Cryptosporidiosis
Summary

Introduction

1. This note provides a brief summary of an analysis prepared by a DISCONTOOLS group of experts on cryptosporidiosis. They reviewed the current knowledge on the disease, considered the existing disease control tools, identified current gaps in the availability and quality of the control tools and finally determined the research necessary to develop new or improved tools. Full details are available on the web site at http://www.discontools.eu/ and can be downloaded by selecting Disease Database, then the specific disease and highlighting the variables of interest. This is completed by selecting "create a report" which can then be downloaded as either a PDF or Excel spread sheet.

Disease profile

2. Cryptosporidiosis is caused by protozoan parasites belonging to the genus *Cryptosporidium*. Currently 31 species have been identified, and over 60 genotypes of unknown taxonomic status. As further genotyping and biological information becomes available, it is likely that some of these will be re-categorised as species. The most common species causing disease in mammalian livestock animals is *C. parvum* whereas both *C. parvum* and *C. hominis* are important human pathogens. A wide range of domestic and wild animals are affected by cryptosporidiosis. The *Cryptosporidium* species affecting non-mammalian host classes are mostly non-zoonotic, with the notable exception of *C. meleagridis*.
3. *C. parvum* has a very wide host range, is predominantly a parasite of young hosts, and is responsible for a substantial proportion of both sporadic and outbreak-related cases of human cryptosporidiosis.
4. *C. hominis* is morphologically identical to, but genetically distinct from *C. parvum.* Many outbreak related cases in humans are due to *C. hominis.*
5. There are good animal disease models for *C. parvum* (neonatal calves and lambs) and infection models (neonatal or SCID mice); however, there are few animal models for *C. hominis* and a symptomatic model is lacking.
6. The oocysts are protected by an outer shell which allows them to survive outside the host for long periods (>6 months) in a moist, cool environment. The oocysts are also very resistant to chlorine-based disinfectants, and there are limited options for effective chemical disinfestation.

Risk

7. Cryptosporidiosis remains a significant public health threat. It usually causes self-limiting but often prolonged diarrhoea in young children but can affect any age group. Cryptosporidiosis is an important cause of moderate-to-severe diarrhoea, and the second cause of diarrhoea-associated mortality, in young children in developing countries. In developed countries with good surveillance systems, it is the 4th most commonly reported cause of infectious gastrointestinal disease and is on the increase.
8. Infection is initiated through ingestion of infective oocysts excreted by infected animals or people or present in contaminated food or water. Animal husbandry practices in relation to housing, feeding, cleaning and disinfection, and birthing patterns and facilities can all have an impact on the spread of disease, as can the movement of animals. Disposal of waste, manure and faeces can lead to contamination of water courses that may be used by animals or humans. Infection by aspiration and inhalation has been reported to occur. There is some evidence for transmission from clinically normal dams to suckling calves or lambs but the precise mechanism remains unknown.

Diagnostics

9. Infection results in both humoral and cell mediated immunity. Local antibody production in the gastrointestinal tract also occurs. Parasite specific antibodies are produced. As the disease generally occurs in the neonate, serum antibodies are not present and as a consequence

serological assays are not helpful in this age group. A variety of antibody-based commercial detection kits are available, all of which rely on the capture of oocyst wall antigens from concentrated or un-concentrated faecal samples, depending on the assay format. These include immunofluorescent microscopy tests, ELISA and immunochromatography based kits. Quantitative real time PCR kits are also available. Acid-fast or fluorescent (such as auramine phenol) staining methods, with or without faecal concentration, are most frequently used in clinical laboratories. The Heine “negative stain” method on concentrated stool is cheap and reliable but requires phase contrast microscopy.

10. There is a need for cheap, reliable, on-site diagnostic tools and for a high throughput PCR assay able to differentiate species or genotypes of interest. Other requirements include sensitive, standardized assays for the early detection of infection, and of asymptomatic carriers, and the development of ISO standards. *Cryptosporidium* should be included in the standard screening of neonates for diarrhoeal diseases.

Vaccines

11. No vaccines are currently available. Since infected animals are a major source of environmental contamination and of infection, an effective vaccine to reduce shedding would be of considerable benefit. The only feasible option is to vaccinate the dams and rely on the transfer of immune colostrum to neonates. There are a range of research needs from the investigation of the potential of adoptive transfer of protective immunity through colostrum from the dam, the *Cryptosporidium* antigens involved in host-pathogen interactions with an evaluation of these as targets for vaccine development along with the identification of a proper *in vivo* model to test vaccine candidates. Identification of potential vaccine candidates, of the appropriate expression system and route of vaccination, and a consideration of regulatory constraints, are all required. Furthermore, mathematical models need to be developed to establish how much the oocyst shedding must be reduced in order to have an impact on transmission and environmental contamination.

Pharmaceuticals

12. Halofuginone lactate is approved for use in new-born calves in Europe, and prevents or reduces diarrhea due to infection with *Cryptosporidium* parvum. Paromomycin is known to be effective in high doses for the treatment of cryptosporidiosis in animal models. This drug is a non-absorbable aminoglycoside which is normally indicated for the treatment of intestinal amoebiasis, however paromomycin is approved at lower doses than required for the treatment of cryptosporidiosis. A number of additional compounds are known to reduce oocyst excretion and to control disease, but are not approved for use in animals. Nitazoxanide (NTZ), an orally administered nitrothiazole benzamide, is licensed by the US FDA for use in humans > 1 year of age and has been found to be effective in immunocompetent individuals. The efficacy in immunocompromised people mainly depends of the level of immunodepression, and treatment modalities have not been well established for this patient group.

13. There may be some potential for development of these or new compounds in animals although the question of parasite resistance remains a potential problem. It is important to evaluate alternative treatment programs (lower dosage, alternate day treatments) with existing compounds (halofuginone, paromomycin) in order to reduce potential side effects in terms of toxicity to the environment, the user and the animal. Recently, parasite specific targets have been identified; however, knowledge of the working mechanisms for the different active compounds are needed in order to better understand and manipulate safety for the environment, target animals and person who applies the treatment. Additional products with emphasis on safety are needed.

Knowledge

14. Animals infected with *Cryptosporidium* act as a potential reservoir for human infection. Wild animals are also infected, or may act as transport vectors, but little is known about their potential role in the epidemiology of infection and whether they play a role in transmitting infection to people or domestic animals. Contamination of animal feed from rodents and other hosts is possible. There has been a human outbreak caused by wild rabbit contamination of a mains drinking water supply in the UK.

15. There are still significant areas of uncertainty in the understanding and knowledge about the disease especially in relation to pathogenesis, immunology, vaccinology, genetics (host and parasite), physiology, influence of the gut microbiome and epidemiology. Research is needed to fill these gaps in knowledge as many of these are closely linked to the research requirements to develop more effective tools for the control of the disease. Full details of the gaps are shown in the Disease and Product Analysis for Cryptosporidiosis on the DISCONTOOLS website.

Conclusions

16. Cryptosporidiosis is a widespread zoonosis of major clinical importance in the developing world, and is of concern in the developed world. As it is a ubiquitous organism, it cannot be eliminated. The discovery of genes, biochemical pathways and protective antigens through mining of the *Cryptosporidium* genomes will help to develop novel therapies and/or vaccines for cryptosporidiosis. The development of vaccines to provide passive immunity to neonatal livestock, and effective biocides, would contribute to animal health and the reduction of the level of oocysts in the environment. Future control strategies could result from passive immunity derived from vaccinated dams and strategic application of therapeutic compounds.