Animal Chlamydiosis and its zoonotic implications

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Objective

A. BACKGROUND

Chlamydiae are widely distributed throughout the world, causing various forms of disease in animals and humans. Several species, particularly Chlamyphila (Cp.) psittaci and Cp. abortus, are known to be transmissible from animals to humans, causing significant zoonotic infections. The unique biphasic lifestyle of these obligately intracellular bacteria, which includes an infective extracellular and a parasitic intracellular phase, renders the respective diseases difficult to control. This is compounded by the specialist growth conditions for the organisms and the lack of a genetic based system for the transformation of chlamydiae, both of which have hampered research on these pathogens. To escape the host immune response these bacteria are capable of transforming into persistent stages of development characterised by a distinct antigenic profile.

(i) Chlamydioses in poultry and other avian species

The most important animal chlamydiosis of zoonotic character is psittacosis, a systemic disease in psittacine birds of acute, protracted, chronic or subclinical manifestation. It is a notifiable disease in Belgium, Germany, Switzerland, the UK and other countries. In Italy, cases of transmission to humans are notifiable. The analogous infection in domestic and wild fowl is often called ornithosis. The economic damage in connection with outbreaks in poultry flocks can be considerable. Although the causative agent, Cp. psittaci, is known to be very widespread in many avian species, not all carrier birds actually show symptoms of disease. Present knowledge about factors contributing to the development of clinical disease, including virulence factors of field strains, is rather limited.

(ii) Chlamydioses in sheep and goats

Another zoonotic disease, enzootic abortion of ewes or ovine enzootic abortion (OEA) caused by Chlamyphila abortus (formerly the ovine subtype of Chlamydia psittaci) has become recognised as a major cause of loss in sheep (and goats) in Europe, North America and Africa. It is the most common infectious cause of lamb loss in several countries of Western, Central and Northern Europe, e.g. accounting for around 50% of all diagnosed abortions in the UK. Economic costs to the farming industry resulting from the disease are considerable and amount to an estimated 15 million (25 million) per annum in the UK alone. OEA is a notifiable disease in Ireland, where its incidence has increased dramatically in recent years. In the UK, cases of transmissions to humans are notifiable.

Although it is well known that enzootic abortion in goats is quite similar to OEA with regard to severity and zoonotic potential, its present spread and economic importance for Europe cannot be assessed for lack of epidemiological data.

(iii) Chlamydioses in cattle, pigs, cats, horses, and reptiles

Over the last few years, endometritis and hypofertility in dairy cattle have sometimes been attributed to chlamydial infections, with clinical manifestations often recurrent in spite of treatment. In a regional survey in Germany, Cp. psittaci/Cp. abortus were detected at a rate of up to 100% in affected herds. Economic losses as a consequence of a drop in milk production and milk quality, as well as abortions and reduced fertility rates were estimated to be Euro 40,000 per year at an average farm of...
Investigations carried out in Italy have shown that the chlamydial organisms involved in these outbreaks belong to the so-called "non-invasive" strains classified as Cp. pectorum. The same agent was also detected in cases of encephalomyelitis in water buffaloes used for milk production.

Chlamydioses in pigs are associated with three different species, i.e. Chlamydia (C.) suis (formerly C. trachomatis), Cp. pectorum and Cp. psittaci/Cp. abortus. A widely held view is that chlamydiae may act in concert with other agents in multifactorial infectious diseases, such as abortions in sows, polyarthritis in piglets, diarrhoea in pigs and genital disorders in boars.

According to veterinary practitioners, clinical manifestations of conjunctivitis in cats are often suggestive of chlamydial infections caused by Cp. felis. Based on an investigation of 462 cats with upper respiratory infections, the prevalence of this agent being 14.3%. Since laboratory diagnosis is seldom conducted the few published data are not representative of the real epidemiological situation.

Occasional evidence of Cp. psittaci/Cp. abortus detected from aborted horse fetuses and Cp. pneumoniae associated with respiratory disorders indicates a role of these species in various equine diseases. Although the former appears to be a commensal germ in many instances, the importance of chlamydial infection in horses is probably underestimated.

In the last few years, several strains of Cp. pneumoniae were isolated from reptiles, some of which are kept as pet animals. It is still unclear whether they form a separate serovar, but it seems certain that the agent represents a pathogen.

Additionally, some recently discovered new members of the order Chlamydiales need to be assessed for their zoonotic potential, for instance the bovine pathogen Waddlia chondriaphila.

(iv) Chlamydiae as causative agents of zoonoses

The zoonotic potential of chlamydiae is illustrated in Figure 1. Avian strains of Chlamyphila psittaci are pathogenic to humans, the symptoms being mainly non-specific and influenza-like, but severe pneumonia, endocarditis and encephalitis are not uncommon. In Germany, the annual average of notified human cases of psittacosis is approximately 100 with several deaths. A study in the United States revealed that, while the average prevalence may be at a few percent, up to 30% of households having purchased pet birds from infected flocks were affected by clinical psittacosis or were serologically positive. The general situation in Europe is believed to be comparable, but comprehensive data are completely absent.

The main group of persons facing an elevated risk of infection includes those having frequent contacts with domestic and companion birds at work or in their spare time. Infections of workers in abattoirs in connection with the slaughtering of ducks, turkeys or geese, as well as cases among bird breeders are regularly reported in several European countries. As a consequence of the increasing habit of keeping parrots, parakeets, budgerigars, etc. as pet birds many more persons are at risk. Feral pigeons are quite commonly found infected in many urban habitats, which also raises serious questions as to the hazards to human health.

In connection with ovine enzootic abortion, transmissions to humans have been repeatedly reported. This serious and potentially life-threatening zoonosis affects pregnant women after contact with lambing ewes and leads to severe febrile illness in pregnancy. With OEA present in Ireland, the UK and other European countries, increased surveillance and improved reporting of human abortions associated with ovine contact are required, even more so as human cases are generally less thoroughly investigated in terms of bacteriology than abortions in animals. It is also necessary to investigate whether outbreaks of chlamydiosis in cattle pose a risk to animal handlers and other contact persons.

A general question that needs to be addressed is the identification of virulence factors of Cp. psittaci and Cp. abortus. The fact that i) only a certain proportion of persons actually develop symptoms of chlamydiosis after contact with infected birds or small ruminants, and that ii) certain geographical areas seem to be more affected than others (e.g. OEA in the UK and Ireland) cannot be explained on the basis of phenotypic or genotypic criteria at present.

The increasing number of reports on the occurrence of the human pathogen Cp. pneumoniae in reptiles and horses raises the question of a potential hazard to human health, particularly among zoo employees and visitors, as well as people keeping such animals.

(v) Chlamydial diagnostics

According to the OIE Manual of Standards for Diagnostic Tests and Vaccines, the most commonly used method for serodiagnosis of animal chlamydioses is the complement fixation test (CFT). It is, however, rather laborious, of limited sensitivity and often impaired by cross-reactions between chlamydial species. More recently developed serodiagnostic tests are mainly based on the two main cross-reactive antigens present in all chlamydial species, lipopolysaccharide and the major outer membrane protein (MOMP), and so are not species-specific for diagnosing animals infected with OEA. Other more specific tests need to be developed and evaluated in a European context, such as those based on specific monoclonal antibodies and recombinant protein fragments.

For antigen detection, cultivation in cell culture is still regarded as the standard. While this time-consuming method is only applicable to cultivable strains, many strains are difficult to grow and not all labs have the facilities and expertise to culture.

The possibilities for diagnostic detection of chlamydiae have considerably improved following the introduction of DNA-based methods, particularly the polymerase chain reaction (PCR), which permits direct identification from clinical specimens and
differentiation of species. A number of tests have been published in the literature, but none of them have been validated in veterinary labs so far.

(vi) Control of chlamydial infection

Currently available vaccines against OEA are based on inactivated whole organisms and a temperature-sensitive live attenuated mutant strain. Although these vaccines offer adequate protection, improvements are necessary to avoid the difficulties associated with bulk chlamydial growth and purification, which result in high production costs and problems with efficacy. In addition, there are safety concerns associated with the inoculation of live zoonotic organisms and with the inoculation of inactivated vaccines with oil-based adjuvants. Recent research has therefore concentrated on sub-unit vaccines, recombinant protein vaccines and more recently DNA vaccination, for which there has been variable success.

Most countries do not currently practise vaccination as a way of controlling infection, instead farms seek to join accredited flock schemes, such as the Premium Health Scheme for Sheep in the UK, through diagnostic testing of flocks. Accredited flocks from farms designated as being ‘OEA-free’ attract premium prices in the market place.

(vii) The rationale for proposing a COST Action

- The zoonotic implications of animal chlamydioses have to be assessed from a European perspective.
- To accumulate epidemiological data on a pan-European scale, harmonisation of diagnostic methods is necessary.
- To highlight changes in disease patterns associated with chlamydioses throughout Europe, a regular exchange of data and expertise between the countries is necessary.
- There is no network to unite all European groups working in the field of animal chlamydioses, which would further the regular exchange of scientific information and practical laboratory expertise.

B. OBJECTIVES AND BENEFITS

The main objective of the Action is to better understand the spread and importance of animal chlamydioses in Europe by using epidemiological data.

The hazards of animal chlamydioses for human public health arising from contact to reared, companion, synanthropic and wild animals will be assessed.

To achieve this goal, effective detection methods based on DNA amplification, monoclonal antibodies or recombinant proteins will be developed and validated by a working group of the Action.

Current research in member countries will increase knowledge on the determinants of pathogenicity of chlamydial isolates, an important prerequisite for the assessment of the zoonotic potential and virulence in general.

The benefits of the Action include the improvement of general diagnostic standards in Europe in connection with animal chlamydioses. Diagnostic data collected during the Action will be the basis for recommendations on improved management, control and prophylaxis of chlamydial infections, such as the psittacosis/ornithosis complex and OEA. The efficacy of currently used vaccines will be assessed and the prospects of novel vaccines can be evaluated. The development of cheaper, safer alternatives, such as recombinant protein or DNA-based vaccines would be a major advance, benefiting animal welfare, reducing the risk of zoonotic infection and reducing the considerable economic losses resulting from the disease.

Generally, the Action will contribute to improvements in animal health and welfare, as well as human health. The negative economic impact on animal husbandry caused by chlamydioses will be reduced.

C. SCIENTIFIC PROGRAMME

Epidemiological investigations

1. DEVELOPMENT AND OPTIMISATION OF DIAGNOSTIC METHODS

As a basis for epidemiological investigations on various animal chlamydioses new methods will be developed and existing protocols will be optimised or adapted to the requirements of routine diagnosis.

DNA-based methods: As a consequence of a major revision of chlamydial taxonomy, specific PCR detection systems for newly introduced species are still required, e.g. the Chlamydophila (Cp.) species Cp. psittaci, Cp. abortus, Cp. felis, and other agents. Moreover, real-time PCR assays will be established in several labs to determine the number of chlamydiae present in clinical and environmental samples, which will be useful in the study of virulence factors and in genotyping. Data from quantitative PCR may help to answer the question whether a certain threshold amount of chlamydial cells is required to trigger clinical disease. Novel approaches to identification, differentiation and detection of chlamydiae based on DNA microarray technology will also be designed and examined. Besides comprehensive transcription analysis, this new technology offers the possibility of rapid multi-site genetic characterisation of organisms, so that chlamydial field isolates could be examined for nucleotide sequence variations in a large number of gene loci associated with potential virulence factors.

Protein-based methods: In an effort to improve the performance of serological diagnostic assays by using recombinant proteins, chlamydial outer membrane proteins from Cp. abortus and different serotypes of avian chlamydiae will be expressed in Escherichia coli and examined for suitability.

Epitope mapping of serotype-specific epitopes will be conducted by using monoclonal antibodies, recombinant protein
fragments and synthetic oligopeptides. The latter will also be tested in serological assays.

2. VALIDATION OF SELECTED DIAGNOSTIC ASSAYS

As isolation in cell culture, mostly regarded as the standard technique, is a time-consuming, costly and cumbersome procedure, it will be appropriate to validate existing and newly developed protocols for molecular diagnosis of chlamydioses. Although quite a number of PCR protocols targeting different genes of chlamydiae have been published in the literature, not all of them will be suitable for routine diagnosis. In the Action, sensitivity and specificity of the tests, as well as accuracy and repeatability will be evaluated in interlaboratory trials. PCR assays will also be assessed in terms of their cost-effectiveness as compared to the "traditional" diagnostic techniques. Protocols will be tested on a range of specimens, namely tissues, swabs, fluids, faeces, as required in laboratories routinely dealing with chlamydial diagnosis.

As to serological diagnosis of chlamydial infections, the objective will be the standardisation of laboratory methods for detection of antibodies to chlamydiae in order to ensure comparability among laboratories.

3. ASSESSMENT OF ZOONOTIC RISKS

Laboratories of the participating countries will carry out diagnostic surveys in flocks of poultry and sheep, herds of cattle, pet animals and others to evaluate the prevalence of chlamydial agents. Previous and present cases of chlamydioses in persons connected with the respective farm, production unit or household will be collected and evaluated.

Pathogenesis and control of chlamydioses

4. RESEARCH ON PATHOGENESIS, IMMUNE RESPONSE AND VIRULENCE FACTORS

Type III secretion (TTS) systems are considered a basic virulence mechanism in many animal and plant pathogens, and the presence of a complete TTS system was recently identified in Cp. caviae, C. trachomatis and Cp. pneumoniae. One of the research topics will be the identification of polymorphism in pathogenicity islands (PIS), which are clusters of genes encoding virulence traits. The obtained evidence will be useful to understand the differences in pathogenicity among chlamydial strains and species.

Other determinants, TTS-associated or not, will also be investigated in order to understand the ability of chlamydiae to disseminate in the host.

As a direct field application, the pathogenicity for humans of Cp. pecorum isolates from various ruminants and of urban feral pigeon strains of Cp. psittaci will be assessed.

To identify virulence markers that distinguish between chlamydial isolates from psittacine birds and those from non-psittacine birds, representative difference analyses (RDA) will be performed. RDA is a multiple-step procedure for the differential comparison of two genomes by restriction analysis and hybridisation.

The immunological mechanisms that control Cp. abortus infection in ruminants and the reasons for abortion are not fully understood. It is of fundamental importance that the disease manifests itself during pregnancy, when persistently infected sheep can abort. In recent years there has been a lot of work published on the effects of cytokines on pregnancy outcome. Cytokines have been found to have either positive or negative effects on placental/fetal survival. Successful pregnancy is considered to be a T helper 2 (Th2)-biased phenomenon. Conversely, unsuccessful pregnancy may be associated with T helper 1 (Th1) type cytokines. In particular, cytokine interferon-gamma (IFN-γ) has been reported to be incompatible with successful pregnancy and has been associated with parturition in humans. In addition, IFN-α has been shown to be a key component of the host response for controlling Cp. abortus growth. Thus, the bias against IFN-γ production during pregnancy in chlamydia-infected ewes may allow for the re-emergence of infection. The re-emergence of infection and concomitant antigenic stimulation may then result in increased IFN-γ production to control infection and this may be responsible for pregnancy failure. The foetal immune response and the attendant inflammatory changes occurring in ovine chlamydial placentitis are beginning to be characterised. Further work will be carried out to improve our understanding of

the relative roles of the infecting organism and the ensuing foetal immune response in determining the resulting pathology. Immune regulation in both infected and non-infected sheep during pregnancy will be studied to elucidate the immunological mechanisms that are important for normal pregnancy and to define the effects of altering the cytokine balance during disease. Comparative studies of Cp. abortus infection in human placental trophoblast cells will give an insight into the pathogenesis of zoonotic infection.

5. ANIMAL MODELS

Greater understanding of the pathogenesis of reproductive tract disease and related immunobiology can be achieved using animal models. Chlamydial infection of the genital tract of sheep is an ideal animal model in which to study chlamydial disease. The sheep represents a unique model for research into chlamydial disease as genital infection with Cp. abortus represents a natural infection in its natural host. Unlike the mouse or guinea pig models of Chlamydial infection, Cp. abortus exhibits true tropism for the ovine reproductive tract. While genetically modified mice have provided invaluable information on the role of particular molecules or cells in the control of immune responses, extrapolations to humans are not that clear cut. Thus studies of disease in their natural host which is both ethical and feasible in domestic animals may be more relevant to human medicine.

Experimental infection of heifers with Cp. pecorum have resulted in cases of salpingitis. Genital infection with C. trachomatis in women is a persistent infection, following infection immunity develops but it is not sufficient to prevent reinfection. Repeated or chronic infections lead to an immune-mediated response which often progresses to fibrosis and scarring and the associated pathology can lead to pelvic inflammatory disease and infertility. The bovine Cp. pecorum model could provide novel insights
6. VACCINATION AND CONTROL OF CHLAMYDIAL INFECTIONS

The generation of new, novel recombinant chlamydial vaccines will depend not only on the identification of relevant antigens but also on ensuring that these antigens are correctly processed and presented to the immune system so that they stimulate the necessary protective immune response. Vaccine research has largely focussed on the predominant protein present in the outer cell membrane of chlamydiae, the major outer membrane protein (MOMP), which still remains one of the most characterised chlamydial antigens. However, in addition to MOMP there are many more antigens that await assessment of their protective efficacy, such as the recently identified polymorphic outer membrane proteins (POMPs) in Cp. Abortus, which are also present in the highly protective outer cell membrane vaccine preparations. The effect of vaccination with some of these antigens (recombinant protein and DNA) on the immune response to challenge with Cp. abortus will be assessed using a mouse model system and in sheep. The mouse model will be used to rapidly assess the efficacy of vaccination through different routes of inoculation, as well as to determine the effect of co-inoculating with cytokines, such as interleukin (IL)-12 which has been shown to enhance a protective Th1 response against many intracellular pathogens. The effect of vaccination on pregnancy outcome will also be assessed. In addition, other novel protective antigens have been recently identified using a DNA vaccination strategy to screen selected open-reading frames from the Cp. pneumoniae genome. This approach will be useful for identifying novel protective antigens in other species such as Cp. abortus, once the sequencing of the genome has been completed.

In an effort to protect poultry against Cp. psittaci infections, a serovar D MOMP and a plasmid DNA expressing serovar D MOMP (pcDNA1::MOMP D) will be tested for their ability to raise an immune response and induce protection against challenge in a turkey model of infection. In order to evaluate the efficacy of both vaccines in the presence of maternal antibodies, vaccination trials will be carried out in one-week old conventional turkeys reared in negative pressure isolators. Maternal antibody titres will be determined by an ELISA using recombinant Cp. psittaci MOMP as antigen.

The immune response will be evaluated by measuring total anti-MOMP serum antibody titres, anti-MOMP specific IgM, IgG and IgA serum antibody titres and by a lymphocyte proliferation assay on peripheral blood lymphocytes. Vaccine efficacy will be determined by examining macroscopic and microscopic lesions, Cp. psittaci replication in the respiratory tract, liver and spleen and by examining Cp. psittaci excretion using both PCR and isolation in BGM cells.

As turkeys can become infected with serovar A, B or D strains, homologous as well as heterologous protection needs to be examined. Experimental pcDNA1::MOMP D or recombinant MOMP D vaccinations and subsequent serovar A, B or D challenge infections will be performed.

D. ORGANISATION AND TIMETABLE

The duration of the Action is 4 years.

The main scientific discussion forum of the Action will be workshops held annually in different member countries. Exchange of scientific information and practical collaboration will be done mainly in the five Working Groups (see below), which will also be responsible for choosing the topics of the workshops. Short-term scientific missions and other exchange of scientists between laboratories will be initiated and prepared at the Working Group level before being proposed to the Management Committee.

i. Working Group 1: New Diagnostic Tools

This Working Group will develop new diagnostic procedures and optimise or adapt existing methods for field applications. The main objectives will include the evaluation of currently used methodologies in each of the countries involved and the evaluation of new tests in terms of specificity, sensitivity, cut-off values, positive and negative predicted values. The exchange of practical expertise, as well as reference material and reagents between the laboratories will be a step towards the harmonisation of diagnostic procedures in the area of chlamydial diagnosis and research. Small and medium-sized diagnostic companies will be invited to take part in these activities.

ii. Working Group 2: Field Survey and Validation

This group will carry out field evaluations of selected diagnostic tests. The main objectives will include validation of the tests with particular emphasis on the reliability, production of recommended protocols for diagnosis from field samples and their use in Premium Health Schemes and other comparable quality assurance systems. The practical results and conclusions will be extremely useful for collection of epidemiological data in member countries and the survey into the involvement of chlamydiae in human infection. Small and medium-sized companies interested in chlamydial diagnostics will be invited to participate.

iii. Working Group 3: Zoonotic Aspects of Chlamydiases

This group is going to focus on cases of transmission to humans and comparison of the epidemiological situation in different countries. Scientists from public health laboratories and research groups in human medicine will be invited to join and exchange their experience with workers from veterinary institutions. The findings of this group will be an important contribution to the general assessment of the risk to human health posed by animal chlamydiases.

iv. Working Group 4: Research on Pathogenesis

This Working Group will deal with the on-going investigations of the research groups in the different participating countries. The research areas to be covered are genetics, molecular biology, biochemistry, physiology, immunology and pathogenesis. Specific research topics will include molecular phylogeny, gene variation, gene cloning and expression, immunogenicity and
variability of surface proteins, virulence factors, humoral and cell-mediated immune responses, disease pathogenesis, host-pathogen interactions, conventional and new approaches to vaccination, impact of the Chlamydia Genome Projects on research.

The members of the group will extensively discuss the impact of their own research, as well as recent data from the literature of the areas covered by the Action.

v. Working Group 5: Development of Vaccines

The efficacy of currently available vaccines and the prospects of novel vaccines will be an important topic. Research scientists will discuss with practitioners the critical aspects of the introduction of recombinant vaccines. The final objective will be the identification of new approaches to controlling chlamydial infections. Small and medium-sized firms involved in vaccine development and production will be invited to participate.

E. ECONOMIC DIMENSION

The following COST countries have actively participated in the preparation of the Action: Belgium, Germany, Ireland, Italy, Switzerland and the United Kingdom. Scientists from France, Greece, The Netherlands and several other countries have indicated their interest to participate.

On the basis of national estimates provided by the representatives of the first six countries mentioned the economic dimension of the activities to be carried out under the Action has been estimated, in 2001 prices, at roughly Euro 4.9 million.

This estimate is valid under the assumption that the six countries mentioned above but no other countries will participate in the Action. Any departure from this will change the total cost accordingly.

F. DISSEMINATION OF RESULTS

The target audience for the dissemination of results will include animal and health officials involved in zoonosis and emerging disease control, researchers in bacteriology and epidemiology, physicians, veterinary practitioners, and also zoo officials and pet owners.

The scientific results of the Action will be disseminated by publication of the proceedings of the annual workshops, articles in international journals, as well as presentations at international and national scientific meetings and seminars. The results of the study on validation of diagnostic methods will be published as recommendations for laboratories and veterinary authorities. This will also contribute to future legislation on chlamydial infections.

In order to make the activities and results accessible to a wider audience and to stimulate scientific exchange contacts will be established to related networks, such as COST 845, COST 854, the Concerted Action on Wildlife Zoonosis and the European Society of Chlamydia Research. In the latter case representatives from human medicine will have knowledge of ongoing activities.

The Management Committee will establish a website to inform the public about the scientific objectives and general organisation of the Action. It will also be used for the preparation and organisation of workshops and meetings, as well as the scientific debate among the participants of the Action. There will be links to the websites of related projects and COST. Additionally the Management Committee will encourage the set-up of small discussion fora (e-groups) dedicated to specific issues as offered by commercial internet providers.

Coordinator

N/A

Administrative contact: N/A

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