COXEVAC suspension for injection for cattle and goats.

**COMPOSITION:** Composition for 1 ml: Inactivated Coxiella burnetii, strain Nine Mile 72 QF Unit Q-fever Unit: relative potency of phase I antigen measured by ELISA in comparison with a reference from Nine Mile 72 strain. Thiomersal max. 120 ug. Stabiliser: Salts for the active immunisation of cattle to lower the risk for non-infected animals vaccinated when nonpregnant to become shedder (5-times lower probability in comparison with animals receiving a placebo), and to reduce shedding of Coxiella burnetii in these animals via milk and vaginal mucus. Salts for the active immunisation of goats to reduce abortion caused by Coxiella burnetii and to reduce shedding of the organism via milk, vaginal mucus, faeces and placenta.

**INDICATIONS:**
- **Cattle**: For the active immunisation of cattle to lower the risk for non-infected animals vaccinated when nonpregnant to become shedder (5-times lower probability in comparison with animals receiving a placebo), and to reduce shedding of *Coxiella burnetii* in these animals via milk and vaginal mucus.
- **Goats**: For the active immunisation of goats to reduce abortion caused by *Coxiella burnetii* and to reduce shedding of the organism via milk, vaginal mucus, faeces and placenta.

**CONTRAINDICATIONS:** None.

**ADVERSE REACTIONS:**
- **Cattle**: It is very common to see a palpable reaction of maximum diameter of 9 to 10 cm at the injection site, which may last for 17 days. The reaction gradually reduces and disappears without need for treatment.
- **Goats**: It is very common to see a palpable reaction of 3 to 4 cm diameter at the injection site which may last for 6 days. The reaction reduces and disappears without need for treatment. It is very common to observe a slight increase of rectal temperature for 4 days post-vaccination without other general signs.

**AMOUNTS TO BE ADMINISTERED AND ADMINISTRATION ROUTE:**
- **Subcutaneous use.** Shake well before use. Administer the vaccine as follows:
  - **Cattle:** 4 ml in the neck region
  - **Goats:** 2 ml in the neck region

Although efficacy studies are based on data from challenge test carried out in goats vaccinated twice 6 and 3 weeks before start of pregnancy, there are indications from a large field trial that it is useful to vaccinate pregnant animals. This information, along with data obtained in cattle, allows recommending the following vaccination program:
- **Cattle**: from 3 months old of age. Primary vaccination: Two doses should be given subcutaneously with an interval of 3 weeks. Under normal conditions, the primary course is completed by 3 weeks before artificial insemination or mating.
- **Goats**: from 3 months old of age. Primary vaccination: Two doses should be given subcutaneously with an interval of 3 weeks. Under normal conditions, the primary course is completed by 3 weeks before artificial insemination or mating.

**WITHDRAWAL PERIODS:**
- **Meat, milk and offal:** Zero days.

**PACKAGING:** box containing 40 ml or 100 ml of solution.

**MARKETING AUTHORISATION NUMBER:** EU/2/10/110/001.

**MARKETING AUTHORISATION HOLDER:** CEVA Sante Animale 10 avenue de la Ballastiere 33500 Libourne, FRANCE.

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**VACCINATION DOSE**

- In cattle: 4 ml
- In goats: 2 ml

---

**PROTOCOLE DE VACCINATION**

**1ER VACCINATION**

3 weeks

**2ÈME VACCINATION**

9 to 12 month

**Booster**

Optimal vaccination period: from 3 months of age

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**2011 - Q FEVER REFERENCE BOOK**

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**QCOXEVAC**

To protect against *Q* fever
Foreword

It is my privilege to introduce you with this book dedicated to Q fever and Coxevac®. It summarizes the latest scientific development and expertise from many well known health professionals from around the world. It also provides more detailed information on what makes Coxevac® a unique vaccine to fight against this zoonosis, illustrating our group’s vision: «together beyond animal health».

Because, they bring solutions to the veterinarians and farmers in the field, the science and innovation that are behind the formulation of Coxevac® are what the researchers from our Ceva Phylaxia Campus are most proud of. Do not hesitate to contact them or your Ceva field representatives. Your experience will help us make further progress.

I hope you will enjoy the reading,

Arnaud Bourgeois
Head of the Biological Business Unit
Q fever

Updated from the 2009 EBF Ceva Symposium "Q fever: an emerging disease"

Coxevac® in cattle

Summary of publications and registration file

Coxevac® in goat

Summary of publications and registration file

Acknowledgements for their contribution

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CNRS, Rickettsia Reference Center, Medicine University of Marseille (France)
Coxiella burnetii, the agent of a worldwide zoonosis named Q fever, is considered a highly efficient pathogen, indeed its infectivity is estimated to range between 1 to 10 organisms for humans.

Coxiella burnetii is extensively distributed in nature and able to infect many animal species including mammals, birds, reptiles and arthropods.

Coxiella burnetii infection in animals can be subclinical, but in mammals it may cause severe reproductive disorders such as abortions and stillbirths. In cattle herds, metritis and infertility are frequently the main clinical signs of infection.

Infected livestock, especially goats, sheep and cattle are considered the main reservoir for human Q fever, as they shed large number of bacteria in placenta and birth fluids after abortion or parturition. Number of bacteria excreted in vaginal mucus, urine, faeces and milk is smaller but shedding via these routes may persist over several months. Cats and dogs may also be a source of human infection especially if they ingest infected placentas and foetuses.

Inhalation of infectious aerosol or airborne dust is the most common transmission route of the disease from animals to human or to other animals. The spreading of manure from infected herds in fields may be an important source of contamination because wind may propagate the disease over large distances.

Vaccination of ruminants with Coxevac® has been proven to reduce clinical signs and shedding of the bacteria. Furthermore, in a recent outbreak of human Q fever in the Netherlands, vaccination of ruminants was implemented as a control measure and the number of reported human cases was, after the implementation of these measures, reduced year after year.
I. The disease: expression in animals and humans

SUMMARY

In cattle, Q fever is mostly subclinical. Economical losses are mainly linked to the impact of Q fever on the reproduction performance of the herds. *Coxiella burnetii* infection has been associated with infertility, repeat breeding, retained placenta and metritis. It has also been linked to weak calf syndrome, respiratory diseases and sub-clinical and clinical mastitis.

In small ruminants, Q fever detection is easier as it causes late pregnancy abortion. In humans, symptomatic illness can be divided into acute and chronic forms. 3/4th of all cases are acute. The incubation period for acute infection is approximately 20 days. Patients display hepatitis, or pneumonia and hepatitis, pneumonia only, or fever only. Chronic Q fever is defined as an infection lasting for more than six months. It occurs in approximately 1 to 5 % of patients infected with *Coxiella burnetii* and may develop insidiously months or years after the acute disease. Endocarditis is the most serious and often fatal form of chronic Q fever. The second most common manifestation is vascular aneurysm and prosthesis infection.

Both acute and chronic Q fever have been described during pregnancy, resulting in obstetrical complications, such as spontaneous abortion, intrauterine growth retardation, intrauterine foetal death, oligoamnios, and premature delivery.

In Animals


**a / Ruminants**

**Cattle**

In cattle, the most frequent clinical signs are repeat breeding, infertility, metritis (most frequently post AI), retained placental, and possibly subacute mastitis.

However, the most visible signs (but not the most frequent) of Q fever are abortion, stillbirth and weak neonates. Aborted foetuses appear normal except in some cases where bronchopneumonia can be detected. Placentas exhibit frequently intercotyledonal fibrous thickening and discoloured exudates but these gross lesions cannot be considered as pathognomonic. Histology allows to retrieve *Coxiella burnetii* inclusions in trophoblast and multinuclear cells. The abortion rate can range from 3 to 80% of pregnant animals. High abortion rates are not frequently observed in cattle.

Chronic infection does not generally lead to endocarditis in animals as it happens in humans. However in an experimental infection of Friesian cows, all the animals developed pneumonia and one cow died of cardiac failure due to degeneration and sclerosis of the myocardium.

**Goats**

Q fever in goats can induce pneumonia, abortions, stillbirth and delivery of weak kids. Abortions take place most of the time near the end of pregnancy. The frequency of occurrence of Q fever abortions in goats is more important than in cattle with very high number of pregnant goats (up to 90%) being affected. Moreover a study has shown that infected goats can abort again during the next pregnancy. These animals develop also more frequently chronic infections with persistence of the bacteria in the uterus and mammary glands.

**Sheep**

Q fever in sheep is very similar to Q fever in goats with a high frequency of abortion. Some differences exist however. Q fever in sheep seldom causes chronic infections. Role of Q fever in ovine mastitis has been evocated but remains questionable.

**b / Swine**

**Pigs**

Exposure has been demonstrated by the presence of antibodies to *Coxiella burnetii* in their serum. However the role played by pigs in the epidemiology of Q fever remains unknown.

**c / Pets**

**Dogs and cats**

Dogs and cats can be infected by Q fever and they can be the source of infection to humans and animals in rural and urban areas. Abortions have been linked to Q fever in different studies however Q fever is usually asymptomatic and remains undiagnosed.
2

In Humans


a / Clinical features

Clinical signs of Q fever are often mild or absent: during an outbreak of 415 cases in Switzerland, 224 (54 percent) were asymptomatic and only 2 percent were hospitalized. The factors which determine whether or not symptoms develop are not well understood. Symptomatic infection is more likely in adults compared to children and in men compared to women. Symptomatic illness can be divided into acute and chronic forms. Chronic Q fever is defined as infection lasting longer than six months.

b / Acute infection

A self-limited flu-like syndrome is the most common manifestation of Q fever. In Spain, this form of the illness accounts for 21 percent of all febrile episodes lasting from one to three weeks. The onset is typically abrupt with high-grade fever (104°F or 40°C), fatigue, headache, and myalgias. Chest radiographs are usually normal.

Pneumonia

Pneumonia is the most common manifestation of acute Q fever in Nova Scotia, the Basque region of Spain, and Switzerland. Most cases are mild, with a non-productive cough, fever, and minimal auscultatory abnormalities; however, some patients display acute respiratory distress. Pleural effusion can also occur. Findings on the chest radiograph are not specific and resemble a viral pneumonia. Symptoms can last from 10 to 90 days with a mortality rate from 0.5 to 1.5 percent.

Hepatitis

Hepatitis can occur in three forms: 1) similar to infectious hepatitis due to viruses, with hepatomegaly but seldom jaundice, 2) clinically asymptomatic hepatitis with fever and an increase in serum transaminases, 3) prolonged fever of unknown origin with characteristic granulomas on liver biopsy. Q fever hepatitis typically has a granulomatous pathology, in which some of the granulomas appear to be «doughnut-like» because they contain a lipidic vacuole surrounded by a fibrinoid ring.

Other

Other manifestations of acute Q fever include: Maculopapular or purpuric rash (10 percent), pericarditis and/or myocarditis (1 percent), severe headache, aseptic meningitis, and/or encephalitis (1 percent). Myocarditis, though uncommon, may be a particularly severe manifestation of Q fever.

c / Chronic infection

Chronic Q fever is defined as infection lasting longer than six months. In this form of Q fever, Coxiella burnetii multiplies in macrophages and produces a prolonged rickettsiaemia. It occurs in approximately 1 to 5 percent of patients infected with Coxiella burnetii (symptomatic or not) and may develop insidiously months or years after the acute disease, especially in patients with underlying diseases. Endocarditis is the most serious and often fatal form of chronic Q fever. The second most common manifestation is vascular aneurysm and prosthesis infection. Other manifestations of chronic Q fever include isolated hepatitis possibly complicated by hepatic fibrosis and cirrhosis, osteoarthritis, and osteomyelitis.

Q fever endocarditis

Endocarditis is the most serious and often fatal form of chronic Q fever (up to 24 percent). Diagnosing Q fever endocarditis is difficult and currently relies on non specific cardiac findings, evidence of liver involvement, and serologic tests. Because the clinical picture of the disease usually does not resemble classic endocarditis, the diagnosis is generally made because of previous valvular damage. The development of Q fever endocarditis in patients with Q fever primarily occurs in patients with previous valvular disease or immuno-compromised. In a retrospective study, of 302 patients diagnosed with Q fever, which investigated the risk factors of developing endocarditis, the following findings were noted: 1) Preexisting valvular disease was much more common in the 102 patients who developed endocarditis (93 versus 3 percent in those who did not develop endocarditis). 2) Among the patients who had preexisting valve disease, the estimated risk of endocarditis was 39 percent. 3) Among the seven patients with endocarditis but no valvulopathy, five had active cancer.

Some patients who develop Q fever endocarditis have clinically silent and previously undiagnosed valve disease. As a result, screening echocardiography may be warranted in patients with Q fever. Q fever endocarditis is a severe and often fatal disease that is the major manifestation of chronic Q fever.

Most patients with Q fever endocarditis are men over the age of 40, but women and children also develop this complication. Most patients with Q fever endocarditis have preexisting valve disease (93 percent in a series of 102 patients). The underlying heart disease may be congenital, rheumatic, degenerative, or syphilitic. The aortic and mitral valves are often involved, but 55 percent of patients in one report had prosthetic valve endocarditis.

There is no typical clinical presentation of Q fever endocarditis. Many patients have previously known valvulopathy and present with symptoms of heart failure or valve dysfunction or with constitutional symptoms, including fever, malaise, weakness, weight loss, fatigue, chills, anorexia, and night sweats.

Peripheral manifestations

Peripheral manifestations of endocarditis are frequently found in Q fever, in contrast to most other causes of infective endocarditis. Common findings include splenomegaly (in approximately 50 percent), digital clubbing (33 percent) and a purpuric rash (20 percent). Hepatomegaly is also frequently seen. The liver is generally hard and often considerably enlarged. Most cases of chronic hepatitis in Q fever are associated with valvular damage; thus, endocarditis should be suspected when diagnosing chronic Q fever hepatitis.

Renal involvement, manifesting as microscopic hematuria, occurs in up to one-half of patients with endocarditis. Embolic manifestations occur in about 20 percent of patients and may be recurrent. The emboli can involve cerebral, arm, or leg vessels, and embolectomy or amputation may be required. Pulmonary and pleural manifestations may be observed as a complication of Q fever endocarditis. Neurologic manifestations are rare except for embolic stroke.

d / Q fever in pregnancy

Both acute and chronic Q fever have been described during pregnancy, resulting in obstetrical complications, such as spontaneous abortion, intrauterine growth retardation, intrauterine fetal death, oligoamnios, and premature delivery. A retrospective study of 53 pregnant women with Q fever was significant for the following findings: 1) Coxiella burnetii infection during the first trimester was associated with an increased risk of obstetric complications and maternal chronic Q fever compared to infection in later trimesters. 2) Long-term duration (35 days) of cotrimoxazole treatment protected against maternal chronic Q fever infection, Placental infection, and obstetric complications, particularly intrauterine fetal death. 3) A strong association was found between placental infection and obstetric complications, including intrauterine fetal death. Because of the high rate of asymptomatic infection in pregnant women, it has been suggested that systemic testing for Q fever should be performed during pregnancy in areas where Q fever is prevalent and when a pregnant woman is febrile or has an abnormal delivery.
II. The agent, the infection and the host’s reaction

Q fever is caused by small Gram negative intracellular bacteria called *Coxiella burnetii*. *Coxiella burnetii* is highly efficient in terms of infectivity and very resistant. The virulent phase, which resists to complement mediated serum killing is phase I.

According to shedding level and pattern, small ruminant herds are often considered as a major source of cattle infection. Abortion and also normal parturition can result in significant environmental contamination and is likely the period at greater risk for transmission of the disease within herds and flocks. The main route by which Q fever spreads in ruminants is via inhalation of aerosols from contaminated material.

In humans, exposure results from:
- Inhalation of contaminated aerosols from parturient fluids of infected livestock, the coats of newborn animals, or the placenta.
- Consumption of infected raw milk.
- Human to human transmission has been reported in sporadic cases following contact with an infected parturient woman.

Humans are incidental hosts in the zoonotic infection caused by *Coxiella burnetii*. Serology is of major interest to diagnose Q fever but will be often performed in conjunction with other tests (PCR), indeed:
- Cows with a history of Q fever can display positive serology up to 15 month post infection, without however displaying, at the time of PCR sampling, any shedding of *Coxiella burnetii*.
- From blood transfusions, intradermal inoculation and transplacental transmission.
- Cows can also shed the bacteria following infection without however developing an immune response. They will therefore be negative to serology.

An efficient vaccine implies reaching a well balanced cellular answer. Indeed, it should stimulate the production of cytokines in order to protect against a virulent challenge. The levels of cytokines produced should however be balanced so as not induce secondary effects such as abortion.
Coxiella burnetii is a small gram-negative intracellular pleomorphic cocobacillus (0.2 to 0.4 μm wide and 0.4 to 1μm long). Historically the bacterium belonged to the order Rickettsiales, however the gene sequence analysis of the complete genome of Coxielia burnetiiNine Mile classifies the Coxielia genus in the order Legionellale, family Coxiellaceae with the genera Ricketsiella and Aquicella.

Coxielia burnetii multiplies by transverse binary fission through a developmental cycle presenting superficial similarities with those of Chlamydia.

**a / Developmental Cycle**

The intracellular developmental cycle of Coxiella burnetii involves three morphological and functionally distinct cell types: large cells variants (LCVs), small-cell variants (SCVs) and small dense cells (SDCs). The LCVs, as the reticulate bodies of chlamydiae are the osmotically sensible intracellular forms that are metabolically active. The SCVs and SDCs are morphologically identical; they are the extracellular forms of the bacteria and are resistant to osmotic pressure.

The SCVs and SDCs enter into the eukaryotic cells by microfilament dependent parasite directed endocytosis. Approximately 2 hours after internalization, the Coxiella containing phagosomes fuses with lysosomes, which acidifies the vacuole. Coxiella burnetii has an absolute requirement of this acidic pH (4.8) to activate its metabolism and to allow the differentiation of SCV to LCV, a process which takes 1-2 days to occur. Over the next 4 days, an exponential growth is observed and LCV forms are predominant. The stationary phase starts approximately 6 days after the entrance into the host cell and coincides with the reappearance of SCV due to a LCV to SCV condensation which is similar to the differentiation of chlamydial reticulate body into elementary body. The number of SCVs increases 8 days after the entry into the host cell.

The morphological differences between the LCVs, SCVs, and SDCs correlate with different protein composition in particular, the major outer membrane protein P1 which functions as a porin. The absence of P1 in SDCs is responsible for the environmental stability of Coxiella burnetii. Since Coxiella burnetii is very resistant, highly infectious, transmissible by aerosol and hardly affected by extreme environmental conditions, it is classified as a category B biological weapon.

**b / Survival**

Coxiella burnetii is able to survive in an extra cellular environment. Survival of Coxiella burnetii for a long period in contaminated foods, buildings and pastures, has resulted in human and animal infections. The microorganism is resistant to outdoor conditions (elevated temperature, ultraviolet light, osmotic, shock and desiccation). For example, Coxiella burnetii can survive during more than 5 months in 20 - 22°C water, more than 6 months in 10% salt solution (9 months at 4°C), 6 months in dried guinea pig blood, 48 months in ticks' faeces.

Coxiella burnetii is also resistant to chemical disinfectant. Formaldehyde gas failed to consistently inactivate 10⁵ Coxiella burnetii in a large room with no humidity control, while Coxiella burnetii seeded onto membrane filters in a small sealed chamber are inactivated by overnight exposition to humidified formaldehyde gas or ethylene oxide. Liquid suspension of 10⁷ Coxiella burnetii is inactivated in 30 min in 70% ethyl alcohol or 5% chloroform. The treatment of slurry by calcium cyanamat 0.6% during one week allows the inactivation of Coxiella burnetii.

Coxiella burnetii may also persist in the environment due to ticks which are considered as the natural primary reservoirs of Coxiella burnetii, the latter is responsible for the spread of the infection in wild animals and for the transmission of Coxiella burnetii from wild to domestic animals. Ticks transmit Coxiella burnetii vertically to their progeny and horizontally to wild animals especially rodents and sometimes ruminants. Coxiella burnetii was identified in more than 40 tick species of 12 genera. The faeces of ticks infected with Coxiella burnetii have very high concentrations of viable organisms which may persist for long periods (at least 586 days) in the environment.

In addition Coxiella burnetii could survive in free-living amoebae.

**c / Phase variation**

Coxiella burnetii has a cell wall similar to that of Gram-negative bacteria. When it is propagated and after several passages on cell culture or embryonated hen eggs, its outer membrane varies. This variation, called phase variation, is similar to the smooth to rough lipopolysaccharide (LPS) transition of Enterobacteriaceae.

Coxiellas expressing a complete LPS (phase I bacteria), are isolated from infected hosts. They are virulent and resist to a complement-mediated serum killing. Phase II Coxiella burnetii are on the contrary, avirulent and have a truncated form of LPS lacking the O-polysaccharide chain, characteristic of phase I LPS. It’s why an efficient vaccine must contain complete LPS obtained from phase I Coxiella burnetii.

**d / Virulence factors**

The ability of Coxiella burnetii to grow in a usually bactericidal environment is likely a crucial virulence factor. Coxiella burnetii is the only known bacterium which replicates in a phagolysosome. Full-length LPS is the only defined virulence factor of Coxiella burnetii.
Potential reservoirs

The potential reservoirs of *Coxiella burnetii* include all domestic and wild mammals, birds and arthropods such as ticks. According to shedding level and pattern, small ruminant herds are often considered as a major source of cattle infection. Nevertheless, areas where sheep and goats are absent frequently show the same apparent prevalence in cattle than areas with high density of small ruminant flocks.

*Coxiella burnetii* is frequently isolated from the mammary glands and placenta of infected animals, and is excreted in large numbers in milk, placental tissue, foetal fluids, urine and faeces. Consequently, abortion but also oral contamination has been suspected in suckling young ruminants but this low risk is not really documented today and, if exists, appears extremely rare.

Transmission to humans

In ruminants, this way of contamination is probably a minor risk but cannot be ruled out. Embryo transfer can also be considered as a route of contamination. The infection of genital tract flushing media and tissues is a risk factor for the transmission of *Coxiella burnetii* from donor to recipient or to the foetus.

In conclusion, according to the potential reservoir of *Coxiella burnetii* and route of transmission, an effective management of manure, water, pets or, may be, of sperm must be carried out in ruminant herds. Nevertheless, as aborted foetuses and contaminated fluids and placenta are of major importance in term of risks, prevention of abortion is one of the most important ways of preventing ruminant Q fever.

Subjects at risk

Q fever can occur in any age group but is more prevalent between the ages of 30 to 70 years of age. Men are more frequently infected than women. After exposure, women and children are more commonly asymptomatic than men and adults, respectively. Because of occupational exposure, subjects at greatest risk of Q fever infection are persons in contact with farm animals, individuals downwind from manure, straw, or contaminated dust from farms, and laboratory workers.

In the largest collected series of cases, 1383 Q fever patients were identified in France from positive serum specimens submitted to a reference laboratory over a 14-year period; 1070 patients had acute disease; risk factors of infection included living in a rural area, consuming cheese made from raw milk, and contact with pregnant or newborn animals. In other studies, HIV-infected patients also appear to be at greater risk for symptomatic Q fever, and pregnant women can develop fever, spontaneous abortion, or premature labour.

Subjects at risk

Q fever can occur in any age group but is more prevalent between the ages of 30 to 70 years of age. Men are more frequently infected than women. After exposure, women and children are more commonly asymptomatic than men and adults, respectively. Because of occupational exposure, subjects at greatest risk of Q fever infection are persons in contact with farm animals, individuals downwind from manure, straw, or contaminated dust from farms, and laboratory workers.

Transmission to humans

In humans, exposure results from inhalation of contaminated aerosols from parturient fluids of infected livestock, which can be present in the environment, the coats of newborn animals, or the placenta. Consumption of infected raw milk is another cause of infection. Sporadic cases of human to human transmission have been reported following contact with an infected parturient woman. Infection also results from blood transfusions, intradermal inoculation and transplacental transmission which cause congenital infection. A case report also favours intercourse due transmission.

Temporal and geographic distribution

The geographic distribution of human Q fever is worldwide. Incidence figures vary widely. Cases of acute Q fever in Europe occur more frequently in spring and early summer. Large outbreaks of Q fever have been reported in the Basque country, in Switzerland, in Great Britain, in Berlin, and in southern France. In North America, Nova Scotia has been the location of a significant number of cases. The most amazing outbreak occurred in the Netherlands. This outbreak began in 2007. Mandatory vaccination and stamping out of infected flocks were implemented to stabilize and finally stop the disease in 2010.
a/ Humoral immunity

The role of antibodies has been overlooked for a long time and their protecting role is still controversial. Despite of all those uncertainties, passive protection of mice and guinea pigs with Coxiella burnetii antisera has been described. Furthermore, specific antibodies accelerate the clearance of the bacteria.

Infection and vaccination with Coxiella burnetii in animals and humans induce significant antibody response against Coxiella burnetii antigens. The antibody response becomes detectable by ELISA 2 to 3 weeks after infection as measured in experimental infection of cows or pregnant goats. The antibody titre gradually increases during 3 to 4 months. Experimentally infected cows remain positive during 15 months.

In naturally infected animals the antibodies may persist several years without the animals displaying reproductive disorders or shedding the bacteria, while other animals can remain seronegative and excrete Coxiella burnetii. This seronegative response of infected animals shedding Coxiella burnetii was also observed in experimental infection. One goat which aborted after being inoculated with the high dose (10⁸ Coxiella burnetii) did not become seropositive.

b/ Cell mediated immunity

Cell-mediated immunity (CMI) seems to be crucial for the elimination of the agent. The immune response interferes locally by a granuloma formation and also by a systemic response which manifests itself by monocytes and macrophages’ interferon-g (IFN-g) activation which results in the end, in the intracellular killing of Coxiella burnetii.

Suppression of CMI in mice by corticosteroid treatment, pregnancy or by gamma irradiation induces the reactivation of persistent Coxiella burnetii infection proving the extent of the cellular immunity for the control of Coxiella burnetii infection.

The role of the CMI in the protection and vaccination has mainly been studied in mice. The transfer of splenocytes from phase I vaccinated mice into naive mice protects them against a virulent challenge indicating that CMI plays an important role in the protective immunity elicited by vaccination.

c/ Immunopathology

In contrast to IFN-g, IL-10 promotes the multiplication of Coxiella burnetii in human monocytes and an overproduction of IL-10 is associated with the development of chronic Q fever in human patients.

IL-10 is a pleiotropic cytokine exhibiting both pro and anti-inflammatory properties, maintaining the balance between immunity to pathogens and pathology. Modification of IL-10 levels can result in an uncontrolled immune response. The modulation of the immune response IFN-g/IL-10 could be related to the host, the Coxiella burnetii strain, and also to the dose of Coxiella.

d/ Inflammatory response and abortion

Phase I Coxiella burnetii can infect and grow in human dendritic cells which usually serve to detect the presence of pathogens. This can result in the absence of maturation and absence of inflammatory cytokine production by these cells.

In contrast to phase I LPS, the truncated phase II LPS induces dramatic maturation and inflammatory cytokines production. This process can also be aggravated by adjuvants in an adjuvated inactivated phase II vaccines. Mice vaccinated with such a vaccine, Chlamyvax FQ (Merial Lyon France) produced 35 times more IFN-g and other inflammatory cytokines than those vaccinated with phase I Coevac® vaccine (CEVA Santé Animale Libourne France).

Such a high level of inflammatory cytokines could explain why, in experimental conditions the goats vaccinated with a classical phase II vaccine aborted earlier than the unvaccinated control goats. Indeed inflammatory cytokines such as IFN-g, produced in response to infection at the maternal-foetal interface have been postulated to predispose to abortions.

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Figure 1. Comparison of cytokine responses of different vaccination groups, 5 weeks post challenge.

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</tr>
</tbody>
</table>

Figure 2. Comparison of cytokine responses of different vaccination groups, 5 weeks post challenge.
III - The diagnosis of Q fever

In ruminants, effective tools for an accurate diagnosis are RT-PCR and ELISA. Positive or negative results must always be interpreted according to the clinical picture and epidemiological risk.

In humans, immunofluorescence assay is the current reference method for the serodiagnosis of Q fever. It is the simplest and one of the most accurate serologic techniques.

**SUMMARY**

In ruminants, effective tools for an accurate diagnosis are RT-PCR and ELISA. Positive or negative results must always be interpreted according to the clinical picture and epidemiological risk.

In humans, immunofluorescence assay is the current reference method for the serodiagnosis of Q fever. It is the simplest and one of the most accurate serologic techniques.

**1. In ruminants**

**MANTECA C., CZAPLICKI G., GUATTEO R.**

**a/ Laboratory diagnostic tools**

Real-time PCR and ELISA on single milk or on bulk milk tank samples are the most effective tools for an accurate diagnosis.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Level of difficulty</th>
<th>Price</th>
<th>Type of test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complement fixation test</td>
<td>+/-</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>qualitative</td>
</tr>
<tr>
<td>IFA</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>qualitative</td>
</tr>
<tr>
<td>ELISA</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>semi-quantitative</td>
</tr>
<tr>
<td>Smears and coloration</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>qualitative</td>
</tr>
<tr>
<td>Histology</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>qualitative</td>
</tr>
<tr>
<td>Culture</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>qualitative</td>
</tr>
<tr>
<td>PCR</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>qualitative for classical PCR, quantitative for RT-PCR</td>
</tr>
</tbody>
</table>

Table 1. Summary of the characteristics of available tools for Q fever diagnosis (Sensitivity: probability that a truly positive animal will be classified as positive using the test, Specificity: probability that a truly negative animal will be classified as negative using the test)

**b/ Quality of samples**

- Pregnant women and susceptible human beings should not be involved in samples manipulation or analytical process.
- All risk of human contamination should be avoided during sampling and transport to the lab.
- Samples from suspicious animals should be collected for PCR purposes within a maximum of 8 days after the onset of clinical signs or after abortion.
- Serum from suspicious animals should be sampled for ELISA purposes minimum 3 weeks after the onset of clinical signs or after abortion and maximum 4 months later.
- Serum and milk for ELISA purposes should be frozen if analysis is postponed.
- Samples for PCR should be frozen if analysis is postponed.
- Sampling of milk must be made according to classical good practice rules.
Interpretation of positive and negative results must be always carried out according to clinical picture and epidemiological risk. This is very important due to the fact that intermittent shedding of Coxiella burnetii or seronegativity and infected animals are frequent.

**Table 2.** Sample processing according to type of analysis

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Samples</th>
<th>Processing</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>Serum</td>
<td>• 5-10 ml of blood should be collected&lt;br&gt;• After clotting and centrifugation, sera are to be refrigerated (2-8°C) or frozen (-20°C) if the test cannot be performed within 5 days.&lt;br&gt;• Samples should not be repeatedly frozen and thawed,&lt;br&gt;• Do not use hyperlipemic or contaminated sera.</td>
</tr>
<tr>
<td>Complement fixation test</td>
<td>Milk</td>
<td>• 5 to 10 ml of individual or bulk milk tank (after shaking) should be collected.&lt;br&gt;• Milk should be refrigerated (2-8°C) or frozen (-20°C) if the test cannot be performed within 3 days.&lt;br&gt;• Samples should not be repeatedly frozen and thawed.</td>
</tr>
<tr>
<td>IFA</td>
<td></td>
<td>An individual negative result is not conclusive as some animals can remain seronegative despite infection.</td>
</tr>
</tbody>
</table>

**Table 3.** Analytical result interpretation according to available tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive result</th>
<th>False negative result</th>
<th>False positive result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual ELISA</td>
<td>Past or recent infection</td>
<td>• No humoral immune answer detectable despite infection&lt;br&gt;• Bad storage condition of samples.&lt;br&gt;• ELISA based on a human strain of Coxiella burnetii</td>
<td>Not relevant under field conditions</td>
</tr>
<tr>
<td>BMT ELISA</td>
<td>Past or current infection in the herd</td>
<td>• Low number of infected animals&lt;br&gt;• No agitation of BMT content before sampling&lt;br&gt;• Bad storage condition of samples&lt;br&gt;• ELISA based on a human strain of Coxiella burnetii</td>
<td>Not relevant under field conditions</td>
</tr>
<tr>
<td>Individual PCR (On abortion products)</td>
<td>Current infection in the herd</td>
<td>• Intermittent shedding.&lt;br&gt;• Excretion via other route&lt;br&gt;• Delay between sampling and analysis&lt;br&gt;• Analysis was not carried out on the day of sampling&lt;br&gt;• Bad storage condition of samples&lt;br&gt;• PCR natural inhibitor in the sample</td>
<td>Exogenous contamination</td>
</tr>
<tr>
<td>BMT PCR</td>
<td>Current infection and presence of shedders in the herd</td>
<td>• Intermittent shedding&lt;br&gt;• Delay between sampling and analysis&lt;br&gt;• Bad storage condition of samples&lt;br&gt;• PCR natural inhibitor in the sample&lt;br&gt;• No agitation of BMT content before sampling</td>
<td>Exogenous contamination</td>
</tr>
</tbody>
</table>

For smears and coloration, placenta foetal tissues, histology, placenta tissues, culture, PCR, it is not possible to predict the shedding route. To avoid risk of false negative results, analysis should be performed as soon as possible and ideally on the day of sampling. If analysis is postponed, samples should be frozen (−20°C).

It is not possible to predict the shedding route, therefore, samples of milk, vaginal mucus and faeces should be analysed. Samples are to be refrigerated (2-8°C).
d / Diagnosis protocols

1. **SINGLE CASE OF ABDORTION, WEAK OR STILLBORN ANIMAL**

   - **Foetus or stillborn animal is available**
     - **Yes**
       - Abomasal foetal content and lungs mixed together
       - Sample
       - PCR -
       - PCR +
     - **No**
       - Vaginal mucus from aborted female and, if possible, milk and faeces eventually mixed together (no more than 8 days after abortion)
       - Sample
       - PCR -
       - PCR +

   - Q fever is not suspected
   - False negative results are possible

   - Q fever is highly suspected

   - PCR if a new clinical case appears
   - Check presence of other pathogens
   - Histology on cotyledons
   - See protocol 2, 3 or 4.

   - Check presence of associate pathogens
   - Vaccination

2. **SPORADIC ABDORTION, WEAK CALF AND/OR REPRODUCTION DISORDERS**

   This protocol is to be used when only one cow shows clinical signs at the time of sampling.

   - **Sample from suspicious cow**
     - ELISA: > 40% of + animals
       - Disorders caused by C. burnetii
         - Check presence of associate pathogens
         - Vaccination
     - ELISA: < 40% of + animals
       - Recent disorder caused by C. burnetii
         - Check presence of associate pathogens
         - Vaccination
     - ELISA: > 0% of + animals
       - Moderate suspicion
         - Check seroconversion 3 weeks later
         - Check presence of other pathogens
         - PCR if a new clinical case appears
         - See protocol 3
     - ELISA: 0% of + animals
       - Low suspicion
         - Check presence of associate pathogens

3. **MULTIPLE ABDORTIONS**

   This protocol is to be used when at least 2 females show clinical signs at the time of sampling, as false negative PCR results are frequent due to presence of inhibitors in vaginal mucus.

   - **Sample of suspicious animal**
     - PCR +/- or -/+
     - ELISA: > 30% of + animals
       - Disorders caused by C. burnetii seroconverted with high antibody titre
       - Check presence of other pathogens
       - Low suspicion
     - ELISA: < 30% of + animals
       - All animals remain seronegative
     - ELISA: > 0% of + animals
       - PCR if new clinical cases appear
       - Check presence of other pathogens
       - See protocol 4 if a high clinical suspicion is made

4. **AN EASY PROTOCOL TO CHECK CIRCULATION OF COXIELLA BURNETII IN PRESENCE OR ABSENCE OF CLINICAL SIGNS**

   **Absence of clinical sign**
   - ELISA on BMT
     - PCR on BMT
     - See protocol 2 and 3

   **Presence of clinical signs**
   - ELISA on BMT
     - PCR on BMT
     - BMT highly +
     - High probability of absence of C. burnetii in the herd at this time
     - Follow-up according to clinical situation and presence of other potential pathogens
   - PCR +
     - Interest of vaccination must be discussed according to clinical signs, epidemiological and economical risk
   - PCR -
     - Circulation of C. burnetii in the herd and suspicion of current or future clinical Q fever
   - BMT -
In Humans


a / Diagnosis of acute Q fever

PCR
A nested PCR assay is useful to test patients with acute infection when they have no antibody, or low level antibodies. A rapid nested-PCR assay using serum as a template and the Light-Cycler as a thermal cycler (LCPCR) might enable the early diagnosis of acute Q fever endocarditis.

Culture
Coxiella burnetii must be cultured in biosafety level 3 containment, due to its extreme infectivity. The microorganism has been isolated by inoculation of specimens into conventional cell culture.

Serology
An immunofluorescence assay (IFA) is the current reference method for the serodiagnosis of Q fever. It is the simplest and one of the most accurate serologic techniques.

Screening is performed with anti-phase II anti-immunoglobulins at a serum dilution of 1:50. Positive sera are then serially diluted and tested for the presence of anti-phase I and anti-phase II IgG, IgM, and IgA. Seroconversion is usually detected 7 to 15 days after the onset of clinical symptoms.

A titer >200 for IgG and >50 for IgM against phase II indicates a recent Q fever infection, while an IgG titer >800 against phase I suggests chronic infection.

b / Diagnosis of chronic Q fever

The most important point in the diagnosis of Q fever endocarditis is to consider Q fever in all cases of blood culture-negative endocarditis. The most commonly used serologic technique is the microimmunofluorescence (MIF) test, which should distinguish between titers of IgG and IgM antibodies.

A titer >200 for IgG and >50 for IgM against phase II indicates a recent Q fever infection, while an IgG titer >800 against phase I suggests chronic infection.

In chronic Q fever endocarditis, there is a high antibody response to both phase I and phase II of the bacterium. An antiphase I IgG antibody titer >800 indicates chronic infection. The titers of IgM are variable, in some cases very high and in some very low. Because Q fever endocarditis is a chronic illness, a single serum specimen may be diagnostic and paired sera are not required. Furthermore, the antibody titers can be used to monitor the course of treatment.

Modifications of the Duke criteria have been made for the diagnosis of Q fever endocarditis, making Q fever serology a major criterion. Detection of Coxibella burnetii by DNA amplification using the polymerase chain reaction has been described, and may enable early diagnosis of acute Q fever endocarditis.

Using immunohistochemical analysis, culture, and PCR of valve tissue in a series of 28 patients with Q fever endocarditis, one group was able to identify Coxibella burnetii for one year after antibiotic therapy. The histopathology of the valves in this series demonstrated significant fibrosis and calcification but not inflammation or large vegetations.

Thus, specific testing for Coxibella burnetii was required to distinguish this from non-infectious endocarditis. Other methods of diagnosis of Q fever endocarditis include the isolation or demonstration of Coxibella burnetii in the tissues (heart valves, liver biopsies, embolic material) using special stains, immunofluorescence with specific polyclonal or monoclonal antibodies, or electron microscopy. Coxibella burnetii can also be isolated using animal inoculation or cultivated in embryonated eggs or cell culture.

c / Follow up

The suggestion that early diagnosis may minimize the clinical manifestations of Q fever endocarditis and the observation that the mortality rate may be as high as 24 percent has led to the proposal of a serologic follow-up strategy in patients with acute Q fever to prevent endocarditis in patients with valve disease and to permit early detection in other patients.

This approach has the following components and is applied to all patients: Once the diagnosis of Q fever is established, transthoracic echocardiography is performed.

- If valve lesions are detected, the patient is treated for one year using the hydroxychloroquine plus doxycycline regimen described below in an attempt to prevent endocarditis, since these patients are at high risk for endocarditis (39 percent in one study) and there is observational evidence that the combined regimen reduces the incidence of endocarditis.

- If transthoracic echocardiography does not detect valve lesions, serologic testing is performed at three and six months:
  - If the anti-phase I IgG titer is <800 at both times, no further evaluation is performed.
  - If the anti-phase I IgG titer is >800 on either measurement, perform transesophageal echocardiography, which is more sensitive than transthoracic echocardiography for the diagnosis of endocarditis, and polymerase chain reaction on serum samples to detect Coxibella burnetii.

- If either test is positive, the patient is treated for Q fever endocarditis.
IV. Means to fight against Q fever

The non medical control actions putatively feasible in infected herds would aim to reduce or prevent the exposure of susceptible animals (and humans) to contaminated aerosols. To reach this goal, several actions can be put forward. Most of these measures are non specific to Q fever control, and the degree of efficacy of each one to reduce the infectious pressure of *Coxiella burnetii* and therefore the transmission between cows and between herds remains unknown.

Control measures should focus on bedding material as an important source of *Coxiella* transmission between animals and from animals to humans. Decontamination of bedding material with, for instance, calcium cyanamide 0.6% has been described for goat manure. However, these results cannot be extrapolated to cattle manure, the latter being very different from goat manure.

Therefore, nowadays, no standardized and efficient protocol for treatment of bedding or manure is available. However, spreading manure on pasture when the wind blows should be avoided.

Due to the existence of vaginal mucus shedders, control measures should focus both on periparturient or aborted cows and all lactating (either susceptible or infected) cows. For periparturient or aborted cows the use of a separate clean and disinfected calving box as well as an adequate management of placentas and aborted foetuses (systematic collection and destruction) is recommended.

Lastly, ticks are also considered to be a reservoir and vector of *Coxiella burnetii* in many countries and should therefore be managed efficiently.

All together, these control measures could limit the risk of transmission among animals and from animals to humans, but medical control actions are necessary to really manage infection in a herd.

Information about the clinical signs relative to human Q fever must be provided to workers at risk (especially farmers, veterinarians and abattoir workers), in order to allow for early detection of the disease.

### Non medical measures

<table>
<thead>
<tr>
<th>Non medical measures</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collection and destruction of aborted foetus and placenta</td>
<td>+++</td>
</tr>
<tr>
<td>Management of manure: long term stocking (&gt; 3 weeks); avoid manure spreading when windy...</td>
<td>+++</td>
</tr>
<tr>
<td>Isolate infected animals specially during calving</td>
<td>++</td>
</tr>
<tr>
<td>Culling of infected animals</td>
<td>++</td>
</tr>
<tr>
<td>Take precautions (gloves...) during obstetrical manipulations</td>
<td>++</td>
</tr>
<tr>
<td>Deep and gentle cleaning and disinfection (avoid spatters caused by pressure washers) of building, materials and machinery</td>
<td>++</td>
</tr>
<tr>
<td>Geographical separation between infected and non infected lots.</td>
<td>++</td>
</tr>
<tr>
<td>Management of the potential risks of contamination from wild animals and arthropods</td>
<td>+</td>
</tr>
</tbody>
</table>

In order to correctly manage the risk Q fever represents, vaccination along with non medical measures such as collection and destruction of aborted foetus and placenta and manure management are to be implemented.

An empiric treatment of animal Q fever in the field consists in the use of oxytetracycline. Some studies have shown reduction in the number of abortions in cattle farms however studies in cattle and sheep have failed to show any effect on *Coxiella burnetii* shedding.

In humans, the preferred treatment of Q fever endocarditis is the combination of hydroxychloroquine and doxycycline.

The treatment of pregnant women with Q fever infection is difficult because many drugs are contraindicated during pregnancy. The recommended treatment is long-term cotrimoxazole therapy.

**Summary**

Antibiotic treatment

Antibiotic treatment The aim of an antibiotic treatment in the field is to reduce the number of abortions and the quantity of Coxiella burnetii shed at parturition. Because of its activity against Coxiella burnetii and its intracellular diffusion, tetracycline is very often the first choice of antibiotic against Q fever. The most used protocol consists of two injections of oxytetracycline (20 mg/kg) during the last month of gestation. The efficacy of this treatment has been shown to be limited, although not accurately assessed. Different groups have tried to evaluate the efficacy of the use of oxytetracycline (OTC). A recent study conducted in Spain aimed to assess the efficacy of an OTC treatment (20 mg/kg two weeks apart at respectively 100 and 120 days of pregnancy) on sheep excreting Coxiella burnetii in vaginal fluids, milk and faeces, from parturition to drying off a commercial dairy sheep flock. At lambing, there was no difference in the proportion of shedder ewes of the treated and untreated groups. These results suggest that OTC treatment on infected shedder ewes neither prevented nor reduced the duration of Coxiella shedding.

Table I. Effect of oxytetracycline on Coxiella burnetii shedding in sheep

<table>
<thead>
<tr>
<th>Number of animals</th>
<th>Regimen</th>
<th>Detection technique</th>
<th>Sample (at lambing or calving)</th>
<th>Number/proportion of positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>Long acting oxytetracycline 20 mg/kg at 10 and 115 days of pregnancy</td>
<td>PCR</td>
<td>Vaginal mucus Milk Faeces</td>
<td>64 % 23 % 53 %</td>
</tr>
<tr>
<td>60</td>
<td>Control group</td>
<td>PCR</td>
<td>Vaginal mucus Milk Faeces</td>
<td>51 % 20 % 55 %</td>
</tr>
</tbody>
</table>

Another study performed in cattle showed a positive effect of OTC treatment on reducing abortion rate but it did not show any effect on shedding of Coxiella burnetii.

Vaccination

In ruminants, vaccination with a phase I vaccine showed the ability to prevent appearance, reduce or stop abortion as well as other clinical signs and shedding in naïve animals. An effective vaccination seems not only to prevent clinical signs but also to prevent infection of susceptible animals. Indeed, in a study under field conditions, a phase I vaccine prevented infection of susceptible heifers despite proximity of naturally infected cows and a high contaminated environment. A vaccine cannot be considered as a classical treatment and indeed a significant reduction of shedding in infected animals was not demonstrated. Nevertheless, vaccination of infected animals allowed to prevent the appearance of clinical signs and to significantly decrease the zootechnical losses due to Q fever at the herd level.

Vaccination is no value to treating patients after spontaneous cure of the disease.

Treatment of Q fever during pregnancy

The treatment of the pregnant women with Q fever infection is difficult because many of the drugs are contraindicated during pregnancy (e.g., doxycycline, fluoroquinolones). The recommended treatment is long-term cotrimoxazole therapy (320 mg trimethoprim and 1600 mg sulfamethoxazole for >35 days) due to retrospective data suggesting a benefit of this approach in decreasing the risk of placental, obstetrical complications, and maternal chronic Q fever infection.

Acute Q fever, abortion and endocarditis are the most frequent picture to be managed by physicians and treatments can differ.

Treatment of acute Q fever

Acute Q fever is usually a mild disease that resolves spontaneously within two weeks. Thus, clinical evaluation of the efficacy of antibiotic therapy is difficult, and comparative studies are scarce. Consideration of therapy is warranted only in patients who are symptomatic. There is no value to treating patients after spontaneous cure of the disease.

Treatment of Q fever endocarditis

• Drug regimens

The preferred regimen for the treatment of Q fever endocarditis is the combination of hydroxychloroquine (650 mg daily) plus doxycycline (200 mg daily). The serum hydroxychloroquine concentration should be monitored in order to obtain a concentration of 1±0.2 microgram/mL; retinal examinations should be performed every six months to detect early signs of ophthalmologic toxicity such as corneal deposits and retinopathy. Serum doxycycline concentrations should be monitored and the level maintained at >5 µgram/mL.

• Treatment monitoring

The serum levels of antibodies decrease very slowly. IgM antibodies, when present, disappear first and then IgG, however, IgG antibodies remain positive for years. After three years, treatment can be stopped if the titer of IgG antibodies against phase 1 antigens is still below 400.

Duration of therapy

The minimum duration of therapy is 18 months with doxycycline and hydroxychloroquine.

Surgical valve replacement

Surgery to replace a damaged valve in Q fever endocarditis is generally indicated for hemodynamic reasons. If possible, at least three weeks of antimicrobial treatment should be given prior to valve replacement. Although not curative, three weeks of therapy should sterilize the blood, minimizing the risk of infection of the new valve.
There are two aims when implementing a vaccination strategy with Coxevac® in cattle. The first one is to decrease the environmental losses due to Q fever by preventing the clinical impact of the disease. The second one is to decrease the risk of contamination for animals and humans by preventing shedding of *Coxiella burnetii*.

Q fever potentially induces an important range of clinical signs. Most of them affect the genital area. Abortion is well-known but in cattle, metritis, retained placenta, infertility, repeat breeding are more frequently recognized on the field. Vaccination with Coxevac® is a tool of major interest to prevent these clinical signs.

Massive shedding of *Coxiella burnetii* is key when studying Q fever epidemiology. Indeed animal and human contamination most often occurs after inhalation of contaminated aerosols which result from *Coxiella burnetii* shedding in infected animals. This shedding induces an important environmental contamination in and around the infected flock and the resulting pressure of infection allows *Coxiella burnetii* to remain present for many years. Vaccination with an efficient phase I vaccine may be a major factor to prevent the spread of Q fever among livestock as well as exposed humans.

Vaccination with Coxevac®, via the subcutaneous route, at the recommended dose, repeated use or overdose doesn’t induce major trouble. Although some cases showed a transient hyperthermia, no impact on general status or weight gain have been observed. Limited local reactions appeared sometimes after vaccination. These reactions disappeared after a short period. No major negative effect on milk yield in dairy ruminants was observed at herd level. Out of label use of Coxevac® in pregnant animals doesn’t induce any major local or general reaction.
Q fever is a well recognized cause of abortions in cattle. *Coxiella burnetii* infection in dairy cattle has been well documented and its association with reproductive problems has been reported in Canada, USA, Cyprus, France, Hungary, Japan, Switzerland and West Germany (To et al., 1998).

For instance, in a recent study (Czaplicki et al., 2008) a significant association has been shown between the herds’ seropositivity and clinical signs of Q fever such as abortion, stillborn, weak calves and repeat breeding.

Moreover, experimental inoculation of *Coxiella burnetii* in cattle induced not only respiratory disorders and cardiac failures (myocarditis) but also frequent abortions and irregular repeat breeding (Plommet et al., 1973).

Reproductive disorders are often polyfactorial. Nevertheless, published field trials proved that use of Coxevac® in infected farms is a tool of major interest to prevent early or late abortion, repeat breeding, anoestrus, silent oestrus, metritis and drop in milk yield when *Coxiella burnetii* is the major cause of these troubles (Wannyn, 2007, Camuset, 2008).

---

**I. Efficacy**

Efficacy of Coxevac® on reproductive performance by preventing the clinical impact of Q fever

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**SUMMARY**

Q fever is a well recognized cause of abortions in cattle. *Coxiella burnetii* infection in dairy cattle has been well documented and its association with reproductive problems has been reported in Canada, USA, Cyprus, France, Hungary, Japan, Switzerland and West Germany (To et al., 1998).

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---

**1.1- APPARENT PREVALENCE OF ANTIBODIES TO COXIELLA BURNETII (Q FEVER) IN BULK MILK TANK OF DAIRY HERDS IN THE WALLOON REGION OF BELGIUM.**

**FROM: CZAPLICKI G.C., HOUTAIN J.Y., MANTECA C. AND SAEGERMAN C. XXV WORLD BUIATRICS CONGRESS, JULY 6-11, BUDAPEST, HUNGARY**

The aim of the present study was firstly, to assess the apparent prevalence in dairy herds and secondly, to identify herd level risk factors and clinical signs statistically associated with bulk milk tank seropositivity in the population of randomly selected Walloon dairy cattle.

**a / Material and method**

Samples

566 herds from the 5086 Walloon dairy herds were randomly selected according to a standard methodology (Jenicek and Cléroux, 1987). Herd level prediction data was collected with a questionnaire on farm demographics and observed clinical signs during the twelve previous months, for calves, heifers and cows. 206 farms answered on a voluntary basis to this questionnaire and submitted a sample of bulk milk tank. The samples of bulk milk were centrifuged and stored at -20°C until testing for antibodies against *Coxiella burnetii*.

Method

Milk Q fever LSI Kit® (Laboratoire Service International, Lissesu, france) is an indirect Elisa test which gives positive result above a prevalence of 10% among lactating cows.

**Statistical analysis**

Apparent prevalence was estimated with 95% confidence intervals (CI) assuming a binomial exact distribution. Statistical analysis of the data was done using a chi square test and the tendency of each parameter to become a risk or protective factor was evaluated by an odds ratio calculation with 95% CI (logarithmic approximation); a P value < 0.05 was considered significant.

**b / Results**

Global apparent prevalence: 54.9% of tested herds showed serological evidence of *Coxiella burnetii* infection. Respectively 40.8%, 14.1% and 0.0% had a result “Positive +”, “Positive ++” and “Positive +++”. Clinical signs with statistical signification are shown (Fig. 1).

<table>
<thead>
<tr>
<th>Exploratory variables</th>
<th>HEIFERS</th>
<th>COWS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abortion</td>
<td>Significant association 1.82 (1.02-3.22)</td>
<td>Significant association 2.04 (1.03-4.05)</td>
</tr>
<tr>
<td>Stillborn or weak calves</td>
<td>Significant association 2.91 (1.29-7.77)</td>
<td>1.74 (0.84-3.60)</td>
</tr>
<tr>
<td>Irregular repeat breeding</td>
<td>1.61 (0.66-3.93)</td>
<td>Significant association 2.40 (1.16-4.97)</td>
</tr>
</tbody>
</table>

**c / Discussion**

This first report on Q fever infection prevalence in Wallon dairy herds indicates a relatively high level of infection. However, seropositivity at herd level doesn’t mean shedding of *Coxiella burnetii*, but identifies herds where at least 10% of lactating animals are seropositive against this bacterium. This critical point could be corrected by testing the same samples with a PCR technique to identify herds shedding *Coxiella burnetii*, aspect which is of capital importance.

**d / Conclusion**

A significant association exists between the herds’ seropositivity and typical clinical signs of Q fever observed such as abortion, stillborn or weak calves and repeat breeding.
1.2- EXPERIMENTAL Q FEVER IN CATTLE


Q FEVER IS RECOGNIZED IN CATTLE TO INDUCE ABORTION AND RESPIRATORY DISORDERS. ONE OF THE GOALS OF THIS STUDY IS TO FOLLOW EVOLUTION OF INFECTION IN TERM OF CLINICAL SIGNS UNTIL END OF PREGNANCY IN INSEMINATED HEIFERS.

a/ Material and method

- Three groups of 4 Friesian heifers, 8 to 11 months old, uninfected by Coxiella burnetii, were inoculated (ID in the neck area) respectively by 0.03, 0.3 and 3 ml of a suspension of 1000 minimal lethal doses (guinea pigs).
- 98 Friesian heifers, uninfected by Coxiella burnetii, were kept as control group.
- Clinical signs following the inoculation were recorded.
- 9 months after inoculation, heifers were inseminated and evolution of reproductive parameters (pregnancy, abortion, repeat breeding) were recorded.

b/ Results

Clinical signs

After inoculation and during at least 3 days, all animals showed a major hyperthermia (Fig. 1) mainly in group 3.

All inoculated heifers showed a significant increase in respiratory rate. During 5 days in group 1 and 2 and 7 days in group 3, animals showed inappetence, rapid, shallow breathing and nasal discharge.

Some respiratory signs persisted during 3 weeks in group 3. 26 weeks after inoculation, one heifer from the group 2 died suddenly. Necropsy showed a dilated heart with extended myocarditis lesions. 32 weeks after inoculation, ECG were performed and 3/11 animals showed different abnormalities.

Fecundity

Fecundity in the inoculated group was 73% (8/11) and 90% in the control group (88/98), abortion rate was 37% in the inoculated group and 1.9% in the control group. Normal pregnancy rate was 55% in the inoculated group and 81% in the control group.

In term of fecundity, impact of inoculation was most impressive in group 3.

Figure 1. Evolution of body temperatures in 12 Friesian heifers inoculated by Coxiella burnetii.

<table>
<thead>
<tr>
<th>Group</th>
<th>Inoculum (mL)</th>
<th>Nb of Heifers</th>
<th>Evolution of pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.03</td>
<td>1</td>
<td>Normal pregnancy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Normal pregnancy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>Normal pregnancy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>No pregnancy, irregular repeat breeding</td>
</tr>
<tr>
<td>2</td>
<td>0.3</td>
<td>1</td>
<td>Abortion (4 months)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Died (myocarditis)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>Normal pregnancy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>Normal pregnancy</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>1</td>
<td>No pregnancy, irregular repeat breeding</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Abortion (3 months)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>No pregnancy, irregular repeat breeding</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>Normal pregnancy</td>
</tr>
</tbody>
</table>

Figure 2. Clinical signs associated with bulk tank milk seropositivity in a random population of dairy cattle (n = 206).

Experimental inoculation of Coxiella burnetii in cattle induces respiratory disorders in all animals. After inoculation, cardiac failure (myocarditis) was detected and caused a sudden death. Q fever induced frequent abortions and irregular repeat breeding.

Figure 2. Clinical signs associated with bulk tank milk seropositivity in a random population of dairy cattle (n = 206).

Figure 1. Evolution of body temperatures in 12 Friesian heifers inoculated by Coxiella burnetii.
1.3- EVALUATION OF REPRODUCTIVE PARAMETERS IN CATTLE HERDS INFECTED WITH Q FEVER AND FURTHER VACCINATED

THE AIM OF THIS TRIAL WAS TO ASSESS THE CAPACITY OF VACCINATION AGAINST Q FEVER TO IMPROVE THE CLINICAL CONTEXT DURING THE FIRST YEAR FOLLOWING PRIMO VACCINATION.

Q fever potentially induces an important range of clinical signs. Most of them affect the genital area. Abortion is well-known but, in cattle, metritis, retained placenta, infertility, repeat breeding are more frequently recognized on the field.

The herds studied were three French herds where Q fever had been diagnosed and where the owner complained about reproductive disorders.

a/ Parameters

These herds were vaccinated and the impact of vaccination on clinical signs was measured and recorded.

- Improvement of silent oestrus: Calving - AI interval (average, in days)
- Metritis: % of retained placenta and % of metritis
- Abortions: % of abortion

PCR on bulk milk tank were performed 6 months to one year following the first injection to assess the capacity of vaccination against Q fever to improve the clinical context during the first year following primo vaccination.

PCR on bulk milk tank were performed 6 months to one year following the first injection to assess the capacity of vaccination against Q fever to improve the clinical context during the first year following primo vaccination.

b/ Vaccination

According to field constraints, vaccination was carried out before mating or during pregnancy with 2 injections of 4 ml of Coxievac®. The booster vaccination was annually performed.

Improvement in Herd A

% of abortion, metritis, retained placenta and calving-effective AI interval are improved. Culling rate remained stable but was not a major farmer complaint.

Improvement in Herd B

The major complaint: metritis and retained placenta were significantly improved. Abortion rate slightly increased but was not a major farmer complaint and analysis didn’t show any role of Coxiella burnetii in these new cases of abortions.

Improvement in Herd C

Culling rate, abortion rate, retained placenta rate and calving-effective AI interval are improved. Metritis rate and retained placenta rate were also improved although not part of major farmer complaints.

Improvement of abortion rate in herd A

Improvement of abortion rate in herd B

Improvement of abortion rate in herd C

**Calving - Effective AI interval (Days)**

<table>
<thead>
<tr>
<th>Herd and major clinical signs</th>
<th>% of abortion</th>
<th>% Retained Placenta</th>
<th>% Metritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-1</td>
<td>N</td>
<td>N-1</td>
<td>N</td>
</tr>
<tr>
<td>Herd A Abortions and silent mortality</td>
<td>16,1</td>
<td>107</td>
<td>8,3</td>
</tr>
<tr>
<td>Herd B Metritis</td>
<td>9,7</td>
<td>132</td>
<td>10,3</td>
</tr>
<tr>
<td>Herd C Abortions and silent mortality</td>
<td>20,6</td>
<td>106</td>
<td>10,3</td>
</tr>
</tbody>
</table>

(c) No evidence of Coxiella burnetii in these cases of abortion - (d) Presence of Coxiella burnetii in only one aborted cow.

d/ Conclusion

Metritis, abortion, retained placenta, repeat breeding, infertility are never explained by only one aetiology. Clinical management, including when Q fever is suspected to be a major cause must include improvement of diet, housing, technical farmer’s level... Despite these limits, according to field data, use of Coxievac® provides a significant improvement to the management of the infectious part of this complex syndrome.
1.4- Q FEVER ERADICATION IN A DAIRY HERD BY USING VACCINATION WITH COXEVA®


The herd is composed of approximately 30 dairy cows in free stall housing producing around 9000 kg of milk per lactation.

**a/ Vaccination schedule**

Since Sept. 2004, the dairy cows and heifers were vaccinated twice 3 weeks apart by subcutaneous route with Coxevac®, preferentially at W2 and W5 post partum (cows) and before first insemination (heifers). A booster injection between W2 and W5 post-partum was yearly planned.

**b/ Recorded parameters**

- Post-partum, an individual diagnostic was initiated with research of the bacteria by real time PCR in the milk at D0, D3, W1, W3 and W8 and in the vaginal mucus between D0 and D3.
- Fertility and fecundity parameters were recorded (% of anoestrus, early and late metritis and abortions).

**c/ Results**

During the first year of study and among the 46 cows studied (10 first lact., 15 cows (2 first lact.) excreted at least once the bacteria, 10 several times. The third year, only 3 cows excreted (no first lact.); among them, two that had excreted several times were culled.

During the study period, only one vaccinated cow, not excreting in the previous lactation, became a new shedder (only in vaginal mucus); on the other hand, four cows seemed healthy again, they had only excreted in a previous lactation around calving always in vaginal mucus, sometimes in milk. They have never re-excreted again.

For fertility and fecundity, the performances (% of anoestrus, early and late metritis and abortions) were improved following the use of vaccination and culling of the excreting cows.

**d/ Conclusion**

Either for human health or for zootechnical and economical reasons, the use of a diagnostic method towards Coxiella burnetii shedding animals, their vaccination then their progressive elimination appears to be a feasible process by using Coxevac®.
Coxevac® is registered for use in non-pregnant cows to reduce excretion of *Coxiella burnetii*.

Under field conditions however, out of label use of Coxevac® in pregnant cows is frequent. Therefore, the aim of this study was to assess the capability of Coxevac® to prevent shedding in pregnant animals in a contaminated environment.

**Efficacy of Coxevac® on *Coxiella burnetii* shedding in pregnant animals in a contaminated environment**


**a / Material and method**

24 heifers were transferred from an uninfected, healthy herd (Simmental breed), where all animals were seronegative for Q fever into an infected dairy herd, where 47% of all cows were seropositive. 12 pregnant heifers (4 to 6 months of gravidity) belonging to the introduced group were vaccinated before the transfer. The 12 others remained unvaccinated and represent the control group.

Vaccinated heifers were considered efficiently protected against a natural Q fever infection if shedding of *Coxiella burnetii* in placenta, colostrum and milk was prevented. To assess the shedding of *Coxiella burnetii* in placenta, colostrum and milk, the samples were intraperitoneally administered to seronegative hamsters examining. Three weeks after the intraperitoneal administration to hamsters a Q fever antibody test was performed.

**b / Results**

6, 13 and 29 months after the seronegative vaccinated heifers were introduced in the infected herd, none were shedding *Coxiella burnetii* in milk.

<table>
<thead>
<tr>
<th>Animals examined</th>
<th>Examination months</th>
<th>Ratio of positively tested animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated heifers</td>
<td>6</td>
<td>0/12</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>0/12</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>0/12</td>
</tr>
<tr>
<td>Non vaccinated heifers</td>
<td>6</td>
<td>3/12</td>
</tr>
<tr>
<td>Naturally infected cows</td>
<td>-</td>
<td>7/12</td>
</tr>
</tbody>
</table>

**c / Conclusion**

In this trial carried out under field conditions, Coxevac® efficiently prevented shedding of susceptible heifers despite proximity of naturally infected cows and a high contaminated environment.
II. Safety

Although Coxevac® is registered for a use in non pregnant cows, the safety and immune answer following vaccine administration in pregnant animals was investigated.

a/ Material and method

236 pregnant cows originating from 6 infected farms were included in this field trial. Some of which were already infected before vaccination.

Group 1 - 116 pregnant cows (1 to 8 months of gestation) were vaccinated subcutaneously with 4 mL of Coxevac®, twice 4 weeks apart.

Group 2 - Control group - 120 pregnant cows (1 to 8 months of gestation) were injected with a placebo.

b/ Parameters recorded

Local and general reactions were recorded after vaccine injection. To assess long-term vaccine tolerance in pregnant animals, calving observations were scored and recorded.

Furthermore, antibody response (ELISA) was tested 15 days after the second injection in 70 cows initially seronegative (35 vaccinated with Coxevac® and 35 receiving a placebo).

c/ Results

No post-treatment local or systemic reaction was observed within 48 hours after treatment.

The number of normal calving was slightly more important in the Coxevac® group (90%) than in the placebo group (83%). There was no significant difference in the incidence of problematic calving between the vaccinated and placebo treated group (Table 1).

Distribution of serological patterns in initially seronegative animals showed that, vaccinated cows seroconverted after injection of Coxevac®. The serological patterns of the control group are dominated by a seronegative status. When seropositivity occurs, it must be considered as a new infection by Coxiella burnetii.

d/ Conclusion

Out of label use of Coxevac® in pregnant cows does not induce any major local or general reaction. Coxevac® induces a seroconversion in vaccinated animals. In infected herds, a slightly more important number of normal calving in the Coxevac® group than in the placebo group was observed. This study highlights the fact that accidental use of Coxevac® in pregnant animals has no impact on pregnancy in cattle.
2.2- EFFECT OF COXEVAC® ON MILK YIELD IN DAIRY CATTLE

THE PRESENT STUDY AIMS AT ASSESSING THE SAFETY OF COXEVAC® ON MILK YIELD IN LACTATING CATTLE.

When milk production suddenly decreases just after use of a vaccine, this injection is often suspected by the farmer to be the cause of this sudden decrease. A sudden drop of milk production can be caused by physiological status, mastitis, infectious diseases as Q fever, treatments, stress, handling... Coxevac® is an inactivated and purified whole-cell Coxiella burnetii vaccine with no adjuvant. Thanks to the purification process and the pure vaccine composition, no major systemic or local reaction is expected.

a/ Material and method

The study was carried out in 2 farms with evidence of Q-fever related clinical disease (e.g. infertility, abortion).

Group 1
Coxevac® group - 64 healthy lactating cows (including 29 seropositive animals) were vaccinated twice 3 weeks apart, with 4 ml of Coxevac® by subcutaneous route.

Group 2
Control group - 50 healthy lactating cows (including 19 seropositive animals) were treated twice 3 weeks apart, with 4 ml of a placebo (Phosphate buffered saline) solution by subcutaneous route.

b/ Parameters recorded

Obvious signs of local or general intolerance were monitored. Daily milk yield results were recorded 7 days before the treatment. Serological patterns (ELISA) were tested at D0, D21 and D38.

c/ Results

No general sign of vaccine intolerance was observed by the farmer throughout the study. Nevertheless, a closer examination by the investigator allowed the detection of mild to moderate local reactions in 17 out of 64 vaccinated cows at D21, by the end of the study this had decreased to 7 out 64 cows. All these reaction resolved spontaneously 5-6 weeks after last vaccination. No negative effect on general health was observed.

d/ Conclusion

No significant effect on milk yield was observed. According to the confidence of statistical tests used, this result means that no more than a 10 % milk drop during 2 days can be attempted at the global herd level (Fig. 1).

A strong serological response was observed in vaccinated infected or not infected cows. Infected cows if not vaccinated showed a quick decline of antibody levels (Fig 2).

No general sign of vaccine intolerance was observed by the investigator.

2.3- SAFETY OF AN OVERDOSE OF COXEVAC® IN YOUNG CALVES

THE OBJECTIVE OF THIS STUDY WAS TO INVESTIGATE THE SAFETY OF THE INACTIVATED VACCINE COXEVAC® FOLLOWING THE ADMINISTRATION OF TWICE THE RECOMMENDED DOSE TO 3 MONTHS OLD SERO-NEGATIVE HOLSTEIN-FRIESIAN CALVES.

To efficiently manage Q fever in a ruminant herd, vaccination should be implemented in young calves as soon collostral immunity disappears.

a/ Material & method

Group 1
10 calves are vaccinated with a placebo solution. This treatment is repeated 3 weeks later.

Group 2
10 calves are vaccinated with a double dose (8 ml) of Coxevac®. The injection is repeated 3 weeks later with the same dose.

d/ Conclusion

The administration of twice the recommended dose of Coxevac® by subcutaneous route was well tolerated by susceptible 3-month-old calves. Weight gain was not influenced by vaccination. No signs of general or local intolerance were recorded.
There are two aims when implementing a vaccination strategy with Coxevac®. The first one is to prevent the clinical impact of the disease, thereby decreasing the economical losses due to Q fever. The second one is to decrease the risk of contamination for humans and animals by preventing the excretion of the bacteria in the environment.

The most important clinical sign due to Coxiella burnetii which can be detected visually is abortion. To assess the efficiency of Coxevac® on this clinical sign, goats are the most relevant specie. Indeed when infected with Coxiella burnetii, the expected number of abortions in goats is high.

Massive shedding of Coxiella burnetii is key when studying Q fever epidemiology. Indeed animal and human contamination most often occurs after inhalation of contaminated aerosols which result from Coxiella burnetii shedding in infected animals. This shedding induces an important environmental contamination in and around the infected flock and the resulting pressure of infection allows Coxiella burnetii to remain present for many years.

A vaccine must be safe to reduce the risk of adverse effects but should also be able to induce an active immune answer in vaccinated animals in order to provide a protection against clinical signs. This is true in all animals but really important in goats where Q fever can induce major economical losses and where local or general post vaccination reactions are unfortunately frequent and important.

The development of Coxevac® took place at the R&D facilities of Ceva Santé Animale. The objectives were to induce an active immunisation of animals without promoting local or general secondary effects. The technology which was used enabled to reach a strong immune stimulation despite the absence of adjuvant in the vaccine formulation. The absence of adjuvant enabled to maximize the safety results including in very sensitive species such as goats.
I. Efficacy on abortion and environmental contamination

Efficacy of Coxevac® to decrease excretion of Coxiella burnetii in adult infected goats under field conditions

FROM: ORSZAGH G., KOVACS F., BENESCH C. CEVA INTERNAL STUDY, 2008

THE AIMS OF THIS STUDY WERE TO EVALUATE THE IMMUNITY PROVIDED BY COXEVAC®, ITS CAPACITY TO DECREASE SHEDDING OF ANIMALS INFECTED PRIOR TO VACCINATION AND EVENTUALLY TO ASSESS THE VACCINE’S SAFETY IN ANIMALS INFECTED.

a / Material & method

During the summer 2006, a Q fever investigation was carried out in a German dairy goat flock following an abortion in a goat and flu-like symptoms in 12 farm visitors later on in the summer, farm employees displayed signs of disease and some needed hospitalisation. Coxevac® vaccination of the flock was implemented in 2007 and immunity and shedding data were recorded during the 2 following years. Eighty six non pregnant goats, 3 months of age and older, were vaccinated subcutaneously with Coxevac® (2 injections of 2ml, 3 weeks apart). The control group was composed of five seronegative goats, which remained unvaccinated and were used as sentinel to check if vaccination allowed a decreased risk of contamination for the uninfected animals in a vaccinated flock. The number of sentinel animals was kept low to allow a high herd protection as obtained under field conditions when all animals in the flock are vaccinated.

b / Recorded parameters

Safety .................................................................

Body temperature, local injection site reactions and general health observations.

Active immunisation...........................................

Individual serology status was checked using an ELISA screening test 25, 7 and 1 week before the first vaccination, at the time of the second injection and 20 weeks after this second vaccination.

Shedding..............................................................

In 2007 and 2008, samples were collected following each kidding and in case of abortion. Presence of Coxiella burnetii was detected by PCR.

c / Results

All goats remained healthy and no immediate adverse reaction was observed. Mild transient local reactions were observed in 2 goats (2%). These reactions resolved within few days.

Mild local reactions and increase in core body temperatures were detected after vaccination with Coxevac® in goats under field conditions. A small rise in rectal temperature measured after both vaccination, with a slightly greater increase following the second vaccination. However, even after the second injection, the mean rectal temperature remained within the physiologically normal range at each observed time point (Fig. 1).

Good serological responses were induced after the first vaccination. All vaccinated animals, which were sampled for serology testing with ELISA were seropositive 21 days following the first vaccination. The serological response to vaccination appears to be long lasting, as even five months after first vaccination, 85% of all tested goats were still seropositive (Fig. 2).

Coxiella burnetii detection by PCR demonstrates that vaccination was indeed able to successfully reduce the circulation of the bacteria in the goat herd. The implemented program together with non-medical measures was able to significantly reduce the number of shedders after kidding or via abortion in the herd (Fig. 3).

All uninfected sentinels remained seronegative and clinically healthy. One year after initiating the vaccination program they still remained none-shedders.

Following the vaccination with Coxevac®, no further human cases (visitors or employees) occurred in 2007.

d / Conclusion

Under field conditions Coxevac®, strictly administered by the subcutaneous route, in both infected prior to vaccination and non infected animals, can be considered as a safe vaccination. A significant active immunisation is obtained in all animals.

In association with other standard non-medical measures used in cases of infectious diseases, vaccination with Coxevac® of all animals present in the flock:

• reduces the number of shedders and the level of shedding of Coxiella burnetii among goats
• prevents infection in animals
• stops subsequent zoonotic Q fever cases.
IMMUNE REACTION AND EFFICACY OF COXEVAC® BOTH ON SHEDDING AND ON ABORTION WAS STUDIED ON NON-INFECTED ANIMALS IN FIELD CONDITIONS AND FOLLOWING AN EXPERIMENTAL CHALLENGE AS PART OF AN EFFECTIVE Q FEVER MANAGEMENT IN INFECTED HERDS. VACCINATION ALLOWS A SIGNIFICANT DECREASE IN ENVIRONMENTAL CONTAMINATION. THIS CONTAMINATION IS CAUSED BY INFECTED ANIMALS WHICH SHED SOMETIMES DURING LONG PERIOD AND ESPECIALLY AFTER ABORTION.

**a/ Material and Method**

3 French caprine flocks constituted of 926 young or adult goats, with high abortion rates were studied before and after vaccination.

Four groups of animals were constituted before vaccine injection:
- non infected young female goats
- infected female goats
- non infected adults goats
- infected adults goats

Fifty percent of animals from each group were vaccinated and 50% remained as control. Effect of vaccination on shedding and clinical signs were recorded.

**b/ Results**

Before vaccination

- 32% of the young goats and 93% of the adults goats were infected (seropositivity and/or shedding).
- Ninety one percent of aborted goats shed more than $10^6$ Coxiella burnetii/mL of uterine liquids and non aborted infected animals shed more than $10^4$ Coxiella burnetii/mL of vaginal mucus every day during the shedding episode. This high level of shedding explains the strong environmental contamination around these infected flocks.

After vaccination

- Adults and young goats already infected before vaccination showed a stable low or moderate level of shedding.
- Young female goats not vaccinated and used as control shed strongly when infection occurred. For this reason, this population is an important group from an epidemiological point of view.
- By contrast, level of shedding in vaccinated young female goats was significantly decreased.
- By contrast, level of shedding in vaccinated young nanny goats was significantly decreased.

**c/ Conclusion**

Coxevac® allowed to significantly decrease the excretion and the risk of contamination. As abortion is a major source of animal and human contamination, prevention of this clinical sign is of major importance. Vaccination and non medical measures, used together enable the efficient management of Q fever in a flock.
Coxevac® induced a highly significant protection in comparison to the unvaccinated controls and to the phase II vaccinated animals (p<0.01).

<table>
<thead>
<tr>
<th>Vaccination scheme</th>
<th>Vaccine</th>
<th>Control (not challenged)</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of goats</td>
<td></td>
<td>27</td>
<td>16</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Duration of gestation (days)</td>
<td>150 (+1.8)</td>
<td>153 (+3)</td>
<td>134 (+15)</td>
<td>141 (+6)</td>
<td></td>
</tr>
<tr>
<td>Abortion (%)</td>
<td></td>
<td>15</td>
<td>6</td>
<td>87</td>
<td>75</td>
</tr>
<tr>
<td>Number of kids per goat</td>
<td>1.9 (+0.88)</td>
<td>1.5 (+0.52)</td>
<td>1.67 (+0.62)</td>
<td>1.75 (+0.87)</td>
<td></td>
</tr>
<tr>
<td>Percentage of goats with contaminated placenta (%)</td>
<td>ND</td>
<td>37.5</td>
<td>93.3</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Duration of gestation, % of abortion, number of kids and % of contaminated placenta depending on the vaccine used.

94% of the animals administered with Coxevac® were seropositive after vaccination. The antibody level between day 14 and 133 in goats administered with phase II vaccine were significantly lower than those administered with phase I vaccine (p<0.01).

After challenge the antibody titers of the goats which received Coxevac® stabilized whereas the titers increased in goats which received the phase II vaccine suggesting a putative higher *Coxiella burnetii* shedding in this last group. Therefore, vaccination with Coxevac® is associated with a strong and fast immune response.

The use of Coxevac® in non infected and further challenged animals was associated with a very strong reduction in vaginal shedding and with the absence of milk shedding when compared to non vaccinated or vaccinated goats with a phase II vaccine.

c / Results

d / Conclusion

Use of Coxevac® induces a strong immune answer correlated with a significant decrease in abortion rate, number of contaminated placenta, and an increase in number of live kids. Furthermore Coxevac® stops or strongly decreases the amount as well as the duration of *Coxiella burnetii* shedding (Fig. 3-6).
II. Safety

Field safety and active immunisation in kids


THE OBJECTIVE OF THIS STUDY WAS TO EVALUATE THE SAFETY OF A PROTOCOL BASED ON REPEATED ADMINISTRATION OF COXEVAC® IN THE MOST SENSITIVE CATEGORY (KIDS) OF THE MOST SENSITIVE TARGET SPECIE (GOATS).

a/ Material & method

The study was carried out on 3 months old seronegative goats.

Group 1

Vaccinated group: 10, three months old seronegative goat kids received one dose of Coxevac® vaccine (2 ml) three times at 3 and 2 weeks intervals respectively.

Group 2

Placebo: 10 goat kids received 2ml of a placebo solution three times at 3 and 2 weeks intervals respectively.

Throughout the study, the following parameters were monitored:

- Body temperature was measured daily starting 2 days prior to the first vaccination and ending 4 days post last vaccination. Individual weighing was performed once a week.
- Thickness of the skin-pleat and size of local reaction was measured every 3 to 4 days.
- Animals were observed daily for immediate reaction following injection (Anaphylactic shock, collapse, tremors...).
- Feeding behavior was observed daily.

b/ Results

Most animals in the vaccinated group did not display any measurable reaction after one, two or three administrations of Coxevac®. Some kids displayed a moderate reaction and the average increase in the thickness of the skin pleat in the Coxevac® group remained very acceptable. The most important reaction (3.2 cm²) disappeared in 2 days.

Following vaccine administration, no general reaction was observed.

Figure 1: Experimental design.

Figure 2. Group mean body temperature (°C) before and after 2nd injection (D21).

Figure 3. Group mean body temperature (°C) before and after 3rd injection (D35).

Figure 4. Average body weight in both treatment groups according to the time.

Figure 5. Thickness of skin-pleat following the 2nd (D21) and the 3rd (D35) vaccination.

C/ Conclusion

The subcutaneous vaccination with Coxevac®, at the recommended dosage, can be performed at two or three weeks intervals with no concern for safety parameters.
COXEVAC® IS AN INACTIVATED AND PURIFIED WHOLE-CELL COXIELLA BURNETII VACCINE WITH NO ADJUVANT. THANKS TO THE PURIFICATION PROCESS AND THE PURE VACCINE COMPOSITION, NO MAJOR SYSTEMIC OR LOCAL REACTION IS EXPECTED. COXEVAC® TARGETS ALL AGE GROUPS OF ANIMALS ABOVE THREE MONTHS OF AGE. THIS INCLUDES ANIMALS IN LACTATION, AND THEREFORE, were observed.

No immediate reaction and no general signs of intolerance were noted. Any clinical sign of disease such as intolerance to the vaccine was not found significant difference in daily milk production of the groups to the median (of the two groups) at none of the investigated time point. Furthermore, there was no significant difference between the average daily milk production of the vaccinated and placebo groups 25 days apart (on D0 and D25).

GROUP 1

Vaccinated group: 40 seronegative, non-pregnant, healthy adult goats were injected SC with one dose of Coxevac® vaccine: 2 mL twice 25 days apart (on D0 and D25).

GROUP 2

Placebo: 40 seronegative, non-pregnant, healthy adult goats were injected with 2 mL of a placebo solution twice 25 days apart (on D0 and D25). The observation period started 7 days prior to the injections (the data collected before the injections were carried out, were used as reference values for milk production) and ended 2 weeks after the last injection. The daily milk production was recorded individually by an in-house computer system. These individual data were then averaged for each group.

Any clinical sign of disease such as intolerance to the vaccine were recorded.

b) Results

No immediate reaction and no general signs of intolerance were noted.

The administration of Coxevac® had no negative impact on the milk production of dairy goats. This vaccine is safe to use during lactation in goats.
COXEVAC® suspension for injection for cattle and goats.

**COMPOSITION:** Composition for 1 ml: Inactivated Coxiella burnetii, strain Nine Mile 72 QF Unit Q-fever Unit: relative potency of phase I antigen measured by ELISA in comparison with a reference from Thiomersal max. 120 μg. **MEASURES:** Cattle For the active immunisation of cattle to lower the risk for non-infected animals vaccinated when non-pregnant to become shedder (5-times lower probability in comparison with animals receiving a placebo), and to reduce shedding of Coxiella burnetii in these animals via milk and vaginal mucus. Goats For the active immunisation of goats to reduce abortion caused by Coxiella burnetii and to reduce shedding of the organism via milk, vaginal mucus, faeces and placenta.

**INDICATIONS:**
- **Cattle:** For the active immunisation of cattle to lower the risk for non-infected animals vaccinated when non-pregnant to become shedder (5-times lower probability in comparison with animals receiving a placebo), and to reduce shedding of Coxiella burnetii in these animals via milk and vaginal mucus.
- **Goats:** For the active immunisation of goats to reduce abortion caused by Coxiella burnetii and to reduce shedding of the organism via milk, vaginal mucus, faeces and placenta.

**CONTRAINDICATIONS:** None.

**ADVERSE REACTIONS:**
- **Cattle:** It is very common to see a palpable reaction of maximum diameter of 9 to 10 cm at the injection site, which may last for 17 days. The reaction gradually reduces and disappears without need for treatment.
- **Goats:** It is very common to see a palpable reaction of 3 to 4 cm diameter at the injection site which may last for 6 days. The reaction reduces and disappears without need for treatment. It is very common to observe a slight increase of rectal temperature for 4 days post-vaccination without other general signs.

**AMOUNTS TO BE ADMINISTERED AND ADMINISTRATION ROUTE:**
- **Subcutaneous use.** Shake well before use.
- **Administer the vaccine as follows:**
  - **Cattle:** 4ml in the neck region.
  - **Goats:** 2 ml in the neck region.

- **Optimal vaccination period:** from 3 months of age.

**COWEVEAC® suspension for injection for cattle and goats.

**PROTOCOLE DE VACCINATION**

**1st VACCINATION**

3 weeks

**2nd VACCINATION**

9 to 12 month

**Booster**

Optimal vaccination period: from 3 months of age

**VACCINATION DOSE**

- **in cattle:** 4 ml
- **in goats:** 2 ml

**WITHDRAWAL PERIODS:**
- Meat, milk and offal: Zero days.

**PACKAGING:** Box containing 40 ml or 100 ml of solution.

**MARKETING AUTHORIZATION NUMBER:** EU/2/10/110/001.

**MARKETING AUTHORIZATION HOLDER:** CEVA Sante Animale 10 avenue de la Ballastiere 33500 Libourne, FRANCE.

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