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CLINICAL ASPECTS AND PREVENTION OF Q FEVER IN ANIMALS[1]

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The natural reservoir of *Coxiella burnetii* encompasses many free-living vertebrates but the major risk of human infection arises through contact with infected ruminant livestock and their contaminated products. The organism has a remarkable affinity for the ruminant placenta and mammary gland but the great majority of naturally-occurring infections are asymptomatic. However, the potential of *C. burnetii* to cause abortion has been demonstrated experimentally and observed in the field while more recent evidence has implied a contributory role in bovine infertility.

Empirical vaccines incorporating inactivated whole cells of *C. burnetii* or derivatives have induced varying degrees of protection of cattle and sheep against both natural and experimental challenge but, in some cases, severe reactions have occurred at inoculation sites. Modifications in processes of antigen preparation seem to overcome this problem. Discrimination between antibodies resulting from natural infection and those induced by vaccination is possible using ELISAs with specificity for individual immunoglobulin isotypes.

The purpose of this communication is to provide a brief overview of *Coxiella burnetii* infection and associated diseases in animals and means for their control or prevention. What is known of the natural history of *C. burnetii* and epidemiology of human Q fever as a zoonotic disease is well documented in the literature and has been the subject of several review articles (1, 3, 4). That by Babudieri (3), written 30 years ago, is comprehensive in its coverage and is recommended.

That Q fever was first detected in abattoir workers (9) reflects the now well recognised link between livestock and human infection, the major reservoirs in most countries being dairy cows, sheep and goats. Wild ruminants, including deer, also harbour *C. burnetii* (3, 10, 22) and with the growth in deer farming that species may add to the reservoir for human infection. In countries where they are used domestically, buffaloes and camels also represent an infection hazard for man. The agent has been identified in arthropods, ticks especially, and a wide range of free-living animal species including fish, birds, rodents, marsupials and in companion animals such as horses, dogs and cats. In nature, *C. burnetii* is maintained in a cycle of infection involving ticks and free-living vertebrates. The organism undergoes massive proliferation in some, but not all species of tick and dried tick excreta contain vast numbers of highly resistant infectious coxiellae. It is from this cycle that *C. burnetii* is transmitted to livestock and companion animals (dogs, cats) either by tick bite or through contact with heavily infected tick faeces.

Tick-independent cycles of infection can develop in herds or flocks of ruminant livestock, the well developed placenta and lactating udder being the organs principally infected. In cycles in which ticks are not involved the virulence of the organism appears to diminish (1).

Animal disease

In contrast to acute human Q fever animal infection with *C. burnetii* is, in most cases, so strikingly

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asymptomatic that the term coxiellosis is considered a more appropriate designation than animal Q fever (13). But by no means are all infections innocuous and the potential for *C. burnetii* to cause or contribute to economically important disease syndromes is probably underestimated. However, it seems that this pathogenic propensity is subject to variation according to country, region, climate, type of animal, system of management and other circumstances, including virulence of the agent.

Even though the infecting doses used may have been large and the routes of administration unnatural, experimental studies have provided unequivocal evidence of the pathogenicity of *C. burnetii* for ruminant animals. Several workers have reported the transient pyrexia in calves and sheep following inoculation of *C. burnetii* by various parenteral routes, occasionally accompanied by sign of mild respiratory disease (3). Although intramammary inoculation of the agent resulted in a local proliferating infection it took a massive inoculum to provoke an acute but short-lived mastitis and a concurrent systemic reaction. An important observation was the brief persistence of *C. burnetii* in tissues other than the mammary gland.

Experimental infection of pregnant animals has confirmed the remarkable affinity of *C. burnetii* for the ruminant placenta, previously suspected from study of natural infections. The high concentrations of infectious organism released at parturition and the remarkable resistance of the organism to environmental extremes underlies the heavy environmental contamination associated with parturient livestock. Given an appropriate combination of dose, timing and host susceptibility, infection of breeding female ruminants can lead not just to placental infection but to abortion, stillbirths and the delivery of weak offspring. For example, of 11 heifers infected before insemination 3 were barren, 2 aborted and 6 had a normal gestation but shed organisms at parturition (21). Abortion and stillbirth was also the outcome of infecting 2 pregnant cows (5). Such experimental evidence of the abortifacient potential of *C. burnetii* reinforces the view that natural infection can sometimes lead to disease, either as sporadic cases of bovine abortion or, in sheep and goats, as more serious outbreaks such as those reported in Cyprus (8, 19, 20) and Hungary (2). It is pertinent that in these latter instances, differential diagnosis excluded other common causes of infectious abortion including *Chlamydia psittaci.*

It must be remembered that *C. burnetii* can infect the human placenta, apparently without compromising the pregnancy. Of interest too, are reports of two outbreaks of human Q fever in Nova Scotia, Canada, separate in time and place (12, 16). In each, the most probable source of infection was identified as a littering cat. One of the queens gave birth to live offspring but the other delivered stillborn kittens. Both cats had serological evidence of infection with *C. burnetii* but in neither case was isolation of the agent attempted. A recent German survey (26) of 1127 dog sera and 108 cat sera revealed prevalences of antibody to *C. burnetii* of 13% and 26% respectively suggesting that feline infection is common.

Prevention

Prevention of animal coxiellosis hinges upon the general principles of reducing or avoiding contact between the infective source and the susceptible host and of stimulating host resistance to infection by vaccination. Particularly in relation to sheep and goat flocks measures for minimising environmental contamination by the products of conception are important, especially under systems of intensive management. In tick-infested areas due attention must also be paid to effective tick control. However, given the nature of the organism and the range of hosts which it can infect, sanitary measures alone, though helpful, will not overcome an enzootic problem. It is necessary therefore to consider vaccination.

Until recently, the primary aim of animal vaccination has been reduction of the risk of human exposure, there being little demand or economic justification for strategic vaccination of livestock against an infection which seldom was associated with serious disease problems. However, over the last decade information has been accruing which indicates that, as well as provoking epizootics of abortion in sheep (8, 19), *C. burnetii* can, under certain circumstances, contribute to complex infertility problems in cattle (7, 25, 28). Thus there is an additional incentive for the availability of effective, safe and affordable animal vaccines for use in countries or regions where coxiellosis is implicated in disease. As the topic of animal vaccines against *C. burnetii* has been dealt with in some detail in a recent review (25) only major points concerning formulation, efficacy, safety and future trends need be addressed here.

Understandably, reports of vaccine studies conducted by various workers over the last 40 years reveal differences in construction and formulation of vaccines. There are variations in the strain and phase of *C. burnetii* used, in the nature and preparation of antigenic material employed and use of adjuvant. An important consideration is which phase of *C. burnetii* to use. Whilst experiments in laboratory animals have demonstrated the superiority of phase I vaccines in affording protection against challenge (18), large scale commercial production of antigen is easier and safer using organisms in phase II (23). The particular strain of the organism would appear to be of little consequence given the proven ability of individual strains to induce complete reciprocal cross protection, at least in guinea pigs (18). However, recent findings indicative of antigenic heterogeneity independent of phase variation suggest that this may be too simplistic a view (2). It was partly for that reason that a recent WHO workshop on Q fever advocated the

establishment of a centralised collection of *C. burnetii* isolates of defined origin and history (2). In general, the antigenic components of animal coxiella vaccines have been inactivated whole cells (WC), with more recent use of particulate residue of chloroform/ methanol extracts of whole cells (CMR) (27) or an enzymically liberated protein of that residue (PE) (17, 25).

However immunogenic and effective vaccines may prove to be in laboratory animals there remains a need to evaluate them in the host species they are meant to protect. In that regard, varying degrees of protection of cattle and sheep against both natural and experimental infection with *C. burnetii* have been reported. For example, an inactivated phase II WC vaccine given to dairy cows before they entered a naturally contaminated environment resulted in a lower infection rate compared to unvaccinated controls and to a correspondingly decreased shedding of coxiellae in milk (15). Comparable findings were later recorded with a Czechoslovakian inactivated vaccine containing phase I WC, provided cows were seronegative when vaccinated (25), and with a similar American vaccine administered to calves or to older animals before the onset of the first lactation (5). In contrast, vaccination of seropositive cows (i.e. already infected) had no effect on the herd shedding rate (25).

Protective efficacy has been demonstrated also following experimental challenge of vaccinated and control animals. In one such study one of two non vaccinated cows infected subcutaneously in mid pregnancy aborted in the 8th month and the other produced a stillborn calf whereas all three vaccinated cows delivered healthy calves (5). *C. burnetii* was isolated from the placentas and colostrum of all cows but in many fold higher titres from the control animals than from the vaccinates. The latter also had a lower index of excretion of the agent in milk. A similar outcome of clinical and relative microbiological protection was obtained when pregnant ewes vaccinated 3 months before mating with a phase I WC or CMR vaccine were challenged at 100 days of gestation (6). Though none of 6 unvaccinated ewes aborted they gave birth to smaller, weaker lambs than 11 vaccinates and in two cases placental necrosis was grossly visible. Compared to controls both groups of vaccinates shed a lower weight of coxiella infection at parturition.

A further reported benefit of vaccination has been the amelioration of chronic infertility problems in cattle herds enzootically infected with *C. burnetii*. In independent trials with a vaccine incorporating inactivated phase II WC of *C. burnetii*, significant improvement in breeding performance followed the institution of a vaccination programme coupled, where appropriate, with oxytetracycline treatment (7, 25). It was acknowledged, however, that concurrent infection with agents other than *C. burnetii*, e.g. *C. psittaci* and bovine pestivirus may have contributed to the underlying problem of impaired reproductive performance. It is relevant that trials conducted since 1983 have used a commercial vaccine which contains inactivated WC of both *C. burnetii* and *C. psittaci* (25). Nonetheless, the clinical improvements achieved by vaccination are noteworthy.

As well as evaluating clinical and microbiological indices of vaccine efficacy attention has to be paid to the induced antibody response not only to determine its value as a possible correlative parameter but also its role as a confounding factor in identifying naturally infected animals. Thus the feeble and irregular complement-fixing (CF) antibody responses evoked by vaccines based on phase II organisms gave little guidance to protective efficacy but did not impede serological diagnosis of infection (7, 15). The reverse trend has been experienced with vaccines incorporating organisms in phase I. Judgements have to take account of the sensitivity and specificity of the test system used and the weaknesses of both the CF and microagglutination tests for detection of antibodies to *C. burnetii* are widely recognised. Of the more sensitive and versatile tests now available the enzyme-linked immunosorbent assay (ELISA) is particularly valuable for routine serum analysis and for isotype analysis of the antibody response. Recently, considerable advances have been made in the development and application of an ELISA for detection and sub-isotypic discrimination of antibodies to *C. burnetii* (23, 24). Notwithstanding criticisms of possible allotypic bias in this type of test (11) it has the particular pragmatic advantage that, in cattle, it can differentiate between the essentially IgG_1 response to infection and the predominantly IgG_2 response to vaccination.

An adverse feature of some vaccines, particularly those incorporating phase II organisms, is that they can provoke quite severe reactions at the site of deposition varying from firm localised swellings to larger sterile abscesses that sometimes rupture (24). In an attempt to overcome this problem phase II vaccines have been prepared from organisms propagated in cell culture rather than embryonating chicken eggs (25). After recovery and purification the cells were extracted with chloroform and methanol and the residue treated with enzyme to release a membrane protein. When tested in mice and rabbits both CMR and PE vaccines expressed a vigorous immunogenicity and vaccinated mice successfully resisted challenge. The CMR vaccine induced a powerful antibody response in a horse without causing an unacceptable reaction at the site of intramuscular inoculation. Together with the success of the phase I CMR vaccine in sheep (6) these findings indicate that empirically derived vaccines can, both safely and effectively, protect ruminant livestock against coxiellosis. It has to be remembered however, that with rare exceptions, vaccination is an adjunct to effective control of disease and containment of infection and should be reinforced by good hygienic standards and other appropriate control measures.

For the future there is likely to be a move towards more sophisticated human vaccines for Q fever of the

subunit, synthetic or recombinant type and that technology may be applied also to develop vaccines to protect cattle, sheep and goats against *C. burnetii.* Methods will need to be devised to optimise antigen presentation and delivery to achieve the ideal of a long-lasting sterile immunity. Scientific attainment of that objective must be based on a thorough understanding of the ruminant immune system at the molecular level, and in that there is still some way to go.

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