

# ApiCOWplexa 2013 – 2nd International Meeting on Apicomplexan Parasites in Farm Animals

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This special issue is dedicated to a group of protozoan parasites of outstanding economic importance, and originates from the 2nd International Meeting on Apicomplexan Parasites in Farm Animals (ApiCOWplexa 2013). The meeting took place in the Pine Bay Hotel in Kusadasi, Turkey, from 31 October to 2 November 2013, and was superbly organized by Prof. Tülin Karagenç and Huseyin Bilgin Bilgiç (Adnan Menderez University, Turkey). ApiCOWplexa 2013 was attended by 114 participants from 27 countries. A total of 48 scientific talks and 40 posters were presented, on different aspects of research on *Toxoplasma*, *Cryptosporidium*, *Eimeria*, *Neospora*, *Sarcocystis*, *Besnoitia*, *Babesia* and *Theileria*. These parasites cause important diseases and economic losses in the production of cattle, pork, poultry, sheep, goats, and are also important pathogens in pets, game and wild animals. Topics of the conference included epidemiology, genetic diversity and phylogeny, food safety aspects, animal models, functional genomics and transcriptomics, host–parasite interactions on the cellular and immunological levels, drugs and drug targets, the development of vaccines, and novel and improved tools for diagnosis. Pathogens mutate and can emerge in regions where they have not been seen before, and it is necessary that the methodologies for detection, surveillance, prevention, control and treatment also continue to evolve. Novel developments in the field allow for productive data mining, innovative laboratory methods, rapid and accurate diagnostics. From the public health point of view, all aspects are crucial to maintain, or even increase, the provision of safe food from farm animals.

The broad attendance of ApiCOWplexa2013 clearly reflects the need for networking between scientists that work on these organisms. Nevertheless, such networking activities, and research on parasitic food-borne diseases in particular, have been grossly underfunded for many years. More recently, the

steady increase in the number of people living on this planet that requires safe and nutritious food, the globalization of the food trade, increased international travel, higher numbers of persons at risk due to changes in culinary habits, all coupled with significant improvements in diagnostic technologies, have contributed to the increased awareness of food-borne diseases (Dorny *et al.* 2009; Broglia and Kapel, 2011; Robertson *et al.* 2014; Torgerson *et al.* 2014).

In the 2012 report of the Food and Agriculture Organization of the United Nations on multicriteria-based ranking for risk management of food-borne parasites the 2 apicomplexans *Toxoplasma* and *Cryptosporidium* were placed fourth and fifth, respectively, on the global ranking of parasites causing food-borne diseases, and *Sarcocystis* spp. was placed at number 24 (Robertson *et al.* 2013). *Toxoplasma* and *Sarcocystis* can be transmitted from animals to humans through the direct consumption of raw or undercooked meat, and *Toxoplasma* and *Cryptosporidium* through shedding of infective stages by parasitized animals into water supplies, leading to contamination of drinking water or plants and vegetables. A major challenge in the future will be the increased demand for animal proteins in developing countries, which will lead to intensified animal production. Thus, the corresponding risk of zoonotic infections needs to be carefully assessed. Increased monitoring and control of food-borne parasites via improved and optimized diagnostic tools must be established and validated, and improved preventive and therapeutic countermeasures will be required to face these challenges. Therefore, to develop these countermeasures it is instrumental to acquire more fundamental knowledge on the biology of apicomplexan parasites, and on the ways they interact with, or modulate, their hosts, and to obtain more information on the strategies whereby they circumvent or manipulate the host's immune and physiological responses to infection.

One result of this meeting was that the quality of the research currently carried out in the field of apicomplexan parasites in farm animals has clearly increased during the last years, and the application of innovative technologies has led to impressive research

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achievements by individual groups. However, the diversity of the diseases and pathologies caused by different apicomplexans and the lack of funding for concerted interactions and networking both contribute to the notion that there is a lot of room for improving the level of coordination between researchers. Cross-species approaches will become increasingly more important, since apicomplexans share many phylum-specific biological features with no counterpart in other groups rather than organisms. Some of these represent potential targets that should be exploited for the development of novel intervention tools with a broader impact.

This special issue of *Parasitology* contains a selection of invited reviews and original research papers, highlighting topics that were presented at ApiCOWplexa 2013. A major topic of this meeting was *Toxoplasma gondii*, which represents undoubtedly the most prevalent apicomplexan parasite worldwide and exhibits a broad host range, affecting pigs, chickens, lambs, goats, as well as approximately 30% of the human population. The original research paper by Geuthner *et al.* (2014) reports on the persistence of *T. gondii* in experimentally infected poultry during an entire fattening cycle, and shows that the edible tissue in turkeys stay infected for at least 16 weeks post-infection, which indicates a potential risk for consumers of undercooked turkey meat. In contrast, chicken appears less susceptible to *T. gondii* infection. The only vaccine against toxoplasmosis currently on the market is Toxovax™, which is licensed for use in sheep in most countries in Europe and New Zealand. Toxovax™ contains live attenuated tachyzoites of the non-persistent strain *T. gondii* S48. An invited review on veterinary vaccines against toxoplasmosis by Hisczyńska-Sawick *et al.* (2014) describes the immunological hallmarks of *T. gondii* infection, discusses selected vaccine trials in different hosts, and highlights the difficulties and the challenges in the development of efficient preventive measures as an alternative to Toxovax™.

Another genus with a wide host range is *Eimeria* with over 1000 species, the majority of which parasitize the intestinal epithelia of vertebrates such as horses, domestic dogs and cats, wildlife species, and economically significant species including rabbits, cattle, sheep, pigs, turkeys and chickens. Most importantly, coccidiosis causes significant economic losses to the poultry industry. Witcombe and Smith (2014) outline here the strategies for anti-coccidial vaccines, including infection with controlled doses of virulent oocysts, reproductively attenuated lines or maternally delivered vaccines that confer protection to newly hatched offspring chicks.

In addition to its leading role as the most successful parasitic organism, *T. gondii* also represents the most successful apicomplexan model system due to its genetic amenability. To identify the novel targets for

intervention, either by chemotherapeutical means or by vaccination, it is of high importance to dissect the functional relevance of such candidate targets on the molecular level. Here Jiménez-Ruiz *et al.* (2014) review different techniques available for the generation of conditional mutants. Comparison of the advantages and pitfalls of the 3 most commonly used regulation systems are discussed, and potential pitfalls involved in the gene-function analysis are explored. *Sarcocystis neurona* is a relative of *T. gondii*, and causes severe neurological diseases in horses and marine mammals. Dangoudoubiyam *et al.* (2014) used a bioinformatics approach to identify purine salvage pathway sequences in the *S. neurona* genome that could be exploited to enhance molecular genetic capabilities and develop transgenic parasites enabling positive–negative selection of stable transgenic mutants for investigating the biology of *S. neurona*.

One disease more frequently addressed than in the previous meetings was bovine besnoitiosis, which is caused by the largely unexplored apicomplexan *Besnoitia besnoiti*. Bovine besnoitiosis is an emerging disease and could develop into a potential threat to productivity in the cattle industry, but its life cycle and definitive host are still unknown, and the modes of transmission have not been entirely resolved. The review paper provided by Cortes *et al.* (2014) presents an insight on the current knowledge of the infection biology, pathogenesis and diagnosis of besnoitiosis, whereas Álvarez-García *et al.* (2014) present an excellent review into the infection dynamics of *B. besnoiti* in cattle and the respective relevant host- and parasite-derived factors. The establishment of a good experimental animal model and the generation of high-quality genomics and transcriptomics data of *B. besnoiti* will be keys to successfully combat this emerging parasite. In addition, more basic research is required on the host–parasite relationship at the cellular level, and Cardoso *et al.* (2014) provide an original research paper, where they show that *B. besnoiti* and *T. gondii* tachyzoites both interact with the host-cell microtubular cytoskeleton following infection, but exhibit marked differences in recruiting the host-cell centrosome and Golgi apparatus. These differences most probably indicate different evolutionary pathways.

*Neospora caninum* is closely related to *T. gondii* and *B. besnoiti*, but causes a different pathology. Reproductive failure in cattle represents the major economic loss of neosporosis, and the review paper by Reichel *et al.* (2014) deals with different aspects on how vaccination and other control measures could be implemented to reduce the economic impact of this parasite. The only commercially available vaccine against neosporosis has been recently withdrawn from the market, and controversial opinions exist with regard to suitable control options, which are presented in this paper. A major drawback in the

development of a vaccine against neosporosis in ruminants is the lack of standardized experimental procedures. Several research groups have developed ruminant models of *N. caninum* infection independently of others, resulting in a high degree of variability. Standardization is greatly needed to advance research in a more collaborative, timely and efficient manner. This aspect is tackled by Benavides *et al.* (2014), and this review summarizes and discusses the different models and parameters currently in use, and identifies knowledge gaps and parameters essential for the development of non-pregnant and pregnant ruminant models. This information could act as the basis to develop a consensus for international standard guidelines for ruminant models of neosporosis.

While boosting acquired immunity to protect against apicomplexan parasites has been an important issue during this conference, the initial host leucocyte–pathogen interactions of the innate immune system have been addressed in a review paper by Hermosilla *et al.* (2014). Polymorphonuclear neutrophils and other leucocytes expel their DNA in a controlled process into the extracellular environment to trap and kill pathogenic microorganisms. This formation of neutrophil extracellular traps (NETs) has recently been recognized as a novel and widely spread effector mechanism of the host innate immune response against microbial infections.

Prophylactic use or post-infection treatments with coccidiostats has been recommended as an economically viable option to combat infections by apicomplexan parasites. However, many drugs currently in use are characterized by either high cost, difficult application, emerging drug resistance and/or adverse side effects. In addition, residues in food destined for human consumption are a major issue. Some exciting news have emerged on that topic. *N. caninum*, *Eimeria tenella*, *Babesia bovis*, *Plasmodium falciparum*, *Cryptosporidium parvum* and *T. gondii* share a class of calcium-dependent protein kinases (CDPKs), and in the paper by Keyloun *et al.* (2014), these are being exploited as targets for drug development. The class of bumped kinase inhibitors (BKIs) is of special interest in this respect, and a comparative analysis of the structural–activity relationships of CDPKs of these organisms was performed by screening against 333 BKIs. BKIs interact with the ATP-binding cavity, and this BKI library was developed for the specific inhibition of glycine gatekeeper CDPKs combined with low inhibition of threonine gatekeeper human sarcoma (SRC) kinase. However, some library compounds exhibit the activity against serine- or threonine-containing CDPKs. Keyloun *et al.* explained this on the basis of differences in the size and orientation of the hydrophobic pocket and the specific variation at other amino-acid positions within the ATP-binding cavity. Overall, BKIs appear to constitute highly

interesting compounds that could be further developed for the treatment of diseases caused by different apicomplexan parasites.

Although BKIs exhibit outstanding activity against *Cryptosporidium*, most other compounds directed against apicomplexan parasites do not. The phylogenetic classification of *Cryptosporidium* is still a matter of debate, but analyses of the SSU RNA place *Cryptosporidium* as a separate branch next to the gregarines. This evolutionary distance to other apicomplexans might explain why cryptosporidia are resistant to the common anti-coccidial drugs. As Lendner *et al.* (2014) highlight in their excellent review on molecular advances in *Cryptosporidium* infections, there are other biological features that clearly distinguish the species of this genus from the typical apicomplexan parasites, including the lack of an apicoplast (although some genes are retained), the mode of entry into host cells, the location of the parasitophorous vacuole, and the absence of tools for continuous culture and genetic modification, among others.

ApiCOWplexa 2013 concluded that an active research community within the field is seeking novel ways to reduce both the medical and the economic burden inflicted by these parasites, and to achieve this goal, networking will be a major issue. More meetings in the field of apicomplexan parasites in farm animals would certainly improve the translation of important findings into practical applications. To contribute to achieving this goal, the next ApiCOWplexa meeting (ApiCOWplexa 2015) will be organized by Lee Innes, Frank Katzer and colleagues from the Moredun Research Institute, and will take place in Edinburgh during 1–3 July 2015.

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