many Higher Education Institutions¹ and often includes student exchange. Students of the Utrecht Faculty of Veterinary Medicine can do parts of their six year educational programme abroad. Options are (a) elective courses (up to twenty weeks full time during six years); (b) clinical externships (up to five weeks in the final year) and (c) the Student's Research Project (between 12 and 22 weeks during the final three years).

Measured over the last five years, annually, an average of 106 of the 1,500 students (7.1%) went abroad for part of their study. Over these years, this number increased from 49 students in 2008 to 169 in 2011, while the average number of credits per student declined from 15.1 in 2008 to 7.5 in 2011.

The Student's Research Projects (SRPs) were analysed in more detail since the students doing their SRP abroad accounted for approximately 50% of the travelling students. Students write a research proposal and a thesis. The objective of the SRP is that the student gets an impression of what research entails. The SRP is in principle part of an ongoing local MSc or PhD research project. This creates a win-win situation: the student gets experience in research and the researcher gets additional assistance, knowledge and skills onboard. All SRPs are executed under co-supervision of staff from Utrecht and the receiving institute, which often is an MOU partner. For this study data were used of SRPs that were assessed during the years 2008 to 2013. Of the total number of 932 SRPs, 232 (25%) were executed outside the Netherlands. In Australia and New Zealand, most SRPs were equine related. In North America dairy cattle prevailed as topic, while aspects of wildlife were mostly studied in Africa.

Apart from the veterinary educational aspects, the international element and personal growth are valuable dimensions of students' international experiences (1, 2).

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Research platform "Production and Conservation in Partnership", promoting collaborative applied research and postgraduate training to study wild/domestic interfaces in southern Africa.

M de Garine-Wichatitsky¹, E Mwenje², A Caron¹, B Mukamuri³, P Mundy², E Etter¹, PH Mugabe³, A Murwira³, H Fritz⁴

¹UR AGIRs, Cirad Zimbabwe, Harare, Zimbabwe; ²National University of Science and Technology, Bulawayo, Zimbabwe; ³University of Zimbabwe, Harare, Zimbabwe; ⁴CNRS UMR 5558, LBBE, Université de Lyon 1, France; michel.de_garine-wichatitsky@cirad.fr

In December 2010 in Harare, Zimbabwe, the agreement to form the Research Platform "Production and Conservation in Partnership" (RP-PCP) has been officially renewed for another 3 year period, following the recommendations of the external evaluation. This platform is a partnership between two Zimbabwean universities and two French research institutes which during the first phase 2007-2010, has significantly contributed to research and training on "wild-domestic interfaces in southern Africa". The RP-PCP provides an integrated multidisciplinary and holistic approach to study and improve management of wild-domestic interfaces in southern Africa, and we believe that the following key points have contributed to this success.

The research platform focuses on a single object of research clearly identified by all the partners, "socio-ecological systems in the context of wild-domestic interfaces", which is of particular interest to Zimbabwe and the southern African region, with blooming transfrontier conservation areas initiatives and pioneer experiences in community based natural resource management. A lot of emphasis was put on multidisciplinary approaches including researchers and students from four main thematic fields (Animal Health and Environment, Ecology, Governance and Natural Resources, and Conservation Agriculture) to promote cross-cutting research projects. The partnership extends to governmental services such as veterinary services and the park and wildlife authorities.

To date, the platform has supported more than 40 postgraduate students at MPhil, MSc and PhD level, and produced more than 40 research papers and book chapters in international peer-reviewed journals.

Posters

Diagnosis, surveillance and control at the interface

1. Awareness of zoonotic tuberculosis among tuberculous and HIV/AIDS patients within Kaduna metropolis, Nigeria

EG Haruna¹, CA Kudi², JU Umoh¹

¹Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria; ²Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria; haruna_godiya@yahoo.com

Bovine tuberculosis caused by Mycobacterium bovis is as pathogenic to humans as that caused by M. tuberculosis but relatively little is known about it (1). The study was designed with the aim of assessing knowledge/ level of awareness about bovine tuberculosis within patients attending and staff of tuberculosis health care centres within Kaduna Metropolis, Nigeria. A crosssectional study was conducted in the six centres using a structured, piloted questionnaire. A total of 172 questionnaires, consisting of both open- ended and closed questions were randomly administered to patients and staff. The result showed that general awareness to tuberculosis, as a disease, amongst the respondent was very high (92%). However, only 16.86% (29/172) of the respondents were aware of the zoonotic effect of bovine tuberculosis. Also very small proportions of the respondents 7.56% (13/172) were aware of BTB as a definite zoonotic disease. Amongst the age groups, the 35 - 54 years age group was found to be the most aware of BTB as a zoonosis. The level of education was found to directly affect awareness of the zoonosis, with 12.20% (10/82) with tertiary education being the most aware and those with non-formal education not aware at all (0%). Based on occupational status, level of knowledge and awareness of bovine tuberculosis as a zoonosis was highest amongst civil servants 11.29% (7/62) and least amongst farmers (0%) with others in between. The increasing incidence of tuberculosis in humans particularly in immunocompromised persons makes bovine tuberculosis increasingly important especially in developing countries, where humans and animals share the same micro environment. Lack of awareness of the zoonotic nature of bovine tuberculosis is one of the major problems affecting the control of tuberculosis in Nigeria (2). There is the need for a combined veterinary and medical effort to enhance active sensitization of the populace on the zoonotic implications of bovine tuberculosis.

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2. Bayesian evaluation of three screening tests for caprine mastitis in Bangladesh

MA Islam^{1,2} D Berkvens¹ AKMA Rahman^{1,2} and E Abatih¹

¹Department of Biomedical Science, Institute of Tropical Medicine, Antwerpen-2000, Belgium; ²Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202; Bangladesh; aminbau14@gmail.com

Mastitis control is of paramount importance for dairy producers worldwide because of the associated economic and public health concerns. Regular screening is essential for control but diagnostic accuracy of tests has not been established. Diagnosis of caprine mastitis being practiced with indirect tests rather microbiological culture under the extensive farming system in agrobased Bangladesh due to logistic and financial consideration involved.

The aim of this study was therefore to estimate the sensitivities and specificities of three screening tests namely, California Mastitis Test (CMT), White Side Test (WST) and Surf Field Mastitis Test (SFMT), for the screening of subclinical caprine mastitis in Bangladesh. Bayesian probabilistic models were constructed using WinBUGS with the assumption of positive conditional dependency among tests and there being intra-cluster correlation. Data were obtained from a cross-sectional study of 484 milk samples from 242 goats of three goat farms screened for subclinical mastitis. Markov chain Monte Carlo simulation technique was used with three chains, prior based generated initial values, 5,000 burn-in and 15,000 post sampling iterations to converge the Bayesian model. Trace plot and time-series plot were checked for model convergence. Prior information was also incorporated in a Bayesian manner to fit the model. The best fitted model was selected based on three statistical indices, the Deviance Information Criterion (DIC), Bayesian P-value and the effective number of estimable parameters (pD).

Bayesian posterior estimation of sensitivities with a 95% Credible Interval (CrI) were 99.42% (97.94-99.98%), 98.39% (95.85-99.91%) and 90.52% (86.17-94.31%), respectively and specificities with 95% CrI were as 98.77% (98.03-99.85%), 99.13% (97.62-99.97%) and 99.21% (97.72-99.96%), respectively for CMT, WST and SFMT. True prevalence of subclinical caprine mastitis was estimated at 42.85% (95% CrI: 38.46-47.28%). Significant conditional dependencies were observed in test specificities. There was no significant intra-cluster correlation within the animal. This model could be beneficial and easily used as a tool for evaluation of existing or newly introduced screening tests for mastitis in other dairy animals without the need of perfect gold standard test.

3. Cell cultures of wild animals as a model to study the reservoirs of emerging viruses that pose a risk to humans and livestock

Savinova IV, Klestova ZS

Institute of Veterinary Medicine, National Academy of Agrarian Sciences Of Ukraine; Reptileviology@gmail.com

The spread of reptilian viruses with potential risk of posing a serious threat to human and animal health has fallen under the spotlight. The results of the isolation of potentially dangerous pathogens indicate that reptilians are the reservoirs of many viruses from different viral families. However, many aspects of the ecology and circulation of these pathogens are still unclear. There is therefore a need for information about the replication, transmission, isolation, detection and spread of these viruses to other animal species and humans. There are currently no continuous cell lines for viral studies like this. For this purpose we have generated primary cell cultures from three different species of reptiles and established the optimal conditions for the maintenance of these cells.

The main aim of this research was to establish sensitive cell cultures for investigations that will facilitate the development of sensitive test systems and new diagnostic methods to study and define viruses present in reptiles.

The animals used in the study were bought from a local company trading in animals and included the veiled chameleon (*Chamaeleo calyptratus*), Russian tortoise (*Testudo horsfieldii*)) and sand lizard (*Lacerta agilis*). These species originated from the Kiev region. Primary cell cultures were established from the kidneys, heart, lungs, testes and ovaria. Cell suspensions were prepared by cold trypsinization. Cells were maintained at 28, 30, & 37°C in Dulbecco's minimum essential medium (MEM) and RPMI 1640 medium supplemented with 10% fetal bovine serum. Chameleon cell cultures (prepared from kidney tissues) were kept 4 days at 28 °C before cell proliferation started. The cells did not form a complete monolayer but survived for 29 days before degenerating due to unknown causes. Cell lines from a Russian tortoise and sand lizard were sub-cultured more than 5 passages in cell culture flasks indicating that DMEM medium was a satisfactory culture medium for the cells. Interestingly, the cells grew better at 37°C than at temperatures below 30°C.

These results may be valuable for future studies on viruses that may have an impact on the wildlife-domestic animal-human interface.

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4. Comparative study of highly pathogenic avian influenza strains isolated in Ukraine in 2005 and 2008

Stegniy BT, Gerilovych AP, Stegniy AB, Muzyka DV

National Scientific Center, Institute of Experimental and Clinical Veterinary Medicine Ukraine, National Agriculture Academy of Science, 83, Pushkinskaya, Kharkiv, 61023; admin@vet.kharkov.ua

Highly pathogenic avian influenza (HPAI) is a widespread avian viral pathogen. Disease caused by the H5N1 subtype has affected many Asian, European and African countries in the last 5 years. The system of effective control and prevention of HPAI is based on surveillance and specific preventive measures. The diagnosis of avian influenza virus infections entails the use of virological, serological and molecular techniques. Phylogenetic studies to establish relationships between virus isolates are also becoming a part of routine diagnostic procedures. The aim of this study was to describe the nature of Ukrainian strains of HPAI viruses isolated during Crimean outbreaks, and to compare them with other HPAI strains from different countries isolated during 2005-2007.

Formalin-inactivated seed of two HPAI isolates - A/Ch/Syvash/02/05 and A/Ch/Krasnogvardeysk/58/08, was used as a source for RNA extraction. The amplification of approximately 300 bp region encompassing the cleavage site of the haemagglutinin (HA) gene was carried out with conventional RT-PCR. For phylogenetic studies, two additional RT-PCRs were performed using primers designed at the National Veterinary Research Institute, Pulawy. All RT-PCRs produced products consisting of three overlapping regions of the total length of approximately 1200 bp. Both real-time and conventional RT-PCRs were performed using Qiagen reagents. Sequencing of amplified products after purification (Qiagen DNA Purification kit) was performed in the Genomed Sequencing Center (Warszawa) by ABI-technology. The phylogenetic tree was constructed by the Neighbour Joining algorithm in Mega 5 software.

The PCR products of A/Ch/Syvash/02/05/H5N1 and A/Ch/Krasnogvardeysk/58/08/H5N1 did not contain any additional nonspecific bands. The amino acid sequences of the HA cleavage site were typical of HPAI (the motif PQGERRRKKR*GLF found in both isolates contained multiple basic amino acids). At the nucleotide level, both Ukrainian isolates shared approximately 97% similarity. The phylogenetic analysis revealed that they clustered on different branches of the phylogenetic tree. The strain A/ Ch/Syvash/02/05/H5N1 located closely to some H5N1 strains isolated in Russia but it was clearly different from other strains isolated in Europe in 2005 and 2006. That would suggest that it does not belong to the lineage that was introduced to Central and Western Europe in 2005/2006. The strain A/Ch/Krasnogvardeysk/58/08/H5N1 grouped together with H5N1 strains isolated in Europe (Poland, Romania, Russia, Germany, Czech Republic) and Middle East (Kuwait, Saudi Arabia) in the second half of 2007.

Strains A/Ch/Syvash/02/05/H5N1 and A/Ch/Krasnogvardeysk/58/08/H5N1 are highly pathogenic. They belong to two separate genetic lineages which imply that there were at least two independent introductions of H5N1 to the Ukrainian poultry flocks.

5. Development of a conventional PCR protocol for West Nile virus detection

AP Gerilovych, BT Stegniy, AB Stegniy, OS Solodiankin, .O Gerilovych, .V Goraichuk

National Scientific Centre, Institute of Experimental and Clinical Veterinary Medicine, Ukraine, National Agriculture Academy of Science, 83 Pushkinskaya, Kharkiv, 61023; admin@vet.kharkov.ua

Background: West Nile fever is a disease of horses, humans and several avian species caused by a Flavivirus. Wild birds act as reservoir for West Nile virus (WNV). Wildlife populations should be monitored for WNV using modern surveillance techniques. Ukraine is located in the migration pathways of wild birds connecting Asia, Europe and Africa. These birds pose a risk for the introduction of WNV into Ukraine. The goal of this work was to develop aprotocol for the detection of viral RNA in the conventional PCR.

Materials: Specific primers were developed for a 280 bp region of the gE gene of WNV using GenBank published sequences of viral cDNA. The amplification reaction was optimized on the basis of gE recombinant template derived from WNV vaccine seed. PCR master mix reagents from a Russian Federation manufacturer were used. The protocol was validated according to the OIE requirements for diagnostics techniques.

Results: The gE gene sequences from GenBank were analyzed for conserved regions, and four of them were selected for primer design. WNV f/r primer pair, flanking 280 bp region of the gE gene were designed. The protocol for the conventional PCR included a 40-cycle reaction, with an annealing temperature of 57 °C. The reaction mix volume was 30 μ l with 5 U of Taq polymerase, 1.5 mM Mg and 20 pM each of the primers. The PCR was able to detect viral cDNA in samples of vaccine virus and plasmid DNA (5-7 pg/ml). The PCRI was validated with cDNA test panels and recognized as 95 % sensitive, 100 % specific, repeatable, and accurate. Further work will include the development of surveillance systems for WNV in wildlife and synantropic birds in Ukraine.

Screening of the blood and blood sera panels from wild birds, collected in Ukraine (n = 63) demonstrated the absence of the WNV in the specimens.



Conclusion: A conventional PCR for WNV detection was developed and validated. Screening of field samples from wild life demonstrated the absence of the virus.

6. Laboratory Surveillance of dangerous pathogens at the Laboratory of the Ministry of Agriculture

M Zakareishvili, M Nikolaishvili, <u>I Beradze</u>, M Donduashvili, N Vepkhvadze, M Kokhreidze, T Tighilauri, K Goginashvili, E Mamisashvili, L Gelashvili, G Osiashvili, L Kerdzevadze

Laboratory of the Ministry of Agriculture, Tbilisi, Georgia (LMA); bakuna7@yahoo.com

Brucellosis and anthrax are widespread bacterial zoonoses in Georgia, resulting in significant economic losses due to reduction in animal populations as well as human death and suffering. Part of the Cooperative Biological Research (CBR) efforts in Georgia is to improve surveillance capabilities and pathogen diagnostics.

This study was undertaken between 2008 and 2011 whereby LMA sampled livestock in three regions of Georgia, namely Kvemo Kartli, Kakheti, and Imereti. They were selected because of reports of high rates of morbidity caused by brucellosis. Animal samples were tested by serology (Rose Bengal), bacteriology and molecular biological (PCR) tests for identification of *Brucella* species. Thirty-two bacterial isolates were recovered and identified as *B. melitensis* (11) and *B. abortus* (21) from 16 regions. Most positive cases (46%) were identified in the Kvemo Kartli region

The brucellosis investigation was continued by LMA scientists; in 2012, 1118 animal samples were received and tested from different regions of Georgia. Of these, 137 (12.2%) were positive, with most obtained from Mtskheta-Mtianeti. During *B. anthracis* surveillance, 130 animals and environmental (soil) samples were tested from multiple regions of Georgia with suspected anthrax cases. According to the bacteriological and PCR tests, 36 *B. anthracis* cultures were isolated. The majority of positive animals and soil cases were obtained from Kvemo Kartli (53%), Kakheti (19%), and the Imereti region (19%). In 2012, 33 of 127 suspect anthrax cases were positive. In 2012, the number of samples received and anthrax-positive cases almost equaled the total number collected during the previous three years. The increased prevalence was most marked in in Kvemo Kartli (73%) and Kakheti (27%).The growth and distribution of the above-mentioned pathogens in different regions presents a risk for the entire country.

Intensive research will support the disease surveillance system in Georgia and significantly improve the country's public health system by enhancing surveillance, detection and research capabilities for various dangerous pathogens.

7. Efficiency of antirabies vaccines and immunoglobulin against rabies street virus isolates belonging to various genetic clusters

SA Nychyk, MU Ivanov, IM Polupan, AP Nikitova, VV Nedosekov

Institute of Veterinary Medicine, Kyiv, Ukraine; snp1978@ukr.net

Background: Taking into account the importance of rabies in human and animals, the Institute of Veterinary Medicine of Ukraine has collected 1 292 isolates of street. Previous studies identified two genetic clusters with clear geographic demarcation with regards to the river Dnieper. After analyzing historical data of the collection, 31 cases in vaccinated animals (13 dogs, 12 cats, 2 goats, 4 cows) were identified. Analysis of data revealed that 29 isolates belong to genetic cluster II (CVS-) which differ from the reference strain (CVS) and vaccine strains in cluster I (CVS+). We therefore conducted a study to identify the levels of protection conferred by anti-rabies vaccines against street isolates of rabies viruses of the two genetic clusters.

Materials: Two commercial anti-rabies vaccines were prepared from derivatives of vaccine strains SAD (Street-Alabama-Dufferin) and PV (Pasteur Virus). Studies of rabies vaccine activity were carried out with a modified method of the U.S. National Institutes of Health using six rabies virus street isolates of both genetic clusters, instead of the CVS. In addition, four isolates of rabies virus (two from each genetic cluster) were studied in the neutralization reaction in white mice using the second International Standard for human rabies immunoglobulin.

Results: The effective dose (ED_{50}) of the commercial vaccines tested with street isolates of rabies virus, regardless of their genetic cluster, were lower in all cases in comparison to the control infection with rabies reference-strain (CVS). Further studies revealed that the neutralising ED_{50} for anti-rabies immunoglobulin against isolates of cluster II were lower in comparison with isolates of cluster I (2.57±0.06, 2.93±0.12) in mice.

Conclusion: Our research indicated genetic and antigenic differences between regional Ukrainian isolates of rabies virus and vaccine strains. These results enable us to assume that a possible reason for development of rabies among vaccinated animals is due to lowered effectiveness of vaccines against the street isolates of rabies virus of genetic cluster II (CVS-).



Currently these results will be used for effective implementation of rabies specific prophylaxis in Ukraine on the basis of allotment of different anti-rabies vaccines depending on the region. These results also emphasize the necessity to develop new regional vaccines against future strains of rabies.

8. Genotyping of Newcastle disease virus strains isolated in Ukraine in 1967-2007

A .P Gerilovych, BT Stegniy, AB Stegniy, VI Bolotin, IO Gerilovych

NSC "Institute of Expirmental and Clinical Veterinary Medicine", Kharkiv, Ukraine; admin@vet.kharkov.ua

Background: Newcastle disease virus (NDV, avian paramyxovirus type 1 (APMV-1)), is taxon of genus Avulavirus, family Paramyxoviridae. NDV causes disease in poultry, associated with different types of disorders and clinical signs. It can vary from a mild or asymptomatic disease to very acute and affecting large numbers of poultry. The list of susceptible species include domestic, synantropic and wild birds, which can be carriers of latent virus.

NDV genotyping provide researchers with data for molecular epidemiology, host-specificity and pathogenicity of NDV strains. This information can be used for the design of effective virus control and eradication strategies.

Materials: Thirty one isolates of NDV were used in this study. Twenty of them were isolated from chickens (8 only RNA detection in clinical samples), 6 from wild birds (only RNA detection), 2 from ducks (only RNA), 2 from pigeons (RNA) and 1 from a turkey. The primers described by Aldous E. for amplification of F gene (partial sequences, ~400 bp region) were used for specific fragment amplification. Sequencing of amplified products after purification (Qiagen DNA Purification kit) was performed by ABI-technology using Big dye terminator kits under normal conditions. Phylogenetic analysis of the sequenced fragments was done including reference nucleotide sequences of each NDV genotype and sub-genotype, using the Neighbor-Joining algorithm in Mega 5 free software.

Results: Analysis of prepared chromatograms showed 12 sequencing variants of PCR products. They belonged to 4 genotypes. Genoype 1 viruses were isolated from turkeys and ducks in 1967 and 2005-2006, and were identical to data of previous researchers. These strains also affected ducks and chicken.

Type 2 isolates were isolated from chickens during 1987 to 2003. We observed a high level of conservatism within the clade. Divergence levels were not more than 5% within the group.

Ukrainian strains of the 4th genotype were subdivided into isolates 4b and 4d and were quite typical for former Soviet Union lineage 4 viruses. Diversity polymorphism of nucleotide sequences were up to 6%, which is typical for this group. The source of the virus from genotype 4b was not typical, because usually it originates from pigeons, but our viruses were from chickens. The 5th virus genotype was represented by 15 viruses of 5a, 5c and 5d subtypes, isolated from wild birds and chickens. Viruses were isolated during 1993-2008, 2003-2004, 2008, and 1993-2007 respectively. These data has some differences with findings of Aldows E., who described earlier isolation of 5d in 1997. Also we observed pigeons as an essential source for 5d viruses. Identity percentages for sequences indicated Asian origin of viruses (Mideast and China). Divergence among isolates of 5th genotype were about 12%.

Conclusion: Analysed viruses (n = 31) were classified as representative of four different types with different origins. All isolates have been characterized as descendants from previously described viruses and genotypes from European and Asian countries.

9. Isolation of uncommon *Pasteurella multocida* strains from cattle in North Central Nigeria

MY Sugun¹, JKP Kwaga², HM Kazeem², NDG Ibrahim², MO Odugbo¹, D Shamaki¹, MS Ahmed¹

¹Bacteriology Department, National Veterinary Research Institute, Vom; ²Ahamadu Bello University Zaria; manasa_sugun@ yahoo.com

The prevalence of *P. multocida*, an aetiologic agent of bovine haemorrhagic septicaemia was studied using a targeted sampling technique in north central Nigeria. A total of 18 positive isolates of *P. multocida* were obtained from 175 tissue samples (lungs, liver, and spleen) examined, giving an isolation rate of 10.3 %. The isolates were characterized using a standard biochemical technique. The eighteen isolates were confirmed as *P. multocida* by Microbact GNB 24E. The study confirmed the presence of the African capsular strain E (511 bp) and a unique capsular F (851 bp). Two of the *P. multocida* strains were somatically typed as *P. multocida* E: 3, 4 and one was E: 2, 5. Also of interest is one capsular group F somatically untypeable that has been identified for the first time from calves in Nigeria. The remaining sixteen positive isolates were capsular group E, somatically untypeable. The significance of this study is the isolation of these uncommon somatic types, which have not been previously reported in Nigeria

or within the West African sub-region. These could redefine the vaccine strategy as the current vaccine used in Nigeria contain *P multocida* B: 3,4 and E: 2. However more work needs to be carried out in other parts of the country to gather more relevant information with regards to capsular and somatic types.

10. Molecular typing of Mycobacterium bovis isolated from cattle tissues in Cameroon

<u>J Awah-Ndukum</u>¹, AC Kudi², G Bradley³, NH Smith⁴, I Ane-Anyangwe⁵, F. Cho-Ngwa⁵ VPK Titanji⁵

¹School of Veterinary Medicine and Sciences, University of Ngaoundere, Cameroon; ²Department of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria; ³School of Biomedical and Biological Sciences, University of Plymouth, UK; ⁴Animal Health and Veterinary Laboratories Agency, Weybridge, Surrey, UK; ⁵Department of Biochemistry and Microbiology, University of Buea, Cameroon; awahndukum@yahoo.co.uk

An epidemiological study was carried out to determine the *Mycobacterium bovis* strains causing bovine tuberculosis (TB) in cattle in North West Cameroon. Isolated mycobacteria agents from suspected cattle tissue were subjected to molecular genotyping. PCR-based genomic deletion typing showed that 54 of 103 tubercle bacilli isolated from cattle tissue were *M. bovis* strains and the African 1 clonal complex was widespread in affected cattle. Spoligotyping analysis also revealed a closely related group of five *M. bovis* strains including SB0953 and four new patterns identified according to www.Mbovis.org as SB2161, SB2162, SB2663 and SB2664. The study revealed overwhelming evidence of the zoonotic risks of bovine TB and significance of *M. bovis* infection to human TB in the North West region of Cameroon. Bovine TB was widespread in cattle destined for human consumption with important implications for the control of TB in animals and humans in Cameroon.

11. Perception of communal farmers on foot and mouth disease control at the wildlifelivestock interface of the Kruger National Park, South Africa

DD Lazarus^{1,5}, R Burroughs¹, L V Schalkwyk², A Mpehle³, G R Thomson⁴, G T Fosgate¹

¹University of Pretoria, Faculty of Veterinary Science; ²Skukuza Veterinary Directorate, Kruger National Park; ³Mpumalanga Veterinary Services; ⁴TADScientific, Pretoria, South Africa; ⁵National Veterinary Research Institute, Vom, Nigeria; lazdav2003@yahoo.co.uk

Communities surrounding the Kruger National Park in South Africa and its environs are considered foot-and-mouth disease (FMD) control areas because of the proximity to wildlife reservoirs. The park is an infected area due to the presence of African buffaloes (Syncerus caffer), which serves as the major reservoir to the South African Territory (SAT) viruses. The aim of this study was to evaluate the perceptions of communal farmers concerning the current FMD control interventions at the wildlife/livestock interface.

A structured questionnaire was administered to farmers as they presented their cattle at dip tanks within the Mnisi Tribal Area. The questionnaire was administered through an in-person interview using the local language. Questions addressed owner demographics, herd management practices, general disease control and knowledge of FMD epidemiology. Cattle were independently selected for serological testing from all dip tanks within the study area to estimate the proportion of cattle with FMDV-specific vaccinal antibodies.

One hundred and four farmers responded to the questionnaire with 73%, (76/104) being cattle owners. The majority of the respondents, (79%; 95% confidence interval, 70% - 80%) indicated a high level of satisfaction with the current programmes at the dip tank. However education level varied over levels of satisfaction with the median education level being standard nine (interquartile range, 2 - 12) for non-satisfied respondents, standard three (0 – 6) for little satisfied and standard seven (2 – 11) for very satisfied respondents (P = 0.036). Non-satisfied respondents were more likely to treat sick animals themselves rather than seek veterinary assistance (P = 0.002). The majority of respondents identified the African buffalo as the source of risk for FMD outbreaks (92%, 95% confidence interval: 85% - 96%).

Relative to an antibody titre of 1.6 logs, 20% (95% confidence interval: 15% - 27%) of sampled cattle tested positive to SAT 1, 38% (95% confidence interval: 32% - 45%) SAT 2 and 24% (confidence interval: 18% - 31%) for SAT 3. Taking into consideration the perceptions and feelings of the communal farmers within the control areas of the interface will enhance FMD control programmes.

12. Rabies in apparently healthy dogs: conflicts and potential risks for spill-over to wildlife in Bauchi State, Nigeria

<u>YJ Atuman¹, AB Ogunkoya², DAY Adawa³, AJ Nok⁴</u>

¹ Diagnostics and Extension Department, National Veterinary Research Institute, Vom, Nigeria; ²Department of Veterinary Surgery and Medicine, Ahmadu Bello University, Zaria, Nigeria; ³Veterinary Teaching Hospital, Ahmadu Bello University, Zaria, Nigeria; ⁴Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria; atumany@yahoo.com

Domestic dogs are by far the most important species in the maintenance and transmission of rabies in Nigeria. For rabies to spill over from a domestic to a wild population, three conditions must be satisfied: susceptibility of the wild species, presence of the disease agent in the domestic population, and contact between the two populations of interest. We investigated the potential of rabies spill over from the domestic dog population to the wildlife population in the Yankari and Lame-Bura game reserves in Bauchi State, Nigeria. Using questionnaires and discussions with residents living at the fringes of the game reserves, we gathered data on demography of dogs including their age, sex, breed, functions, and health management. Finally, 202 heads of dogs were collected from dog meat markets to determine the presence of rabies antigen using the fluorescent antibody test. The majority of the dogs owned were local breeds (86.95%) used mainly for security (40.62%) and hunting (40.78%). Dog owners reported low vaccination coverage (9.53%), a level considered insufficient to prevent rabies transmission. There was high population turnover of dogs with frequent opportunities for contacts with humans, domestic animals and wildlife species with conflicts at the level of the interface. Also, 46 (22.8%) of the brain samples tested were positive for rabies. Rabies in domestic dogs is a threat to wildlife conservation and of increasing public health risk in Bauchi State, Nigeria.

13. Specific prophylaxis and therapy of actinobacillosis of cattle stock in Ukraine

VP Rizhenko, S.A. Nychyk and A V Rudoĭ

Institute of Veterinary Medicine of NAAN, Ukraine, Kyiv; rud_spass@ukr.net

Background: Actinobacillosis was first described by *Lignières and Spitz* in Argentina in 1900. Sporadic and enzootic outbreaks of actinobacillosis occur in different continents of the world. Actinobacillosis is a new disease in Ukraine (legionellosis) that was first diagnosed in 1996 amongst imported cattle. Initially it was diagnosed as actinomycosis and treatment was ineffective and costly.

Objective: To improve diagnostic methods and develop specific protective actions against bovine actinobacillosis in Ukraine.

Material: Epidemiological surveillance of bovine actinobacillosis was performed during 2001-2012 and approximately 28 500 cattle were inspected, 1 983 specimens were collected and from which 20 isolates of *Actinobacillus lignieresii* were obtained.

Having estimated the epizootic situation, economic and social impact of actinobacillosis, we have used biotechnology methods to produce *A. Lignieresii antigens*. As a result we have produced and successfully approbated inactivated vaccine "Aktinosan".

Results: For the period 2001-2012 bovine actinobacillosis was detected in 76 locations in 13 regions of Ukraine. The infection became enzootic in these areas affecting from 10 to 70 % of livestock on farms Vaccine "Aktinosan" has been used for prophylaxis and treatment together with sanitary measures.

Conclusion: A highly effective inactivated vaccine against bovine actinobacillosis has been developed for the first time in Ukraine and Europe. The use of the vaccine on affected farms together with implementation of sanitary measures had a significant improvement on the occurrence of actinobacillosis in cattle within one calendar year.

Drivers of emerging and re-emerging diseases

14. Bluetongue and epizootic haemorrhagic disease viruses in Reunion Island

C Cêtre-Sossah¹, L Rieau¹, R Matthieu¹, C Sailleau², E Bréard², C Viarouge², S Zientara², O Esnault³, E Cardinale¹

¹CIRAD, UMR Contrôle des Maladies, CYROI, CRVOI, 2 Rue Maxime Rivière, 97490 Sainte Clotilde, La Réunion, France; ²CIRAD, ANSES/INRA/ENVA-UMR Virologie 1161, 23 Avenue du Général de Gaulle, 94700 Maisons Alfort, France; ³GDS, 1 Rue du Père Hauck, PK23, Bâtiment E/F/G, 97418 La plaine des Câfres, La Réunion, France; catherine.cetre-sossah@cirad.fr

Bluetongue virus (BTV) and epizootic haemorrhagic disease virus (EHDV) are two members of the *Orbivirus* genus of the *Reoviridae* family, and are transmitted between ruminants via the bites of *Culicoides* midges. Both viruses encode seven structural proteins (VP1 to VP7) which are presented within two nested capsids, the outer capsid being composed of the VP2 and VP5 proteins. VP2 is the main component of the outer capsid and determines the viral serotype, of which twenty-six BTV variants and 7 EHDV variants have been identified to date. BTV went undetected in Reunion Island between its first documented emergence in 1979 and a series of outbreaks in 2002. Additionally, several severe EHDV outbreaks occurred in 2009 with infected animals displaying clinical signs including hyperthermia, anorexia, congestion, prostration and nasal discharge. Co-circulation of BTV serotype 2 and EHDV serotype 6 was also observed in 2009.

Serological studies were performed to assess the presence of BTV and EHDV in indigenous ruminants of Reunion Island in 2011. Cross-sectional studies were undertaken on 67 farms, including a total of 254 cattle (142 sheep and 71 goats). The seroprevalence for BTV and EHDV were estimated at 50% (70% for cattle, 27% for sheep and goats) and 40% (65% for cattle and 4% for sheep and goats) respectively, indicating elevated levels of circulation of both viruses. Additionally, three suspected outbreaks occurred during the analysis period, one BTV/EHDV negative, one BTV specific and one BTV/EHDV outbreak. In total, 14 EHDV positive cases and 1 BTV/EHDV co-infection case were identified. Isolations of EHDV were successful resulting in identification of a Reunion-specific EHDV serotype. The results confirm that BTV and EHDV are highly prevalent and likely to be in active circulation, with cattle being infected from an early age. The origins of a novel EHDV serotype in Reunion Island are still subject to investigation.

15. Brucellosis in wild animals in Ukraine

B Stegniy, O Obukhovska, A Zavgorodnii, A Babkin

Dept. of Tuberculosis and Brucellosis, National Science Center "Institute of experimental and clinical veterinary Medicine" (NSC IECVM), Kharkiv, Ukraine; olgaobukhovska@gmail.com

Background: Brucellosis is a dangerous contagious disease affecting animal (domestic and wild) and human. The presence of natural reservoirs of the pathogen in wild fauna is one of the main characteristics of brucellosis. In Ukraine, porcine brucellosis is of special relevance. Five outbreaks of the disease related to wild boars were recorded in pig farms in Eastern Ukraine during the period 1973-2011.

Goal: The goal of our investigations was to determine the relationship between brucellosis outbreaks and the circulation of *Brucella suis* in wild fauna.

Methods/materials: The study was conducted in pig farms and populations of wild boars, elks, and deer in 9 regions of Ukraine. Serum samples for studying with the Rose Bengal test (RBT), serum agglutination test (SAT), and complement fixation test (CFT) were taken from selected animals. Livestock farms, breeding animals, pedigree sires were examined once a year in spring. Wild animals were tested once a year during the autumn hunting season. In risk areas, livestock was examined twice a year (in spring and autumn). Infected farms were subjected to stamping out. Morphological, cultural, biochemical and antigenic properties of cultures were also studied. All selected *Brucella spp.* strains were stored in the laboratory collection.

Results: Porcine brucellosis outbreaks during 1981-1993 years in 5 pig farms in the East of Ukraine (Lugansk region) were investigated. Clinical manifestations were typical: abortion, endometritis, orchitis, lymphadenitis. Employing RBT, SAT, and CFT, about 14.0% young pigs, 27.3% of sows and near 14% of boars were identified seropositive. *Brucella suis* biovar was isolated from 2 samples of biological materials of animals. Pig farms were cleared by the change of all livestock. Relapse of the disease was not observed.

Serological studies were conducted in wild animals in Lugansk region. About 15 % of wild pigs and near 1% of elks and deer were investigated. About 32.0% seropositive individuals were found in wild pigs and near 0.02% in deer. It should be pointed out that sexual contact was reported for pig farms between domestic sows and wild boars (evidence of this was the birth of striped piglets). Serological studies in wild pigs in this region were continued during 1993-2011. In total, 7.7% of seropositive individuals were identified. This fact confirms the circulation of *Brucella suis* in wild fauna population.

Conclusion: It has been proved that the outbreak of porcine brucellosis in pigs was caused by the contact of domestic pigs with wild boars. *Brucella suis* biovar 2 has been found to circulate in wild animals in Eastern Ukraine. Wild boars and deer can be a source of the pathogen for domestic pigs, other animals, and humans.

16. Rabies in Georgia

K Dzamashvili¹, <u>N Vepkhvadze</u>², L Ninidze¹, S Menteshashvili¹, M Donduashvili², M Kokhreidze², MSoselia², I Gilels²

¹National Food Agency of the Ministry of Agriculture, Tbilisi, Georgia (NFA); ²Laboratory of the Ministry of Agriculture, Tbilisi, Georgia (LMA); imereli7@gmail.com

Rabies is an acute, especially dangerous, widespread zoonotic infection, which has been known since ancient times. The infectious agent is a neurotropic virus that severely damages the central nervous system, ultimately causing death. In order to protect human and animal health, constant surveillance of the disease is required. Rabies virus belongs to the family *Rhabdoviridae*, genus *Lyssavirus* and is highly infectious to warm-blooded animals including humans. Infected dogs, cats, foxes, and bats represent the greatest risk to humans since these animals are reservoirs of the infection and reside in close association with humans. Surveillance and monitoring as well as tracking of suspicious and confirmed cases of rabies is vital and requires epidemiological studies for the disease. The measures against rabies shall be carried out fully and different institutions shall be involved in this process. The epidemiological situation of neighbouring countries shall be taken into consideration as well. Rabies is endemic in Georgia and is characterized by recurrence and seasonality. The number of cases increases during spring and summer.

Diagnosis of rabies takes place *post mortem* via fluorescence microscopy or mice bioassay investigation. Identification and laboratory diagnostics of the circulating strain are not carried out. There are some circumstances in Georgia that dramatically complicate the fight against rabies: pet owners usually do not carry out planned prophylactic vaccination of their pets on time, and oral immunization of wild animals is not carried out properly. Animals' registration and identification are not accomplished appropriately. Mandatory animal vaccination is carried out only if the disease has been already detected in order to create immune-buffer zones.

According to the data provided by the Rabies Department of the Laboratory of the Ministry of Agriculture starting from 2003 through 2011, it is clear that the situation is very serious. Over this period, the Laboratory of the Ministry of Agriculture received 2,883 samples and 806 (28%) of them, from a diversity of animal species, were positive for rabies. Especially disturbing was the situation noted in the capital city: 555 dog samples were received, of which 186 were positive (34%). Out of 8 samples from suspect cattle 7 were positive (88%), and from 210 cat samples 16 were positive (8%). Statistics for other regions and towns are as follows: 338 out of 900 dog samples were positive (38%); 105 out of 109 cattle samples were positive (96%); 13 out of 124 cat samples were positive (10%), 2 out of 42 pig samples were positive (5%); and 2 out of 5 small ruminant samples were positive (40%). We recorded two cases of rabies in wild animals. In 2001, 10 people died following bites from infected dogs and cats; in 2002 - 10 people; in 2003 - 8 people; in 2007 - 1 person and in 2009 - 1 person.

In 2012 LMA received 416 suspect samples and 102 of them were positive for rabies (24.5%). Recording the data and managing it will enable the identification of disease foci and allow for a timely response by veterinary services, which will facilitate protection of the general public, animals and the environment.

17. Risk factors of bovine tuberculosis and bovine brucellosis in Mnisi area of Mpumalanga Province, South Africa

Molefe CK^{1,2}, A Michel²

¹Department of Agriculture, Limpopo Province, Maruleng; ²Department Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria; molefeck@gmail.com

The study involves investigating the risk associated with unpasteurized milk in the transmission of bovine tuberculosis and brucellosis from cattle to humans in Mnisi area in Mpumalanga.

Literature review reveals that raw milk is the most important role player in the transmission of tuberculosis and brucellosis. Unpasteurised milk poses a threat to the health of human beings. There is inadequate control of bovine tuberculosis and brucellosis as well as animal by-products in Mnisi which could facilitate the spread of tuberculosis and brucellosis among livestock and people.

Structured interviews were used in the collection of data from selected households. The questions related to risk factors associated with socio-economic information, farming details and awareness of brucellosis and tuberculosis as zoonotic diseases. In total, 300 heads of households were interviewed in three study areas of the Mnisi community, including 150 cattle owners and 150 non - cattle owners (50 of each category per study area).

The following three study sites were selected for conducting the structured interviews:

- (i) Hluvukani town which is at the centre of Mnisi Local Municipality;
- (ii) Communal area of defined size bordering the Manyeleti Game Reserve with a high likelihood of wildlife contact;
- (iii) Communal area along the main road between Hluvukani and area with high accessibility by road and frequent movement of people and cattle.

Preliminary analysis of the study revealed that 36% of cattle farmers interviewed were selling fresh milk to the local community. In terms of milk consumption, 55.70% of all respondents affirmed that they preferred fresh milk over boiled or soured milk, but only 29.1% was aware of the zoonotic character of bovine tuberculosis and brucellosis. Regular contact between cattle and wildlife was reported by 63.75% of respondents, while cattle movement into Mnisi appeared to be negligible as only 6% of farmers introduced cattle t Mnisi from outside.

18. Risk assessment of bovine tuberculosis and bovine brucellosis in Mnisi area of Mpumalanga, South Africa

CK Molef

Maruleng Municipality State Vet Services; molefeck@gmail.com

The study involves investigating the risk associated with unpasteurized milk in the transmission of bovine tuberculosis and brucellosis from cattle to humans in the Mnisi area in Mpumalanga.

Literature review reveals that raw milk is the most important role player in the transmission of tuberculosis and brucellosis. Unpasteurised milk poses a threat on the health of human beings. There is inadequate control of animal and animal by-products in Mnisi. That could facilitate the spread of tuberculosis and brucellosis among livestock and people.

Bovine tuberculosis and brucellosis eradication scheme is not implemented in the Mnisi State Veterinary area due to intuitional shortcomings of the State Veterinary Services.

In total 300 householders were interviewed, including cattle owners and non - cattle owners *i.e.*150 cattle owners (50 per target area) 150 non-cattle owners (50 per target area). Structured questionnaires were used in the collection of data from selected respondents.

Three different sites were selected for conducting structured interviews:

- (i) Hluvukani town which is at the centre of Mnisi Local Municipality;
- (ii) Communal area of defined size bordering the Manyeleti Game Reserve with a high likelihood of wildlife contact;
- (iii) Communal area along the main road between Hluvukani and area with high accessibility by road and frequent movement of people and cattle.

The study was done in the following phases:

Phase 1: Meetings were convened with the community of Mnisi area to explain the research project and household participants were selected;

Phase 2: The study areas were mapped with regard to type and number of outlets where milk is sold;

Phase 3: Cattle owners and non-cattle owners were interviewed using a structured questionnaire.

19. The role of the grasscutter (*Thryonomys swinderianus*) as a zoonotic factor in the tropical humid rain forest of south-eastern Nigeria

MN Opara¹, JA Maxwell², TC Iwuji¹, <u>CC Ugwu¹</u>, IC Okoli¹, BO Fagbemi³

¹Tropical Animal Health Research group, Department of Animal Science and Technology, Federal University of Technology, Owerri, Nigeria; ² Department of Health services, Federal University of Technology, Owerri, Nigeria; ³Department of Veterinary Microbiology and Parasitology, University of Ibadan, Nigeria; dozieugwu@yahoo.com

The grasscutter (*Thryonomys swinderianus*) is a wild hystricomorphic rodent widely distributed in the African sub-region and exploited in most areas as a source of animal protein. The gastrointestinal tract and blood of 274 captive-reared grasscutters (CRG) and 1 020 live-trapped wild grasscutters (LWG) in Imo State, south-eastern Nigeria were examined within a period of 32 months for helminths and haemoparasites using standard parasitological procedures. The identification of haemoparasites was confirmed using polymerase chain reaction (PCR) technique and data analysed using descriptive statistics and ANOVA. One hundred and thirty one CRGs were infected with helminths. Of these, 73.3% were nematodes (*Ascaris, Trichuris species* and strongyles), 22.1% were trematodes (*Fasciola* and *Schistosoma species*) and 4.6% were cestodes (*Moniezia* and *Taenia*)

species). One thousand LWGs were helminth infected, with nematodes (*Ascaris, Haemonchus, Strongyloides, Toxocara* and *Trichuris species*) accounting for 71.2%, cestodes (*Moniezia* and *Taenia species*) 13.0%, trematodes (*Fasciola, Paramphistomum* and *Schistosoma species*) 12.7% and acanthocephalan (*Moniliformis species*) 3.1%. Captive-reared grasscutters were naturally infected with trypanosomes (29.5%), *Plasmodium species (62.8%)* and *Babesia species* (7.7%), while LWGs were infected with trypanosomes (33.4%), *Plasmodium species* (56.6%) and *Babesia species* (10.0%). Mixed infections of all haemoparasites were observed in CRG (42.4%) and LWG (80.0%). The PCR analysis of trypanosomes in the blood samples of LWG confirmed *T. simiae* infection, with a prevalence of 2.5%. The arrays of GIT and blood parasites from this study suggested that they might affect productivity in the grasscutters. Occurrence of *T. simiae* in the grasscutters is of great concern, because it is not naturally found in grasscutters. The identification of *Moniliformis sp* is of public health significance, especially in rural areas of tropical rainforest of southeastern Nigeria. *Moniliformis* is a rodent parasite of cosmopolitan distribution, which has been reported in man in different parts of the world, including Nigeria. Of worry again, is the involvement of rodents and their allies in emergence or re-emergence of human and animal disease pathogens such as *Babesia, Plasmodium and Trypanosoma* species

Food safety and food security

20. A five-year retrospective study on the prevalence of bovine tuberculosis in slaughtered cattle in Adamawa State, Nigeria

S Danbirni¹, SO Okaiyeto², JAA Ababa² and <u>AC Kudi¹</u>

¹Veterinary Medicine Department, Ahmadu Bello University Zaria Nigeria; ²Veterinary Teaching Hospital, Ahmadu Bello University Zaria Nigeria; calebkudi@hotmail.com

A retrospective study was carried out in 20 Local Government areas of Adamawa State to determine the prevalence of bovine tuberculosis over a five year period from 2008 to 2012. Different levels of prevalence were recorded in 16 Local Government areas. Slaughtered cattle from each of the Local Government abattoirs were thoroughly examined for tuberculous lesions. Suspected tissues were then confirmed by simple acid fast staining, using an impression smear technique. The results indicated three Local Government areas (Mubi South, Yola and Mubi North) with the highest mean prevalence of 14.4%, 9.4% and 8.4%, respectively, of *Mycobacterium bovis*, while two Local Government areas (LGAs) Fufore and Ganye recorded a mean prevalence of 6.6% and 4.0% respectively. Eleven LGAs (Demsa 0.3%, Ganye 4.0%, Jada 0.9% Hong 0.8%, Madagali 2.1%, Girei 2.7%, Toungo 1.6%, Larmorde 2.7%, Mayo-belwa 1.5%, Michika 3.5% and Gombi 0.4% observed low mean prevalences of *M. bovis*. This finding is of prime public health significance; given the fact that pasteurization of milk is most often not adequately done and abattoir workers hardly wear protective clothing when handling carcases. Most of these cattle belong to the nomadic fulanis that consumed raw fermented milk which is a common practice by the herdsmen. With the spread of droplet nuclei from infected animals to susceptible in-contact animals and humans, there is the need for proper control measures and characterisation of the Mycobacterium organism. This study established that tuberculosis is still a problem in the study area.

Animal health and production

21. African swine fever: Peculiarities of manifestation in Ukraine

SA Nychyk¹, MP Sutyuk ¹, OM Nevolko², LV Marushchak²

¹Institute of Veterinary Medicine of NAAS, Kyiv, Ukraine; ²National Research Institute for Laboratory Diagnostics and Veterinary-and-Sanitary Expertise, Kyiv, Ukraine; snp1978@ukr.net

Background: African swine fever (ASF) is the most dangerous epizootic infectious disease in swine farming. ASF affected countries in 2012 include 18 countries in Africa, the Caucasus region, the island of Sardinia and Ukraine.

Objective: To characterize ASF outbreaks in 2012 in the territory of Ukraine with the use of epidemiological, clinical, postmortem and laboratory research methods.

Materials and methods: The following diagnostic studies have been used: epidemiology (comparative historical description, inspection of ASF affected locations); clinical symptoms of illness in swine; postmortem changes; laboratory (haematologic – estimate of leucocytes amount in blood, virologic – immunofluorescence test, ELISA and haemadsorption test, molecular – real-time PCR and phylogenetic analysis of sequences of ASFV DNA fragments). Tissues (blood, spleen, lymph nodes, kidneys) taken from swine that died from ASF was used in the study.

Results: ASF was confirmed by MFA and real-time PCR in tissues taken *post mortem* from domestic swine in Zaporizhia Province, Ukraine in 2012. Haematology studies confirmed leukocytopenia (4,0-4,2/mcl). ASF virus was confirmed by haemadsorption on initial culture of leucocytes, real-time PCR, and ELISA (IFA-antigen). No antibodies were found in spleen. ASF virus titer in the hemadsorption test was >108 HAD₅₀/ml. Sequencing results displayed 100% homology of ASF virus in Ukraine in 2012 with Caucasian and Russian isolates, belonging to genotype II with appropriation of collection number UKR/2012 Zapo (Madrid, Spain). ASF cases in 2012 showed an acute course and duration of illness of 2-3 days. Timely measures against infections allowed control of the outbreak within 3 days.

Conclusion. Diagnosis of ASF in Zaporizhia Province, Ukraine in July 2012 was made on the basis of epidemiological, clinical, postmortem and laboratory research methods. Sequencing results in the ASF reference center, Madrid, Spain showed 100% homology of isolated material of ASF virus in Ukraine in 2012 with Caucasian and Russian isolates, belonging to genotype II.

22. Assessing dairy cattle welfare in different housing systems in Bangladesh using animal- based measurements and farm records

MA Jabbar¹, MR Alam², JM Regenstein³, AH Shaikat², MB Hossain², SKMA Islam², Monalisha¹; MM Hassan²

¹Research Associate, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh; ²Department of Physiology, Biochemistry and Pharmacology, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh; ³Department of Food Science, Cornell University, Ithaca, New York, USA; jabbar_cvu@yahoo.com

Although farm animal welfare has been of considerable concern in most developed countries, the concern for animal welfare in Bangladesh is just emerging. The drive to increase dairy cattle production is being done while the animals' wellbeing is being overlooked. However, interest in dairy cattle welfare is slowly growing, consistent with the rising quality of life and education level. Moreover, to gain access to global markets, satisfy consumer concerns, produce healthy food and ensure the good health of both people and animals, it is vital to ensure the optimum welfare of production animals.

The present study will assess the welfare status of dairy cattle in urban and peri-urban dairy farms in Bangladesh. A total of 30 dairy farms will be selected randomly around Dhaka and Chittagong City and will be visited at least four times during the year. The study is designed to measure compliance with the five freedoms for animals as described by the UK Farm Animal Welfare Council. The research involves direct observation of animals, housing conditions and farm records. Housing conditions will be evaluated by examining floor structures, sloppiness of the facilities, cleanliness, space allowance per cow, ventilation systems, waste management systems, water sources (qualitatively and quantitatively), feeding trough size and fly infestations. Farm records will be examined along with asking farmers about average milk yield, the prevalence of mastitis, milk fever, dystocia, lameness and sudden death, and the number of animals sold for slaughter in the previous year. Finally, animals will be observed *in situ* to determine variables such as respiration rate, rumen status, BCS, body temperature, number of fresh bruises and injuries, dirtiness scores, hock lesions, claw lesions, and lameness scores. Data will be analyzed using statistical software.

This study will serve to benchmark the current welfare status of dairy cattle in Bangladesh and to provide a basis for potential advice that can be given to the farmers and scientific community to improve dairy cattle welfare, including legislation for improving dairy cattle welfare.

23. Comparing the potency of two killed and a live infectious bursal disease vaccine in commercial chicks

MT Angani¹, PA Abdu², L Sa'idu³, HM Kazeem⁴

¹College of Veterinary Medicine, Federal University of Agriculture Makurdi; ²Department of Veterinary Medicine, Ahmadu Bello University; ³Veterinary Teaching Hospital, Ahmadu Bello University, Zaria; ⁴Department of Veterinary Microbiology, Ahmadu bello University, Zaria; maryangani@gmail.com

This study evaluated and compared the potency of two killed and a live commercial infectious bursal disease (IBD) vaccine in chicks. Two hundred and eighty, day old chicks were divided into four groups of seventy chicks each. Group 1, 2 and 3 were vaccinated with live vaccine, killed vaccine (A) and killed vaccine (B) respectively while group 4 was not vaccinated. Vaccination was conducted at 9 and 16 days of age with Group 1 vaccinated orally and groups 2 and 3 vaccinated intramuscularly via the leg muscles. Blood was collected from each group weekly from 2 to 44 days of age.

Five chicks from each group were sacrificed weekly and the bursa to body weight ratio (BBWR) were determined from 2 to 37 days of age. Twenty chicks from each group were challenged with a very virulent infectious bursal disease virus (vvIBDV) at 30 days of age. Agar gel precipitation tests done on the sera revealed that the group vaccinated with live vaccine had the highest percentage of chicks with antibody followed by group 2 (killed vaccine A). Antibodies were not seen in chicks in groups 3 (killed vaccine B) and 4 (unvaccinated). Enzyme-linked immunosorbent assays performed on the sera revealed that vaccination with live vaccine induced the highest antibody titre (2 757 \pm 897) followed by group 2 (893 \pm 458) then group 3 (404 \pm 179) and

group 4 (331 ± 168) had the least. The chicks vaccinated with live vaccine did not come down with clinical disease, mortality and gross lesions when challenged at 30 days of age with vvIBDV. Killed vaccine A also protected chicks against clinical disease, gross lesions and mortality. Vaccination with killed vaccine B failed to protect chicks against clinical disease, gross lesions and mortality. The unvaccinated and challenged chicks were also susceptible to clinical disease, gross lesions and mortality. The mortality rate of groups 1 to 4 were 0, 0, 55, 65 per cent respectively. The bursal weights and BBWR of chicks in the four groups were not significantly different from 2 to 16 days of age. From 23 to 37 days of age, there was significant difference in bursal weight between group 1 and 2, group 1 and 3 and group 1 and 4 at P value 0.0035, 0.0001 and 0.002 respectively. The bursal weight and BBWR in group 1 was significantly lower than that of other groups. From day 23 the BBWR were significantly different between group 1 and 2, group 1 and 4 at P value of 0.0001.

In conclusion, the live vaccine was potent but caused bursal atrophy. Killed vaccine A was potent and did not cause an adverse effect on the bursa of Fabricius (BF) while the killed vaccine B did not fully protect the chicks against IBD but did not cause an adverse effect on the BF. Killed vaccine A is recommended for the control of IBD in Nigeria and the potential of live vaccine to cause immunosuppression should be investigated.

24. Current status of avian influenza in Ukraine

AM Golovko¹, <u>VO Postoienko²</u>, MA Sapacheva¹

¹State Research Institute of Laboratory Diagnostics and Veterinary-Sanitary Expertise , Kyiv, Ukraine; ²State Scientific Control Institute of Biotechnology and Srains of Microorganisms, Kyiv, Ukraine; vpostoenko@ukr.net

Background: Avian influenza is a dangerous highly contagious disease with an acute course characterized by clinical signs of septicaemia and lesions of the respiratory and digestive systems. It occurs in more than 50 countries and causes significant economic losses. According to classification of the World Organisation for Animal Health (OIE), avian influenza virus belongs to the category of dangerous diseases of poultry.

The epizootic situation in the world relating to avian influenza remains problematic. According to the OIE there were outbreaks of highly contagious avian influenza (H5N1) recorded in five countries during early 2012. Therefore, continuous monitoring of avian influenza in individual countries is an important element in the control of this pathogen.

Goal: To study the prevalence of avian influenza in Ukraine for the period 2005-2012.

Methods: The study was performed on specimens that were submitted to the state veterinary laboratories of Ukraine and the Laboratory for Diagnostics and Veterinary-Sanitary Expertise at the State Research Institute.

Serological testing of blood sera from birds was performed by the haemagglutination inhibition test as recommended by the OIE. We used the "Set of antigens and sera for diagnosis of avian influenza in the haemagglutination inhibition assay" from JSC "Pokrovsky plant biologics" (Russia).

Specimens collected from birds were tested by polymerase chain reaction (PCR). Commercial kits from both domestic and foreign manufacturers, namely Bird-Flu-PCR (Ukrzoovetprompostach, Ukraine), avian influenza, A-5, A-7 (Amplisense, Russia) and Quagen (USA) were used.

Results: Several populations of birds (n = 1 486 087) were sampled during the period 2005-2012. These included birds from poultry farms (n = 595 050), birds from the private sector (n = 791 491), wild migratory birds (n = 47 139), synanthropic birds (n = 51 299), and zoo birds (n = 1 108).

In 2005, specific antibodies to different serotypes of influenza virus were detected in the blood serum of birds from 18 regions in Ukraine. Avian influenza virus was detected by PCR in gees from three poultry farms and 10 wild ducks in the Vinnytsia region. Antibodies to H5N1 were detected in the Odessa region. Antibodies to the H5N1 virus were also detected in six samples of geese blood serum from poultry farms in the Poltava region. According to data obtained in 2006, 2 172 samples tested positive to specific antibodies of different strains of avian influenza. In 2008, 99 samples tested positive of which 26 birds were from poultry farms, 59 bird from the private sector, and 14 were wild birds.

Influenza strain H5N1 was detected in poultry farms of Crimea, as well as birds from the private sector of Crimea and Sumy region, in wild birds in the Crimea, Kherson and Odessa, in synanthropic birds in the Crimea. During the period 2009 to present avian influenza virus has not been identified in Ukraine.

Conclusions:

- 1) The results shows that for the period from 2005 to 2008 outbreaks of highly pathogenic avian influenza virus occurred in Ukraine.
- 2) Surveillance studies have shown that from 2009 to present Ukraine is free from avian influenza.
- 3) Continuous monitoring of avian influenza virus is an important element in the control of this pathogen in Ukraine.

25. Development of a PCR protocol for food safety estimation through the detection of *Salmonella* spp.

A P Gerilovych, B T Stegniy, I O Gerilovych, V L Arefiev, O S Solodiankin, K V Glebova

National Scientific Center, Institute of Experimental and Clinical Veterinary Medicine (NSC IECVM), Ukraine, National Agriculture Academy of Science, 83, Pushkinskaya, Kharkiv, 61023; admin@vet.kharkov.ua

Salmonellosis is a worldwide medical and veterinary problem. The main risks for humans associated with *Salmonella* are associated with contaminated food. This research was aimed at the establishment of effective means for pathogen detection in raw materials of animal origin through the development of genus- and species-specific oligonucleotide systems for *Salmonella* detection. Primers for the detection of bacterial DNA of the genus *Salmonella* and serotypes of *Salmonella enterica* enteritidis and typhimurium were created and tested.

The study was carried out employing bioinformatic methods. Oligonucleotides were created after the analysis of major genes and entire genomes of different species of *Salmonella* obtained from GenBank nucleotide sequence databases. The sequence analysis of conserved regions was obtained using BioEdit (v.7.2.4). The oligonucleotides were designed according to the principles of species and interspecific stability, sequence specificity, and specificity to certain taxa using AmplifX 1.5 software. The primer sequences were tested by the methods of local and global comparison developed in NSC IECVM.

Analysis of the nucleotide sequence databases showed that the greatest breadth of sample homogeneity and characteristic genes sequenced parts InvA and 16S rRNA, containing conserved fragments for all bacteria of the genus *Salmonella*. The oligonucleotide sequence search resulted in 22 potential primer pairs for the *Salmonella* InvA gene in different regions with a length of 230-720 bp. Microanalytic study of these oligonucleotide pairs showed 100% compliance of one of them (named Salm3_4) in a 380 bp region. For S. serovariant *enterica enteritidis*, the SefA gene was selected as the target. Primers for the 420 bp specific region for S. serovariant *enterica enteritidis* were found. These oligonucleotides were named SentF_R.

The FliC gene was determined as the target for the detection of S. serovariant *enterica typhimurium*. The calculation of the oligonucleotides showed the presence of six primer pairs, one of which called StypF_R, targeted a gene fragment with a length of 320 bp.

The quality of the oligonucleotide pairs showed that degenerate and palindromic areas as well as signs of secondary structure formation were absent. The difference in the melting temperature of the primers was designed to be less than 1 °C. They were 100% complementary to the *Salmonella* DNA template and a had low pair and individual match (<65% and <85%) in relation to similar and heterologous matrices.

The primer systems Salm3_4, SentF_R and StypF_R, flanking fragments with the length 380, 420, and 320 bp correspondingly, have been developed for *Salmonellas* detection in multiplex PCR. They are characterized by high specificity providing satisfactory detection and PCR-quality.

26. Effect of a supplementation with *Euphorbia heterophylla* on nutritional meat quality of guinea pigs (*Cavia porcellus L*.)

N'GDV Kouakou¹, J-F Grongnet², NE Assidjo³, E Thys⁴, P-G Marnet², D Catheline⁵, P Legrand⁵, M Kouba²

¹Département de Formation et de Recherche Agriculture et Ressources animales, Institut National Polytechnique Félix Houphouët-Boigny, B.P. 1313 Yamoussoukro, Côte d'Ivoire; ²Unité Mixte Recherche (UMR) PEGASE INRA-Agrocampus Ouest, 65 rue de Saint-Brieuc, 35042 Rennes cedex, France; ³Département de Formation et de Recherche Génie chimique Agro-alimentaire, Institut National Polytechnique Félix Houphouët-Boigny, B.P. 1313 Yamoussoukro, Côte d'Ivoire; ⁴Institut of Tropical Medecine, Department of Animal Health, Nationalestraat 155-B-2000 Antwerp, Belgium; ⁵Laboratoire de Biochimie-Nutrition Humaine, Agrocampus Ouest-INRA 65 rue de Saint-Brieuc, 35042 Rennes cedex, France; kwayki@ yahoo.fr

Euphorbia heterophylla is a very good forage for guinea pigs (1). Its high availability, palatability and digestibility and low fiber content (22% of dry matter) makes this weed one of the most ingested by guinea pigs in Africa (2)(3). The aim of the present work was to study the effect of dietary supplementation with *Euphorbia heterophylla* on the quality of the guinea pig meat. Forty guinea pigs were divided into two groups and fed *ad libitum* for 46 days, a *Panicum maximum* diet (Panicum diet) or a mixed diet (75% *Panicum maximum* + 25% *Euphorbia heterophylla*; Paneuphorbia diet) to compare their effect on performance and composition of guinea pig tissue and carcass.

Daily weight gain, liver weight, carcass yield, and the lipid content of both the carcass and the perirenal fat were significantly increased by the Paneuphorbia diet. Feeding Paneuphorbia diet increased (P<0.05) the omega 3 polyunsaturated fatty acids (PUFA) content in perirenal fat, muscle, liver and in the carcass, and decreased (P<0.05) the omega 6 to omega 3 ratios in all these tissues and the carcass.

This study shows that *Euphorbia heterophylla* is a source of omega 3 fatty acids which can improve significantly the omega 3 PUFA content of guinea pig meat and carcass (4).

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	Diets	MUFA ^{1,2}	PUFA ^{1,2}	C18:3 n-3 ^{1,2}	C18:2 n-6 ^{1,2}	Σ n-3 ^{1,2}	Σ n-6 ^{1,2}	Σ n-6/ Σ n-3 ^{1,2}
Muscle tibialis cranialis	Panicum	10,9 ±0,7	50,5 ± 1,5	6.1 ± 1.4	24.6 ± 0.7	16.3 ± 2.0	34.2 ± 0.7	2.1 ± 0.3
	Paneuphorbia	10,3 ±1,8	51,7 ± 2,6	7.7 ± 1.1	24.9 ± 1.1	18.2 ± 1.6	33.4 ± 1.7	1.8 ± 0.2
Liver	Panicum	6,4 ± 0,7	53,6 ± 0,8	6.9 ± 0.9	32.3 ± 0.7	12.7 ± 1.3	40.8 ± 0.6	3.2 ± 0.4
	Paneuphorbia	6,4 ± 0,5	53,3 ± 2,9	9 ± 0.7	30.4 ± 2.6	15.3 ± 1.3	37.8 ± 2.4	2.5 ± 0.3
Perirenal adipose tissue	Panicum	15,3 ± 0,6	50,4 ± 1,1	29.7 ± 1.8	19.6 ± 1.3	30.4 ± 1.9	20.1 ± 1.4	0.7 ± 0.1
	Paneuphorbia	13,5 ± 1,3	53,3 ± 2,2	35.6 ± 2.8	16.6 ± 0.9	36.2 ± 2.9	17.1 ± 0.8	0.5 ± 0.1
Carcass	Panicum	15,4 ± 1,3	51,1 ± 1,5	25.2 ± 0.6	20.0 ± 0.3	28.2 ± 1.0	22.9 ± 0.6	0.8 ± 0.0
	Paneuphorbia	15,2 ± 1,5	51,9 ± 1,4	31.2 ± 1.4	16.9 ± 0.7	33.4 ± 1.8	18.4 ± 0.5	0.5 ± 0.0

¹ mean ± SEM; (n = 20 per diet)

² Fatty acid composition (% of total fatty acids)

27. Effect of improved macro-economic regime on small-scale farmers' capacity to access and manage cattle in Sengwe Communal Land, Zimbabwe

S Zishiri¹, <u>M De Garine-Wichatitsky</u>^{2,3}, A Caron^{2,3}, B Mukamuri¹

¹Centre for Applied Social Sciences, Uni. Of Zimbabwe, Harare, Zimbabwe, ²Cirad-Zimbabwe, Harare, Zimbabwe, ³Cirad, Montpellier, France; michel.de_garine-wichatitsky@cirad.fr

This study evaluated the effect of changes in the macro-economic regime on small-scale farmers' access to cattle and overall livestock management, especially disease control, in the South East Lowveld of Zimbabwe. Zimbabwe's economy suffered from hyperinflation since the late 1990s. The economic situation improved when the government adopted a multicurrency regime in 2009, which may have changed opportunities and strategies regarding livestock keeping because of the high monetary and cultural values of cattle. Our hypothesis was that the relative macro-economic stability following the adoption of a multi-currency system in 2009 resulted in a change of local farmers' perspective regarding cattle ownership and management, including social, economic and financial opportunities and constraints related to veterinary service provision. We implemented two quantitative surveys, in 2006-7 and 2011-12, using structured questionnaires (n = 150 in and n= 180) and key informant interviews, followed by focus group discussion in three villages in the Western part of the Chiredzi district.

Our results indicate that the establishment of the multicurrency system had a positive impact on the way farmers in the area accessed and managed cattle. More local markets, fewer middle men, a slightly easier access to credit and more farmer associations revitalized cattle trade, even reducing apparently the illegal export of cattle to Mozambique. Increased support to farmers by active veterinary services and a better access to veterinary drugs also improved the control of livestock diseases (as perceived by farmers). We conclude that the multi-currency system improved cattle management and access to animal market through a self-organisation of farmers adapted to the new market and economic conditions, and more effective agricultural extension services.

28. Epizootic Monitoring of Aujeszky's disease in population of wild boars in Ukraine

SA Nychyk, <u>MP Sytiuk</u>, IM Kalabekov

Institute of Veterinary Medicine (IVM) NAAS of Ukraine, Kiev, Ukraine; snp1978@ukr.net

Background: Aujeszky's disease is a contagion of all types of agricultural animals, furred animals (rabbit, fox, polar fox), wild animals (boar, badger, rodent, insectivorous bat) and poultry (turkey, chicken, pigeon etc.). The virus infects the cerebrum and spinal cord with clinical signs of severe itching and scratching, of which the latter is not specific for swine. Laboratory methods used for detection and identification of the virus include the polymerase chain reaction (PCR) technique, virus neutralization assays and enzyme-linked immunosorbent assays (ELISA). The ELISA and virus neutralisation assays are widely used to assist in the diagnosis of Aujeszky's disease and are prescribed tests by the World Organisation for Animal Health (OIE).

Objective: To determine the seroprevalence of Aujeszky's disease in a population of wild boar in Ukraine and to detect the DNA virus in collected specimens using molecular techniques.

Methods: Blood serum (n = 3 462) and organ samples (lymph nodes and spleen) (n = 328) were collected from killed wild boars during the hunting seasons between 2001 and 2011. Blood sera were tested by virus neutralisation assay for specific antibodies to the virus of Aujeszky's disease. Real-time PCR was used for the detection of the DNA virus in the lymph node and spleen specimens.

Results: During 2001 to 2010 the wild boar population in Ukraine increased 1.8 times, with the total number of wild boars at the end of 2010 estimated at 61 800 animals. Positive cases of Aujeszky's disease were detected annually during this 10-year surveillance period with an average seroprevalence of 13,46%. Six of the lymph node and spleen specimens were positive for virus DNA and we were able to isolate virus from all six the specimens. These were further characterised by phylogenetic analysis. Conclusion: Our surveillance confirms the presence of Aujeszky's disease virus in the wild boar population of Ukraine. These animals could act as a reservoir for the virus and further studies are needed to determine the role of the wild boar in the epidemiology of Aujeszky's disease amongst domestic swine in Ukraine.

29. Evaluation of berries of *Phytolacca dodecandra* for growth inhibition of *Histoplasma capsulatum var. farciminosum* and treatment of cases of epizootic lymphangitis in Ethiopia

NM Asrese¹, E Makonne², N Aklilu³, G Ameni⁴

¹Department of Biomedical Science, College of Veterinary Medicine, Haramaya University, PO. Box 138, Dire Dawa, Ethiopia; ²Department of Pharmacology, School of Medicine, Addis Ababa University, PO Box 1176, Addis Ababa, Ethiopia; ³Society for the Protection of Animals Abroad (SPANA), School of Veterinary Medicine, Addis Ababa University, PO Box 34, DebreZeit, Ethiopia; ⁴Aklilu Lemma Institute of Pathobiology, Addis Ababa University, PO Box 1176, Addis Ababa, Ethiopia; mnegesse@yahoo.com

Epizootic lymphangitis (EL) is a contagious, chronic disease which mainly affects horses, mules and donkeys. It is caused by *Histoplasma capsulatum var. farciminosum* (HCF). The disease is highly prevalent and economically important in Ethiopia. Currently there is no effective treatment option in Ethiopia, which demands the need for other approaches including the use of traditional remedies. In this study the effect of *Phytolacca dodecandra* (*P. dodecandra*) was evaluated, for its effect on HCF and for the treatment of cases of EL on horses from September 2010 to July 2011.

Samples were collected from unruptured nodules of cases of EL at Debre Zeit and Akaki (Central Ethiopia) and cultured at Aklilu Lemma Institute of Pathobiology (ALIPB). Phytochemical screening was done for n-butanol extract of *P. dodecandra* to detect alkaloids, saponins, phenolic compounds and flavonoids. The minimum inhibitory concentrations (MICs and minimum fungicidal concentrations (MFCs) of aqueous and *n*-butanol extracts of *P. dodecandra* against HCF were determined by agar dilution assay. For the *in vivo* trial, 5% simple ointment was prepared from *n-butanol* extract and applied topically to 24 horses (12 early and 12 moderate cases) of EL.

Phytochemical screening showed that *n*-butanol extract of *P. dodecandra* was positive for alkaloids, saponins and phenolic compounds but negative for flavonoids. The MICs of *n*-butanol and aqueous extracts of *P. dodecandra* were (0.039%-0.078%) and (0.625%-1.250%), respectively. The MFCs of *n*-butanol and aqueous extracts of *P. dodecandra* were (0.078%-0.156%) and (1.25%-2.5%), respectively. The MIC and MFC of ketoconazole (positive control) was (1.2 x 10^{-5} %-2.5 x 10^{-5} %) and (5x 10^{-5} %-1x 10^{-4} %), respectively while growth was observed on free medium (negative control). From the total of 24 treated cases of EL, 14 (58.3%) responded to treatment; however, 10 (41.7%) did not respond to treatment. There was no significant difference in the degree of response to treatment between early and moderate cases (χ^2 = 0.686; P=0.408). It can be concluded that *n*-butanol extracts of *P. dodecandra* demonstrated antifungal effects while the aqueous extracts showed no antifungal activity. We recommend pursuing further studies on the *in vitro* antifungal effect of the fractions of N-butanol extract, an *in vivo* study in

larger populations, possible mechanism(s) of antifungal action, and pharmacokinetic and adverse reactions of the extract when used systemically.

30. Evaluation of the prevalence and economic impact of bovine tuberculosis at SABAN-Nyabugogo abattoir, Rwanda

<u>G Habarugira</u>¹, J Rukelibuga², M Opiyo Nanyingi³, B Mushonga⁴

¹Umutara Polytechnic, Veterinary Medicine, Nyagatare/RW; ²CDC Rwanda, Avian Influenza Specialist, Kigali/RW; ³University of Nairobi, Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, Nairobi/KE; ⁴University of Fort Hare, Department of Department of Livestock and Pasture Science, Faculty of Science and Agriculture, Alice/ZA; gervaish@gmail.com

The prevalence of human tuberculosis (normally caused by *Mycobacterium tuberculosis*) in Rwanda was estimated to be 0.08% in 2008 (1). It has been demonstrated that cattle tuberculosis (caused by *Mycobacterium bovis*) plays an important role in human tuberculosis prevalence (2, 3). However, there are no records of the nationwide prevalence of cattle tuberculosis in Rwanda.

The objectives of this study were to assess the prevalence of bovine tuberculosis, to review the role of mycobacterial species in the transmission of bovine tuberculosis and to evaluate the economic impact of bovine tuberculosis (BTB) in the meat industry in Rwanda.

Routine meat inspection was conducted on 16753 animals slaughtered at SABAN Nyabugogo abattoir in Kigali, Rwanda, over a period of 11 months to determine the prevalence of bovine tuberculosis. At the same time, laboratory examination of 36 samples randomly obtained from the condemned organs was also conducted. The samples comprised of 31 lymph nodes (*retropharyngeal, mediastinal, tracheobronchial*), 3 samples of lungs and 2 samples from the liver. Microscopy with Kinyoun stain, culture on Löwenstein-Jensen and Colestos media and biochemical tests for species identification were used to confirm suspected cases of bovine tuberculosis.

The results of our routine gross examination study showed that the prevalence of tuberculosis was 0.9%. The major risk factors were age, sex and province of origin. Animals of 2 or more years had a higher prevalence (1.6% vs. 0.6%, P<0.05, n=16 753). The prevalence is higher in females than in males (1.4% vs. 0.6%, P<0.05, n=16 753) and the Eastern province had the highest prevalence (1.3% vs. 0.9%, P<0.05, n=16 753). Only about 72.2% of the samples picked up by routine gross examination as being positive for tuberculosis tested positive with the Kinyoun laboratory staining technique and mycobacteria were isolated in only 63.9% of the 36 samples. Mycobacteriam bovis was present in 91.3% of cases of bovine tuberculosis transmission while 8.7% were due to atypical mycobacteria. In the 11 months of the study bovine tuberculosis caused condemnation of 1683.5 kg of meat and thereby resulting in Rwanda losing US\$ 4,810.

The results of this study show that the prevalence of bovine tuberculosis in Rwanda is relatively low and the methods of control of this disease are working fairly well. However, there is concern that atypical *mycobacteria* may be playing an important role in human *tuberculosis* in immune-compromised patients as their prevalence of 8.7% in positive bovine cases is high. This study has also shown that none of the diagnostic methods used was accurate because of the variation in the sensitivity from a test to another. More information can be obtained if more sensitive techniques like RT-PCR are employed. Since there is no information about prevalence of BTB in Rwanda, our results can serve as baseline for further studies.

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31. First evidence of Schmallenberg virus infection in exotic and indigenous cattle in Nigeria

DO Oluwayelu¹, CA Meseko², Al Adebiyi¹

¹Department of Veterinary Microbiology and Parasitology, University of Ibadan, Ibadan, Nigeria; ²Virology Division, National Veterinary Research Institute, Vom, Nigeria; ogloryus@yahoo.com

Infections with Schmallenberg virus (SBV), a recently emerged orthobunyavirus of the Simbu serogroup, are associated with fatal congenital malformations in ruminants. The virus is transmitted by *Culicoides* biting midges of which several species that feed

on domestic ruminants have been identified in Nigeria. Cases of abortions, stillbirths and malformed fetuses which are typical of SBV infections have been recorded in cattle, sheep and goats nationwide. It is hypothesized that SBV infection is prevalent in Nigeria and its diagnosis might have been missed due to similar clinical signs and pathological lesions caused by other known infectious agents. We investigated the presence of SBV antibodies in a limited number of exotic and indigenous cattle as a first step towards determining the status of SBV infection in Nigeria.

Blood was collected from 120 exotic and indigenous cattle, namely 60 Friesians, seven Jerseys and 53 indigenous cattle. The Friesians were located in north-central Nigeria while the Jerseys and indigenous cattle were in the southwest region. The 67 exotic cattle and most of the indigenous cattle were managed intensively on farms. A small number of the indigenous cattle were transported from the northern part of the country for slaughter in major cattle markets in southwest Nigeria. Test sera were assayed for SBV antibodies using a commercial indirect ELISA kit (IDVET, Montpellier, France) that detects antibodies against recombinant SBV nucleoprotein in bovine, ovine or caprine sera.

Anti-SBV antibodies were detected in 22 (32.8%) exotic cattle sera: Friesians 26.9% (18/67), Jerseys 6.0% (4/67), and indigenous cattle 23 (43.4%). Overall, 45 (37.5%) of the 120 tested sera were positive for anti-SBV antibodies, 71 (59.2%) were negative while 4 (3.3%) yielded doubtful results.

This study provides the first evidence of SBV infection in ruminants in Nigeria. The detection of anti-SBV antibodies in exotic and indigenous cattle indicates natural exposure of these animals to SBV since there is no vaccine available against the virus. Moreover, the fact that seropositive animals were detected among exotic and indigenous cattle located in different geographical regions of the country suggests widespread exposure to SBV when *Culicoides* vectors are most active. It can be concluded therefore that SBV infections may be responsible for some of the abortions, stillbirths and malformed fetuses observed in ruminants in Nigeria. Thus, SBV poses a threat to naive populations of ruminant livestock in Nigeria. While vector control strategies are advocated, there is also a need to isolate and characterize circulating SBV strains in cattle, sheep, goats and *Culicoides* in Nigeria.

32. Frequency of dehydration and metabolic depletion in cattle and water buffalo transported from India to a livestock market in Bangladesh

MR Alam¹, NG Gregory², MA Jabbar³, MS Uddin³, JP Widdicombe², ASMG Kibria¹, MSI Khan⁴ and A Mannan¹

¹Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh; ²The Royal Veterinary College, University of London, Hatfield AL9 7TA, UK; ³Research Associates, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh; ⁴Patuakhali Science and Technology University, Patuakhali, Bangladesh; jabbar_cvu@yahoo.com

The effects of presumed long distance transport on blood parameters were examined in cattle and water buffalo exported from India to Bangladesh. The aim was to assess the welfare of the animals in terms of the frequency of dehydration, metabolic depletion and muscle injury or activation when they reached their destination. The physiological indicators were total plasma protein, serum sodium, plasma glucose, serum non-esterified fatty acids (NEFA) and serum creatine kinase (CK).

Ninety-eight cattle and 57 water buffalo were randomly selected from 24 trucks as they arrived at the market between 0700h and 1000h each day during the hot season (April). In the majority of animals, all five blood variables were higher than published normal values. In all the cattle, total plasma protein concentration, serum NEFA concentration and serum CK activity were higher than the normal range and these values were higher than normal in over 84% of the buffalo. Seventy-two percent of all the animals were hypernatraemic. About 90% of the buffalo and 86% of the cattle were hyperglycaemic. There were no differences in total plasma protein and serum sodium between cattle and water buffalo. By contrast, cattle had significantly higher NEFA and CK and lower glucose than water buffalo.

It was evident that this long distance export trade was associated with dehydration, lipolysis and muscle injury or activation. It is recommended that both cattle and water buffalo are given adequate feed and water whenever they are off-loaded from vehicles during the course of journeys.

33. Interactions between ticks, hosts and pastures in subequatorial zones

A G Zoffoun^{1,3}, S Salifou², M Houinato³ and A. B. Sinsin³

¹National Institute of Agricultural Researches of Benin (I.N.R.A.B.), 01 PO Box 884 Cotonou, Benin; ²Polytechnic School of Abomey-Calavi, University of Abomey-Calavi (UAC), 01 PO Box 2009 Cotonou, Benin; ³Laboratory of Applied Ecology, Faculty of the Agronomic Sciences, University of Abomey-Calavi (UAC), 01 PO Box 526, Cotonou, Benin; zofalex@yahoo.fr

On the Kpinnou ranch in subequatorial zone of Bénin, Girolando dairy cattle (imported from Brazil) grazing Panicum maximum var. C1 appeared to be more susceptible to infestation of ticks, leading to abandonment of grazing this highly productive forage.

Therefore, the purpose of this study was to highlight the interactions between ticks and their bovine hosts on two types of artificial grazing pastures.

Nineteen young Girolando dairy cattle were used during the big rainy season. The following parameters were used to constitute two relatively homogeneous groups of ten and nine animals: age, weight, gender, coat color and loads in ticks. One group of ten animals (Group 1) was grazed on Panicum maximum while a second group of nine animals (Group 2) grazed on Panicum maximum var. C1. During the two months of grazing, weekly monitoring of the tick burden was conducted, comprising a count of all ticks on the animal by bodily region (head, ears, neck, back, declivitous region, ano-genital region, paws and tail).

Four genera of ticks of eight species have been identified: Amblyomma (1 species): A. variegatum; Hyalomma (2 species): H. truncatum and H. rufipes; Rhipicephalus (2 species): R. sanguineus and R. evertsi; Boophilus (3 species): B. decoloratus, B. geigy and B. annulatus. The Boophilus genus is the most abundant (P<0.05), most likely due to the monophasic cycle in this species, unlike the other three genera (Amblyomma, Hyalomma and Rhipicephalus), which have a biphasic or triphasic cycle.

The animals grazed on Panicum maximum var. C1 were more infested than those that grazed Panicum maximum. This could be explained by fundamental differences noted in their characteristics. A variation of the rate of infestation of the animals was observed in relation to the different body areas. The colour of the hide also had an effect on the tick burden, with dark cattle being more infested (P<0.05) than those of a lighter colour

34. Modeling of the replacement of *Rhipicephalus (Boophilus) decoloratus* by *Rhipicephalus (Boophilus) microplus,* an exotic emerging tick in Ivory Coast

M Boka¹, M Madder², D Berkvens²

¹Department of Animal Health, Veterinary Services, Ministry of Livestock, BPV 84 Abidjan, Ivory Coast; ²Department of Biomedical sciences, Institute of Tropical Medecine (ITM), Nationalstraat 155, 2000 Antwerp; marcelboka2@yahoo.fr

Considered as one of the most important arthropoda harmful to livestock, the tick *Rhipicephalus (Boophilus)* microplus is known for its resistance to most acaricides, its high invading potential and enormous economic consequences associated with its infesting cattle. This tick, discovered for the first time in west Africa, in Azaguié, in the south of Ivory Coast, is replacing other species of the same genus, as has been reported by several researchers in southern Africa. This phenomenon, reported in 2008 with 96% of the replacement of *R. (B.) decoloratus* around Azaguié, is a real obstacle for the development of breeding as this exotic species is reputed to be more destructive than the other species and its emergence could be very harmful to cattle health.

In order to better understand the replacement of *R*. (*B*) decoloratus by *R*. (*B*.) microplus, a statistical model has been developed in R to determine the period necessary for this and the impact of the resistance of the ticks to acaricides in the south of Ivory Coast.

This study showed that the replacement of *R* (*B*) decoloratus by *R* (*B*) microplus is quick due to climatic conditions favouring the development of ticks (year-round high and relative temperature and humidity) and allowing at least four generations per year. Estimates indicate for different levels of resistance, a period of 18 to 45 months and nine to 27 months for a respective replacement of 100% and 96% respectively. *R.* (*B.*) microplus could yield 3.3 times more offsprings than *R.* (*B.*) decoloratus and its increased resistance could be a factor in the shortening of the period of replacement.

35. Monitoring of classical swine fever in wild boars in Ukraine

A Golovko, O Nevolko, L Marushchak, L Dedok, O Litvinchuk

State Research Institute of Laboratory Diagnostic and Veterinary Sanitary Expertise, Kyiv, Ukraine; olegnevolko2010@ukr. net

Problem definition: Classical swine fever (CSF) is recorded in different parts of the world. CSF has been almost eradicated in Ukraine, though lately it has randomly emerged amongst wild boar. Economic losses caused by CSF outbreaks in swine farms are severe and characterized by high morbidity and mortality in all age groups of swine, and by losses connected with long-term quarantine and other disease control measures.

Objective: To control CSF in wild fauna, and to assess risks of CSF in wild boars in Ukraine.

Methods: Applied epidemiological and clinical data, postmortem changes, and information from laboratory diagnostic studies. We used the diagnostic kit for ASF, CSF MFA (Russia) for immunofluorescence, and IDEXX CSVF Ab (IDEXX, USA) for ELISA. For real-time PCR we used the CSF test system, produced by the Russian Agency for Health and Consumer Rights, Moscow, and for immunochromatography we used the Rapid CSFV Test (USA).

Results: Annual CSF monitoring is conducted (during the hunting season) amongst wild boars in Ukraine, with the intent to control CSF in wild fauna and to assess risks. For the period 2009-2012 the Sate Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertize (SRILDVSE) studied 4,616 serum samples from wild boars by ELISA. In 2009 1,284 samples were tested with 103 positive; in 2010 1,282 samples were tested with 83 positive; in 2011 199 samples were tested with 7 positive; in 2012 1,851 samples were tested with 110 positive. In 2012 383 samples of biological material taken from wild boars were tested by real-time PCR, with 1 sample positive. In September 2012 a dead wild boar was identified in the village of Bily berig, Kyiv Province. CSF positive results were obtained by MFA (RIF) method, real-time PCR, ELISA and immunochromatography at SRILDVSE.

Conclusion: Surveillance of wild boars populations in different provinces of Ukraine shows evidence of circulation of CSF virus, that may pose a risk to unvaccinated domestic swine.

36. Occurrence of tick-borne haemoparasites in the African buffalo (*Syncerus caffer*) from northern Botswana

D Eygelaar¹, F Jori^{2, 3}, M Mokopasteso⁴, EM Debeila¹, NE Collins¹, I. Vorster¹, M. Troskie¹, MC Oosthuizen¹

¹Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria; ²Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria; ³RP-PCP, CIRAD, UPR AGIRs, Montpellier, France; ⁴FAO-ECTAD Office for Southern Africa, Gaborone, Botswana; hillfox@gmail.com

Introduction: The African buffalo (*Syncerus caffer*) is host for many pathogens known to cause economically important diseases and is considered an important wildlife reservoir for livestock diseases. Theileriosis, heartwater, babesiosis and anaplasmosis are the most important tick-borne diseases (TBD's) of livestock in sub-Saharan Africa, resulting in extensive economic losses to farmers in endemic areas. There are no official reports on the presence of *T. parva* in Botswana and information on detrimentally significant tick-borne haemoparasites especially in Northern Botswana is scarce.

Aim: The aim of the study was to screen buffalo samples for the presence of *Theileria*, *Babesia*, *Ehrlichia* and *Anaplasma* species using the reverse line blot (RLB) hybridization assay. Samples were also specifically screened for the presence of *T. parva* using a *T. parva* specific 18S rRNA real-time PCR assay.

Methods: DNA was extracted from buffalo blood smear samples obtained from two geographically different areas in Northern Botswana; the Chobe National park (n=64) and the Okavango Delta (n=57). A fragment of the 18S ribosomal RNA (rRNA) gene was PCR amplified using genus-specific primers and amplicons were identified by RLB hybridization to species-specific probes. Samples were also screened using a *T. parva*-specific real-time PCR assay (1).

Results: The RLB results revealed the presence of *Theileria, Babesia, Anaplasma* and *Ehrlichia* species, either as single or as mixed infections. Of the *Theileria* spp. present, *T. parva* (60%) and *T. mutans* (36%) out of a total of 121 samples were most prevalent. Other species of interest were: *Anaplasma centrale* (30%), *A. marginale* (20%), *Babesia occultans* (23%) and *Ehrlichia ruminantium* (6%). Real-time PCR results indicated that 80% of the samples tested positive for the presence of *T. parva*.

Discussion: This is the first report of *T. parva* in the buffalo population from Northern Botswana. Buffalo act as asymptomatic reservoirs for these haemoparasites. When these infected buffalo share the same home ranges with cattle, haemoparasites can be transmitted to cattle through infected tick bites. This information on the circulation of parasites that cause TBD can contribute to raise awareness among veterinary officials and cattle owners so that control measures (prevention of wildlife-cattle contacts, regular dipping) can be implemented to mitigate their economic impact.

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37. Participatory assessment of animal health constraints and husbandry practices in the pig production system in three districts of Uganda

MM Dione, E Ouma, K Rosel, D Pezo,

International Livestock Research Institute, Nairobi, Kenya; M.Dione@cgiar.org

Pork has become increasingly important in Uganda. Whereas pork accounted for only 1-2 % of the 11-12 kg/yr per capita meat consumption in the 1960s, it now accounts for at least a third of the current 10 kg/yr. Pigs help both rural and urban households to improve livelihood security and also serve as a source of cash in times of need. However, animal health constraints have been identified as one of the major limiting productivity factors, but there is limited information on the relative incidence of different diseases and their impact on the pig production. The main purpose of the assessment was to identify animal health and management constraints and opportunities for intervention in the smallholder pig value chains.

Focus group discussions were held with 1400 pig farmers in 35 villages in Masaka, Kamuli and Mukono districts using a semiqualitative interview checklist in order to gain an understanding on the role of pig farmers in the livestock value chain with respect to animal health. Some quantitative data were obtained through group consensus. Findings were further triangulated with secondary data during the exercise.

The findings show that pig housing are dominated by tethering (66 %) and free range/scavenging systems (17 %) in rural settings. In peri-urban and urban settings, 66 % and 87 % of farmers, respectively, confine the pigs in houses. African swine fever (ASF) is the first cause of pig mortality with epidemics commonly occurring during dry season. Worms and ecto-parasites namely mites, lice, midges, ticks and jiggers are endemic and lead to stunted growth which causes reduced market value of pigs. Diarrhea and malnutrition are common in piglets and are associated with poor hygiene and feeding, respectively. Swine erysipelas, Foot and Mouth-Disease are sporadic and appear once or twice a year; 93% of farmers declare practicing deworming of their pigs; 37% practice ecto-parasite spraying and 77% castrate their pigs. Indigenous treatments are sometimes used in replacement of commercial drugs. These treatments include human urine and decoctions of local herbs for ASF and the used disposed engine oil or tobacco extracts for ecto-parasites.

The main constraints identified are poor housing and feeding, weak veterinary services, faked drugs and lack of knowledge on piggery management. However, actions are already being attempted by farmers to solve these constraints. These include formation of groups for capacity building and utilizing professional health workers. To better tackle these constraints, farmers think that government authorities, research and development institutions need to provide them with training on better husbandry practices and health management, and improve their access to funding to enable them improve on the management. Animal health is a limiting factor in the pig production system in Uganda; therefore, there is a need for building capacity of technology service providers and farmers to tackle these problems.

38. Serological monitoring of leptospirosis amongst bovine and swine in Ukraine

VG Skrypnyk¹, <u>VV Ukhovsky²</u>, AA Kucheryavenko²

¹State Scientific Control Institute of Biotechnology and Strains of Microorganisms; ²Institute of Veterinary Medicine of NAAS; ukhovsky@ukr.net

Leptospirosis (icterohemoglobinuria, leptospiral jaundice) is an infectious natural focal zooanthroponotic disease with specific short-term fever, display of anemia, ochrodermia, necrosis of mucus membranes and skin, bloody urine, atony of gastrointestinal tract and weight loss, abortions and birth of unviable fetuses.

Constant interest in leptospirosis infection is stipulated by its role in the infectious pathology primarily in people and animals. Leptospirosis is one of the most significant zoonoses, as the infection causes substantial economic and social losses worldwide (WOOH, 1985). Leptospirosis is annually registered in Ukraine both as sporadic and grouped disease, with severe clinical picture in a substantial percentage of cases and frequent fatal cases. Human mortalities due to leptospirosis in Ukraine are not reducing and over the last years stand at 10-11% of human leptospirosis cases are fatal.

Currently there are over 250 Leptospira serovars combined in 26 serogroups. However, the number of serological groups and Leptospira variants is constantly growing. New strains have been researched and identified by isolation from animals, humans and ambient environment. Leptospirosis cases in animals caused by pathogenic strains of Leptospira which were not previously identified within the country have been registered more frequently.

Objective: To conduct serological monitoring of bovine and swine leptospirosis.

Material and methods: Sera from bovine and swine of various regions of Ukraine were used during this research. Presence of antibodies to Leptospiras was identified by microagglutination test with living antigens of different serogroups.

Results: Serological monitoring was conducted between 2007-2011. During that period, 56761 positive sera of bovine and 32130 sera from swine have been tested. The number of positive sera of bovine varied from 16618 animals in 2007 year to 8006 animals in 2011 year. The number of positive sera of swine varied from 9 278 in 2009 year to 4 551 in 2011 year.

On average 3,0% of bovine sera and 25,0% sera of swine reacted to serogroup *Icterohaemorragiae*, while 1,1% and 4,3 % reacted to serogroup *Canicola*, , 3,2 and 1,4 %, to *Grippotyphosa*, 1,3% and 3,0 % to *Pomona*; 4,9% and 4,1 % to *Tarassovi*, 14,4% and 0,6 % to *Hebdomadis* 11,0% and 1,2 % to *Sejroe*, 2,9 and 31,4 % to *Australis*. In the meantime, 58,2% of bovine sera and 29,0% of sera of swine reacted with 2 and more antigens.

Conclusion: Bovine and swine livestock have considerable levels of sero-positivity to leptospirosis agents in Ukraine.

The majority of cattle sera reacted with antigens of *Hebdomadis* (14,4%) and *Sejroe* (11,0%), and several antigens simultaneously (58,2%). The majority of sera of swine reacted with antigens of *Icterohaemorragiae* (25,0%) and *Australis* (31,4%), and several antigens simultaneously (29,0%).

39. Seroprevalence of Newcastle disease and other infectious diseases in backyard chickens at markets in Eastern Shewa zone, Ethiopia

H Chaka^{1,2}, F Goutard³, SPR Bisschop¹, PN Thompson¹

¹Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, Pretoria, South Africa; ²National Animal Health Diagnostic and Investigation Center, P.O Box 04, Sebeta, Ethiopia; ³Centre de Cooperation Internationale en Recherche Agronomique pour le Developpement Departement ES, Unite AGIRs, Montpellier 34398 Cedex, France; hasscha@yahoo.com

This study was conducted to estimate the seroprevalence of Newcastle disease (ND), *Pasteurella multocida* (PM) infection, *Mycoplasma gallisepticum* (MG) infection and infectious bursal disease (IBD), and to assess the level of concurrent seropositivity during the dry and wet seasons of the year 2010. A total of 234 and 216 sera were collected during the dry and wet seasons respectively from unvaccinated local breed backyard chickens at four live poultry markets in two woredas (districts) of Eastern Shewa zone, Ethiopia, and were tested using commercial enzyme-linked immunosorbent assay (ELISA) kits. The overall seroprevalence of ND, PM, MG and IBD was 5.9%, 66.2%, 57.7% and 91.9% respectively during the dry season, and 6.0%, 63.4%, 78.7% and 96.3% respectively during the wet season. The seroprevalence of MG was significantly higher (*P*<0.001) during the wet season than during the dry season and significantly higher (*P* = 0.002) in Adami-Tulu-Jido-Kombolcha (ATJK) woreda (74%) than in Ada'a woreda (60%). Area and season had no significant effect on the seroprevalence of ND, IBD and PM, indicating the widespread presence of those pathogens throughout the year in the study area. Of all the chickens tested, 85.6% had antibodies concurrently to more than one of the pathogens investigated. Out of the four diseases evaluated, birds were concurrently seropositive to more diseases during the wet season (median = 3) than during the dry season (median = 2) (P = 0.002). As serology is not able to distinguish between strains, further studies are warranted to better understand the circulating strains, their interactions and their economic effect on backyard poultry production in Ethiopia.

40. Standardisation and validation of diagnostic test-kits for enzootic bovine leucosis

OF Blotska

Sector on Control and Industrial Strains, State Scientific and Control Institute of Biotechnology and Microorganism Strains, Kyiv, Ukraine; blotskaya@ua.fm

Background: Enzootic bovine leukosis (EBL) is a viral disease of cattle caused by bovine leukaemia virus (BLV) that belongs to the family *Retroviridae*. Bovine leukaemia virus has a worldwide distribution. If the animal is infected with BLV, it remains infected for life. Animals with EBLV have altered metabolic and biochemical pathways and as a consequence, there is a change in the quality of their milk and meat with accumulation of harmful metabolic products, including tryptophan metabolites witch can be carcinogenic.

There is no treatment or effective vaccine for EBL. Serological tests like agar gel immunodiffusion (AGID) and enzyme-linked immunosorbent assay (ELISAs) have formed the basis for the successful control of EBL. The State Science-Control Institute of Biotechnology and Strains of Microorganisms (SSCIBSM) in the Ukrain is responsible for the harmonization and standardization of methods for diagnostic testing. Reference Standards are used for the verification and quality assurance of different commercial BLV antibody detection kits.

Goal: To develop national reference standards which are calibrated against international standard reagents to assist in the quality control of different serological assays for BLV.

Methods: The calibration of the National Reference Standards was based on the accredited OIE Reference Serum E05, which has been validated against the former Reference Serum E4. The positive serum for manufacturing the National Reference Standard Serum comes from naturally infected cattle. The negative serum comes from healthy cattle.

The dilution of the positive serum, that gives a weak positive result in the test, was an indicator of its sensitivity. Results: We have prepared a set of National Reference Standard Serums that consist of freeze-dried positive (weak-positive in dilution 1:10 in AGID) and negative serums.

We collaborated with Prof. Jacek Kuzmak - Head of OIE Reference Laboratory for EBL (Poland, Pulawy, PIWet). Together with experts from OIE we have examining specificity and sensitivity of our fabricated National Reference Standards of Serums for EBL and obtained positive assessment for their sensitivity and specificity (100%).

National Standard Reagents are now routinely used for the verification of commercial EBL - kits .

Conclusions: We developed a Set of National Reference Standards of Serums for EBL, which is used for the verification of different AGID- and ELISAs commercial EBL kits. The results of our work improved the diagnostics for EBL.

41. Study of introduction of new variants of bird emergent infectious agents in Ukraine

BT Stegniy, DV Muzyka, AB Stegniy, AP Gerilovych

NSC "Institute of Expirmental and Clinical Veterinary Medicine", Kharkiv, Ukraine; admin@vet.kharkov.ua

Background: Avian flu and Newcastle disease belong to emerging infections of agricultural and wild birds. Wild birds are the natural reservoir of these agents. Because they fly thousands of kilometres during seasonal migration they play an important role as viral reservoir in the epidemiology of infectious diseases of humans, animals and birds. During seasonal transcontinental migration, birds gather in big flocks, and fly thousands of kilometres, crossing over different regions. They can easily carry infectious agents from one geographic region to another.

Goal: To study the prevalence of especially dangerous pathogens in populations of wild birds in Ukraine, the risk of introduction of new variants of infectious agents to new geographical regions, and to study the biological properties of these agents.

Methods: During the period of 2006 to 2011 we sampled 66 species of wild birds in the Azov and Black Sea region of Ukraine. Biological material was collected from wild birds at locations of mass gathering in the AR of Crimea, Kherson and Zaporizia oblasts. Specimens were collected from *Pelecaniformes, Ciconiiformes, Anseriformes, Galliformes, Gruiformes, Charadriiformes, Coraciiformes*, and *Passeriformes*. Virus isolation was performed according to the OIE guidelines.

Results: Geographically Ukraine is located in the centre of Europe, with many of the migration routes of wild birds crossing over the area. Ornithological fauna of Ukraine consists of 414 species, of which 207 nest here. Three of the 14 transcontinental global migration flows come through this area with birds from North Asia and Europe to the Mediterranean, Africa and South-West Asia. Flying routs of birds from the Baltic Sea and Caspian Sea to the Black and Mediterranean seas, from West Siberia and Kazakhstan to West Europe and North Africa pass through this area. During 2006 to 2011 we obtained 79 virus isolates, 59 of which were avian flu viruses of various subtypes and 20 were paramyxoviruses. Based on sequencing results of avian flu viruses they were all from European origin. These viruses were mostly associated with wild migrating ducks. Sequencing of the paramyxoviruses revealed that the majority of them belonged to avian paramyxovirus type 2 and only one virus was from type 1. One virus isolated from a pied goose belonged to genotype XIV. Viruses of Newcastle disease of genotype XIV originated from Africa and caused massive outbreaks amongst poultry in 2008. The sequence data for protein F indicated that the virus was highly pathogenic while intra-cerebral pathogenicity index of the virus indicated that it was not pathogenic and did not causes disease in chickens.

Conclusion: Results of our research indicate potential introduction of virus with new biological properties to new territories including Ukraine. This emphasises the need for continuous surveillance and research.



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