DISCONTOOLS

Symposium

Filling the knowledge gaps in animal disease control

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DISCONTOOLS expert group on peste des petits ruminants

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Peste des petits ruminants (PPR) is one of the most economically important diseases in developing countries. There is now a global effort to eradicate PPR. Filling the gaps in our knowledge of the disease and development of new diagnostic tools and new vaccines will increase our chances to reach this goal. Importantly, regional coordination and involvement of all stakeholders will be paramount to success. The PPR situation in countries bordering the EU emphasises the importance of implementing and maintaining appropriate control measures with regard to illegal imports and animal movements to mitigate risks. Significant areas of uncertainty in the understanding and knowledge about PPR exist especially in relation to pathogenesis, immunology, vaccinology, and epidemiology. Information is needed in areas such as the identification of factors involved in the variation of host susceptibility, determinants of PPRV pathogenicity, the importance of animal species other than sheep and goats in the epidemiology of PPR, the potential importance of indirect transmission of virus, and the transboundary transmission dynamics of PPRV, notably in complex multispecies systems. Research is needed to fill these gaps in knowledge. Key new advances concerning PPR research gaps will be presented.

Evaluation of safety and efficacy of an IBR marker vaccine in buffalo (*Bubalus Bubalis*) against *Bubaline alphaherpesvirus 1* (BuHV1)

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The breeding of the Italian Mediterranean buffalo (*Bubalus bubalis*) in Italy is an important economic resource, mainly due to the production of a typical product called "Mozzarella di Bufala Campana DOP". This species is sensitive to both bovine (BoHV-1) and buffalo (BuHV-1) alphaherpesviruses. Currently, there is no specific vaccine for buffalo species against BuHV-1. Therefore, the purpose of this study was to evaluate the safety and efficacy of a gE-deleted marker vaccine. Ten buffalo calves, seronegative for BoHV-1/BuHV-1 were selected and divided into two groups (five calves/group). Five animals were used as control, whereas the remaining were vaccinated by an inactive gE-delete marker vaccine administered by intramuscular route. The vaccine did not induce any clinical signs or adverse reactions. After thirty days following the first immunization, all calves were challenged with a virulent BuHV-1 strain. Clinical examinations, nasal swabs and blood samples were carried out at 0, 2, 4, 7, 10, 15, 30, 63 days post-infection (DPI), to set up both viral isolation tests and virus neutralisation tests against BoHV-1/BuHV-1. After the challenge, only control buffaloes showed nasal lesions (pustules) and excreted BuHV-1 2 DPI. On the day of the challenge, only immunized animals had neutralizing antibodies (1.87 log against BoHV-1; 1.70 log against BuHV-1). These antibodies increased until the end of the experiment (3.25 log against BoHV-1; 3.22 log against BuHV-1). On the contrary, the control group seroconverted 15 DPI (1.24 log against BoHV-1; 1.76 log against BuHV-1). These results demonstrated the safety and efficacy of the vaccine tested.

(words 250/250)

DISCONTOOLS expert group on Henipavirus Infections of Farm and Companion Animals

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Nipah and Hendra virus of the genus Henipavirus are both highly pathogenic zoonotic viruses. Hendra virus, first detected 1994 in Queensland, Australia, has been shown to cause severe respiratory and central nervous system symptoms in horses, which may be transmitted to humans and cause fatal infections. A recombinant vaccine has been approved for horses in Australia. In 1998, severe respiratory symptoms in farmed pigs in Malaysia were reported for the first time after infection with Nipah virus. Farm workers which had been in close contact with these animals developed severe respiratory and neurological symptoms with fatality rates of 36%. In both cases, pteropoid fruit bats had transmitted the virus via contaminated fruits. Since 2001, repeated outbreaks of human Nipah virus infections have been reported in India and Bangladesh with fatality rates up to 70%. Here, indirect transmission events from fruit bats to humans via contaminated date palm sap or contaminated fruits are the cause of infection.
To enable us to detect previous henipavirus infections in pigs, horses and fruit bats, we developed ELISA protocols targeting antibodies raised against the attachment proteins or the nucleoprotein of both viruses. Using the Nipah antibody detection protocol for pigs, we have been able to prove a past exposure of domestic pigs in Uganda to henipaviruses, thus allowing us to further assess the henipavirus distribution in Africa. For the analysis of horse sera, we developed a DIVA (**D**iscriminating **I**nfected from **V**accinated **A**nimals) assay which is important for the testing of horse sera originating from Australia.

MONOCLONAL ANTIBODIES AND RECOMBINANT PROTEINS: PROMISING TOOLS FOR THE SEROLOGICAL DIAGNOSIS OF THE LUMPY SKIN DISEASE

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Lumpy skin disease (LSD) is a transboundary disease of cattle caused by LSDV, a virus belonging to the Capripoxvirus genus of the Poxviridae family. It is currently widespread in Africa, the Middle East, and recently expanded into part of Eurasia and South-East Asia; LSD was introduced within UE in 2015 causing thousands of outbreaks during 2016-17 in southeastern Europe, where it was controlled by mass vaccination. While molecular tests are available for disease laboratory confirmation, reliable high-throughput serological tests useful for surveillance are still missing.
In this study, we produced and characterized biotechnological reagents potentially strategic for the development of serological ELISAs. These included 73 Monoclonal Antibodies (MAbs) raised against the LSDV Neethling strain and two recombinant proteins selected for their known immunogenicity, namely the p32 (rp32) and a protein located on LSDV internal envelope, codified by the ORF LSDV060 (rLSD060).
Two indirect ELISAs were developed using the rp32 or the unpurified inactivated LSD virus, both captured by a p32-specific MAb, while a third indirect ELISA used the purified rLSDV060 antigen directly adsorbed onto ELISA microplates. A panel of sera from 18 cattle experimentally infected with different strains of LSDV and collected weekly up to the fourth week post-infection were tested.
All the assays evidenced seroconversion from 14 days post-infection and maintained positivity throughout the experiment.
These results proved that rp32 and rLSDV060 reproduced the antigenic properties of the native viral proteins and the developed ELISAs are promising serological tests to support active LSD surveillance.

Areas for further research in *Mycoplasma hyopneumoniae*

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Mycoplasma hyopneumoniae (*M. hyopneumoniae*) is the primary agent of enzootic pneumonia in pigs. The pathogen-host interaction is complex and the virulence mechanisms are not yet fully understood. *M. hyopneumoniae*-specific serum antibodies are not correlated with the degree of protection. Hence, cell-mediated and local humoral immunity might play a more important role. Vaccination against *M. hyopneumoniae* is often practiced to control disease. Current commercial *M. hyopneumoniae* vaccines are mostly inactivated, whole-cell vaccines reducing clinical signs but not protecting against infection nor preventing transmission. Many adhesins like P97 and P102 are important virulence factors, as adhesion to the ciliated epithelium of the respiratory tract is the first step of infection. Furthermore, some of these adhesins trigger a cascade causing production of reactive oxygen species and pro-inflammatory cytokines. Because of its importance, the P97 antigen has been frequently included in experimental subunit or vector vaccines. However, this did not lead to vaccines giving a better protection than the commercial vaccines. *M. hyopneumoniae* can also evade the immune system of the host explaining the chronic character of an infection. To inform vaccine design, further research is necessary to elucidate the complex pathogen-host interaction and to identify bacterial factors responsible for the survival of the pathogen in the host. Furthermore, *M. hyopneumoniae* attenuated vaccines should be investigated as these vaccines might induce a good local immune response upon mucosal administration and might prevent colonization and transmission.

MOLECULAR EVOLUTION OF SWINE VESICULAR DISEASE VIRUS IN ITALY FROM 1992 TO THE ERADICATION

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Abstract (max 250 words):
Swine Vesicular Disease (SVD) virus was detected for the first time in 1966 in Italy; from then on, four consecutive genomic-antigenic variants occurred in Europe and the fourth one, firstly identified in 1992, persisted in Italy for more than 20 years until the last outbreak recorded in 2015. In the present work, we studied the molecular evolution of this fourth variant by analyzing a total of 166 isolates selected as representative of the time frame considered (1992-2015). Almost complete sequences with a length of 7163-7400nt were obtained by using the Miseq platform. The Bayesian phylogenetic analysis of the polyprotein coding portion confirmed the presence in Italy of two main sub-lineages, with a putative common ancestor dating back to 1990-91. The first sub-lineage includes strains that evolved in Italy from 1995 to 2007 and the second comprises two distinct clusters, of which one composed of viruses isolated in the most distant period (1992-98) and the other composed of viruses isolated from 2004 onwards, probably derived from a re-introduction in Italy. A unique recombination event, dated back to 2007, occurred. The recombination breakpoint was found within the nucleotide segment 3761-3821 of the genome. The recombinant strains, clustering in a distinct group, present the regions 5'UTR and P1 belonging to the sub-lineage 2 and the remaining part of the genome to the sub-lineage 1. Synonymous nucleotide mutations are uniformly distributed along the genome, the ratio between the number of nonsynonymous substitutions to the number of synonymous substitutions indicates that the viral population underwent a stabilizing evolution.

DISCONTOOLS expert group on rabies

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Rabies virus (RABV), a lyssavirus transmissible to all mammals and almost invariably fatal from the onset of symptoms, kills an estimated 60,000 people each year. Although most dog-transmitted human cases occur in Asia and Africa, also wildlife rabies represents a matter of concern given its impact on animal and public health. Failure to control dog-mediated rabies on a large scale will result in continued high mortality each year. More generally, uncontrolled rabies circulation in any host species is likely to create possibilities for further spillover events and viral adaptation to new hosts. In addition, the risk for disease introduction into free areas through natural movements or illegal importation remains real.
Adequate surveillance tailored to regional or local circumstances and field diagnostic tools need to be implemented. Validation of field tests might improve rapid follow-up of biting and suspect animal cases, especially in areas where dog mediated rabies is endemic. Although a variety of animal and human rabies vaccines are available, they do not confer protection against divergent lyssaviruses known to circulate in Eurasia and Africa. Combined rabies and contraceptive vaccines would maximize herd immunity in dogs in principle, but this still needs to be demonstrated. As for rabies vaccines for veterinary use, the replacement of the current *in vivo* assays for vaccine potency testing by a combination of *in-vitro* testing and consistency monitoring should also be addressed. Basic studies on disease mechanisms that trigger spillover and adaptation of lyssaviruses to new hosts are required. Applied research is needed to investigate reservoir population dynamics, with a focus on understanding dynamics in dog populations. This would improve the efficacy of control measures and lower their cost.

DISCONTOOLS expert group on Echinococcosis: control actions needed in Europe

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Abstract (max 250 words):

Human cystic echinococcosis (CE), caused by *Echinococcus granulosus* s.l., and alveolar echinococcosis (AE), caused by *E. multilocularis,* are important public health threats globally including large parts of Europe. The gradual increase of *E. multilocularis* infection pressure due to the increasing fox populations and the spread to Western, Northern and Eastern Europe is causing concern. Some areas in Europe have an incidence of CE in humans among the highest in the world. There is an urgent need to begin new, active steps to reduce this burden on human health. The necessary tools and knowledge are available to start control actions in Europe. CE is a preventable zoonosis and the best control measure is to interrupt the domestic life cycle of the parasite. This can be achieved with well-designed, integrated control programs based on deworming of dogs and vaccination of lambs over the long-term. What is needed is the European registration of the EG95 vaccine for livestock and the political will and funding to undertake control programs. Improved diagnostic tests to use in monitoring of control programmes for livestock and for the individual rapid diagnosis in dogs should be developed and made available. Control of *E. multilocularis* is more complex because the cycle occurs mainly within wildlife including voles and red foxes. Foxes can be dewormed but only in defined areas whilst control in voles is currently not feasible.

DEVELOPMENT OF TWO MULTIPLEX LATERAL FLOW DEVICES FOR ON-FIELD IDENTIFICATION AND SEROTYPING OF FOOT-AND-MOUTH DISEASE VIRUS

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Foot-and-mouth disease (FMD) is a livestock viral disease with severe socio-economic impacts. Rapidity in detection and serotyping is crucial to identify circulating strains/serotypes and to implement proper control measures. In endemic regions, which often lack of equipped laboratories, Lateral Flow Devices (LFD) represent the simplest tool for rapid on-site diagnosis. This study describes the development of two multiplex LFDs (LFD1-*Eurasia* and LFD2-*Africa*), based on monoclonal antibodies (MAbs), for FMD diagnosis and simultaneous serotyping. The assays exploit serotype-selective capturing MAbs and gold-conjugated detectors designed to identify two different groups of FMDV serotypes. LFD1-*Eurasia* combines four reactive lines (pan-FMDV, O, Asia1, A) and a pan-FMDV MAb as detector, while LFD2-*Africa* has three reactive lines (SAT1, SAT2, pan-FMDV) and the same capture MAbs in pool for detection. An initial evaluation of the analytical performances showed that the reference strains were correctly classified with absence of cross-reactions. The detection limits of the LFD1-*Eurasia* were comparable to those of Antigen-ELISA: approximately 10^3 $TCID_{50}$ for types A and O, and 10^4 $TCID_{50}$ for type Asia1. LFD2-*Africa* was less sensitive, requiring 10^3-10^4 $TCID_{50}$ for type SAT1 and 10^5 $TCID_{50}$ for type SAT2 to provide positive reactions. These FMDV concentrations are often present in vesicular lesions, indeed also a few positive epithelium homogenates, representative of four serotypes, produced type-specific reactions combined with the pan-FMDV detection. These novel LFDs are promising tools for on-site detection and typing of the five most common FMDV serotypes in endemic countries and potentially for decentralized confirmation of secondary outbreaks following incursion in free-areas.

KNOWLEDGE GAPS IN PREVENTION, CONTROL AND DIAGNOSIS OF CAMPYLOBACTERIOSIS

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Campylobacteriosis is an important, worldwide public health problem with significant socio-economic impact. Campylobacteriosis is the most commonly reported notifiable disease in many countries. In Europe, 200 000 -250 000 human cases have been reported annually since 2010. *Campylobacter* is frequently isolated from the faeces of healthy food-producing, wild and companion animals. *Campylobacter* can be transferred from animals to humans directly after contact with animals, or through consumption or handling of contaminated food products and water. In humans, clinical signs of campylobacteriosis include diarrhea, abdominal pain, fever, headache, nausea and vomiting. Most cases of campylobacteriosis are sporadic and self-limiting, but post-infection complications occur. Poultry and poultry products are considered important sources of human infections. Poultry meat can become contaminated with *Campylobacter* during slaughter if live chickens are intestinal carriers. Although much research has been performed there are still gaps in knowledge. A DISCONTOOLS group of experts on *Campylobacter* and campylobacteriosis identified the most important gaps and areas for further research as: (i) knowledge of the true number of infected humans; (ii) mechanisms of pathogenicity; (iii) effective methods to prevent transfer of *Campylobacter* from raw to ready-to-eat food; (iv) development of effective vaccines; (v) understanding transmission routes to broiler flocks; (vi) knowledge of bacteriocins, bacteriophages and antimicrobial peptides as preventive therapies; (vii) feed composition as a preventive measure at farm level; (viii) development of kits for rapid detection and quantification of *Campylobacter* in animals and in food products; and (ix) development of more effective antimicrobials for treatment of humans with campylobacteriosis.

DISCONTOOLS expert group on Swine *A. pleuropneumoniae*

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Actinobacillus pleuropneumoniae (*A.pp.*) is a primary porcine respiratory pathogen leading to high economic losses worldwide by peracute, acute or chronic courses of disease. In swine dense regions most swine farms are endemically infected and sudden outbreaks occur in pigs colonized on their tonsils. Disease is triggered by environmental factors and coinfecting agents, which are not understood in their interaction so far. Up to date 19 serotypes are known, providing limited cross-protection after infection. Commercial vaccines are available, but they do not provide full protection against disease outbreaks. In most labs in Europe no molecular typing by determination of the capsule genes is established, although this is the basis for production of autologous vaccines. Swine pleuropneumoniae is controlled by antibiotic treatment as tiamulin, tetracycline and ampicillin. Quinolones are very effective but belong to the highest priority critically important antibiotics. Up to now there is lacking knowledge in pathogenesis and immune reactions especially in the transition from colonization to infection. Innate immunity is decisive for the course of disease but is not addressed by vaccine candidates. A marker as correlate of protection is not known. Eradication is hampered by strategies with a high success rate, especially in swine dense regions. The verification of animals being free from *A.pp.* is difficult due to too low sensitivity of detection methods in healthy carrier pigs. The interaction with coinfecting organisms as influenza virus A or *Mycoplasma hyopneumoniae* and the persistence of *A.pp.* in multispecies biofilms has been described, but pathomechanisms are not elucidated so far.

DISCONTOOLS - A review of the prevalence, characteristics and antimicrobial resistance of *Campylobacter* species from meat and meat products from diverse geographical regions

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Foodborne *Campylobacter* are causative agents of campylobacteriosis, which is a zoonotic infection of global concern. Campylobacteriosis is characterized by abdominal pain, bloody diarrhoea, and fever. Consumption of contaminated meat and meat products, particularly poultry has been linked to campylobacteriosis in human beings. The challenge of managing campylobacteriosis is exacerbated by antimicrobial resistance (AMR) among *Campylobacter* species that has been reported to be increasing and proliferating. The AMR has been attributed to a clear association between antimicrobial usage in food-producing animals for treatment, prophylaxis or growth promotion and the resistant *Campylobacter* that cause foodborne campylobacteriosis in human beings. For instance, it is well documented that there is a link between *Campylobacter* resistance in human beings and the administration of fluoroquinolones in food-producing animals. In this regard, antimicrobial resistance may be considered a food safety issue due to the potential spread of resistant *Campylobacter* and associated genes along the food-value-chain. The resistance poses socio-economic challenges due to relatively high risk of adverse outcome. The data from different geographical regions vary with respect to the availability of information about the prevalence, characteristics and antimicrobial resistance among *Campylobacter* species. Therefore, the aim of this review is to compare and discuss the prevalence, characteristics and antibiotic resistance among foodborne Campylobacter from meat and meat products from different geographical regions and identify gaps in knowledge.

Keywords: Foodborne *Campylobacter*; antimicrobial resistance; zoonosis; prevalence; meat and meat products

Application of gamma interferon test in Mediterranean buffaloes

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Traditionally, bovine tuberculosis TB eradication programs are based on the slaughterhouse surveillance and intradermal tests (IDT); moreover, the gamma-interferon test (IFN-γ) has been introduced in cattle and recently used also in buffalo to enhance TB surveillance. Particularly, in Campania region (Italy), the population of water buffalo (*Bubalus bubalis*) consists of 1,272 herds, with a total of 299,039 bred animals representing the 71.34% of the whole buffalo population in Italy (Italian National Livestock Database). Since 2017, a special regional buffalo tuberculosis-surveillance program, based on a first screening with single IDT, followed, in case of positive reactions, by a comparative IDT in parallel with the IFN-γ test, was applied in TB officially free buffalo herds. Then, starting from 2019, the IFN-γ test replaced the comparative IDT. From 2017 to 2020, 32,040 animals of 855 farms were tested with the IFN-γ test, and 4,895 resulted positive. TB outbreaks were then confirmed by post-mortem examinations. This diagnostic approach (IFN-γ test combined with IDT), could have detected an increasing number of outbreaks. Indeed, the buffalo bTB prevalence in Campania region increased from 2.27% in 2017 to 6.22% in 2019 and the bTB incidence increased from 1.68% to 5.95% in the same period. Thanks to the large number of outbreaks detected between 2017-2019, in 2020 TB prevalence and incidence levels decreased significantly, 1.74% and 1.65% respectively. Based on our preliminary data, the new diagnostic approach seems to have improved the sensitivity of the buffalo TB surveillance system in Campania region.

DISCONTOOLS expert group on disease VARROOSIS

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The mite, *Varroa destructor* is the most important ectoparasite of *Apis mellifera* that feeds on the preimaginal host stages within the sealed brood cells and penetrates the intersegmental skin between the abdominal sclera of adult bees to ingest haemolymph and fat body tissues. Without treatment of the honey bee colony, the number of parasites steadily increases with the growth of the bee population and the brood leading to the collapse of the colony. The clinical signs of infestation mainly occur late in the season. The mite can survive from some days to a few months. Currently the control of Varroa mite infestation (Varroosis) consists of application of veterinary medicinal products to the colonies, often combined with zootechnical control techniques. The veterinary medicines are traditionally based on hard chemicals (i.e., pyrethroids, organophosphates, amitraz) but due to pharmacoresistance and contamination of beeswax in particular, active substances such as organic acids (formic and oxalic) and essential oils (thymol) have caught on thanks to their chemical and efficacy characteristics. *V. destructor* is a major problem for apiculture and the search for novel control methods is an essential task for researchers. The needs for research are understanding of ecological interactions of this parasite; selection for resistance; improvement of application methods and reduction of side-effects of organic acids/essential oils-based veterinary medicines; development of new drugs alternative or complimentary to the existing ones; and optimization and standardization of zootechnical control techniques. All of these are necessary for creating strategies to sustainably manage the parasite.

Comparison of three Serological Tests for the Diagnosis of Canine Brucellosis Caused by Brucella canis in Italy

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Canine brucellosis caused by *Brucella canis* is an emerging and underestimated phenomenon in EU and in Italy. The agent is responsible for abortions, infertility and chronic systemic infections in dogs, as well as a potential source of infection in humans. Current diagnostic serological tests available for disease surveillance suffer from lack of knowledge in accuracy, having only limited data available in the international literature. In this study, we compared the performances of three serological tests, microplate serum agglutination (mSAT), complement fixation (CFT) and immunofluorescence (IFAT), using sera from culture positive dogs as gold standard. Analyses were performed on sera collected from 61 naturally infected dogs during an outbreak occurred in a commercial breeding kennel in Italy, while 143 negative sera from randomly selected dogs, not involved in the outbreak, were collected. Sensitivity, specificity and level of agreement (Cohen's kappa value) were calculated. IFAT shows a sensitivity higher than both mSAT and CFT. mSAT is lower in specificity than IFAT. The Cohen's kappa correlation index indicates a strong correlation with the gold-standard test. The highest degree of correlation is recorded between IFAT and CFT, while the lower is between IFAT and mSAT. Concordance between mSAT and CFT, show an intermediate value. Our results suggest the use of mSAT and CFT as screening test and IFAT as confirmatory test. A diagnostic protocol based on the use of these serological tests in series would represent a good strategy for a better control of canine brucellosis due to *Brucella canis* in infected kennels.

Evaluation of three marker vaccines against Bovine alphaherpesvirus 1 (BoHV-1) in calves

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In this study, we evaluated safe and efficacy of three different IBR marker vaccines. For this purpose, twelve calves devoid of BoHV-1 neutralizing antibodies (NA) were used. The animals were divided into four groups of three animals each. Group A, was immunized with a modified-live (MLV) gE, tk-deleted marker vaccine via intramuscular route; Group B was inoculated with an MLV gE-negative vaccine via intranasal route; Group C was administered with an MLV gE-negative vaccine via intramuscular route. Two doses of each vaccine at 2 mL were given to each animal 21 days apart. Group D represented the control group. Thirty-five days following the first immunization, all animals were challenged with a virulent BoHV-1 strain. Rectal temperatures and blood samples were taken at different experimental times. The vaccines did not induce any clinical signs or adverse reactions. A progressive increase in the BoHV-1 NA titre was detected in all vaccinated animals from a mean titer of 1.51 $\log_{10}$ to 2.71 $\log_{10}$ on post-vaccination day (PVD) 28. The animals in the control group showed clinical signs 3 post-challenge days (PCD). A progressively increased NA titre until 28 PCD was observed in all vaccinated groups with a mean titre from 3.21 $\log_{10}$ to 3.46 $\log_{10}$. Differently, group D evidenced NA 7 PCD with a mean titre of 1.35 $\log_{10}$, and at 28 PCD, this value increased to 3.16 $\log_{10}$. In all groups, a positive signal for gE was detected only on 14 PCD. In conclusion, the results of this study indicate that the vaccines are safe and efficacy.

Knowledge gaps for Bluetongue prevention and control

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Bluetongue (BT) is an OIE-notifiable viral disease of wild and domestic ruminants caused by the Bluetongue virus (BTV). In recent years, in addition to the classical 24 serotypes, several other serotypes regarded as atypical have been described. Some of them have been officially recognized, some others are still to be formalized. These atypical BTV serotypes have been exclusively detected in small ruminants where they cause only subclinical infection. They are growing in number and, at least for some of them (BTV-25-27) the in-contact transmission has been demonstrated. Gene reassortment is a frequently observed phenomenon in bluetongue virus. The possibility that atypical and typical BTV strains could reassort and generate a virulent progeny capable of being transmitted horizontally can't be discarded and should be investigated. Classical BTV, however, are transmitted by midges. As vector borne pathogens, it is expected that their incursions in Europe will increase in the next coming years due to the setting of new and favourable environmental and climatic conditions. For these reasons, BT remains a major health and trade problem for livestock industry. Efforts to fulfill knowledge gaps and set up cost-effective control measures are still crucial. In respect to prevention, vaccination is considered fundamental in contracting BTV emergence and spread. Unfortunately, for some serotype (e.g., the recently reported BTV-3) there is an urgent need for efficacious cost-effective vaccines. Moreover, although some promising research is ongoing, next-generation DIVA vaccines/DIVA assays are still commercially unavailable, and their implementation is desirable.

DISCONTOOLS expert group on zoonotic Toxoplasmosis: many gaps identified, new diagnostic developments and vaccines are necessary for effective intervention

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Toxoplasmosis is a relevant zoonotic disease, caused by the protozoan *Toxoplasma gondii*. Congenital toxoplasmosis can cause abortion or birth of severely affected children. Postnatally acquired toxoplasmosis can have severe consequences in particular in humans with immunosuppression. *Toxoplasma gondii* is pathogenic to livestock, for example an important abortifacient for small ruminants. Cats are definitive hosts, able to shed oocysts via faeces, causing environmental contamination. In addition to infections by oral uptake of food or water contaminated with oocysts, humans can become infected by consuming meat or other animal products that contain infectious *T. gondii*. Serological diagnostics plays a key role in detection of *T. gondii* infections in animals and humans and commercial tests are available. However, for some livestock species the existing serological tests seem unreliable in identifying animals with viable *T. gondii*. Parasite stage-specific serology could inform targeted interventions addressing the main sources, and point-of-care tests could be useful to detect shedding cats. For humans no vaccine exists. In the veterinary sector there is a live vaccine for use in sheep, commercially and seasonally available in a few regions of the world. It has a short shelf life and is potentially infective for users. More user-friendly and safer vaccines against toxoplasmosis are needed. A transmission blocking vaccine for young cats could reduce the oocyst contamination in the environment and could probably be commercialised with other well accepted cat vaccines. New developments in the field of diagnostics and vaccines represent promising options to reduce *T. gondii* infections in animals and humans.

DISCONTOOLS expert group on mammal coccidiosis

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Coccidia are ubiquitous species-specific intracellular enteric protozoa highly prevalent in pigs, cattle, sheep, goats, rabbits and dogs, featuring a one-host lifecycle. Most domestic mammals harbour several different species, frequently at the same time. The genus mainly involved is *Eimeria* for all animal species except of pigs and dogs which mostly are affected by species of the genus *Cystoisospora*. Infection is being acquired orally from the environment where oocysts can persist for long periods. Coccidiosis produces acute gastrointestinal disease and may predispose to secondary infections. High morbidities and significant mortalities may be observed. Clinically important infections with significant oocyst shedding are usually seen in young animals. However, not all species of coccidia present in a host are pathogenic, thus, the presence of large numbers of oocysts in the faeces by itself is not necessarily indicative of disease. For disease diagnosis, faecal samples from clinically ill animals should be analysed for both identity (i.e., oocyst speciation which is necessary to define pathogenicity) and number of excreted oocysts. Protective immunity, preventing repeated development of disease and intense oocyst shedding, establishes after primary infections for most coccidia, especially the pathogenic species. Effective coccidiosis control relies heavily on both management measures (especially effective cleaning and disinfection) and control by anticoccidial drugs. Drug resistance should be considered a significant potential threat to effective coccidiosis control (e.g., already reported for *Cystoisospora suis* and ovine *Eimeria* spp), therefore available drugs should be used strategically, and alternatives, such as plant secondary metabolites (e.g., essential oils, condensed tannins) should be considered where applicable.


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