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Intracellular bacteria and adverse pregnancy outcomes

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Abstract

This review considers the role of intracellular bacteria in adverse pregnancy outcomes, such as miscarriage, stillbirths, and preterm labour. The cause of miscarriage, stillbirth and preterm labour often remains unexplained. Intracellular bacteria that grow either poorly or not at all on media used routinely to detect human pathogens could be the aetiological agents of these obstetric conditions. For example, *Listeria monocytogenes* and *Coxiella burnetti* are intracellular bacteria that have a predilection for the fetomaternal unit and may induce fatal disease in the mother and/or fetus. Both are important foodborne or zoonotic pathogens in pregnancy. Preventive measures, diagnostic tools and treatment will be reviewed. Moreover, we will also address the importance in adverse pregnancy outcomes of other intracellular bacteria, including *Brucella abortus* and various members of the order *Chlamydiales*. Indeed, there is growing evidence that *Chlamydia trachomatis*, *Chlamydia abortus* and *Chlamydia pneumoniae* infections may also result in adverse pregnancy outcomes in humans and/or animals. Moreover, newly discovered *Chlamydia*-like organisms have recently emerged as new pathogens of both animals and humans. For example, *Waddlia chondrophila*, a *Chlamydia*-related bacterium isolated from aborted bovine fetuses, has also been implicated in human miscarriages. Future research should help us to better understand the pathophysiology of adverse pregnancy outcomes caused by intracellular bacteria and to determine the precise mode of transmission of newly identified bacteria, such as *Waddlia* and *Parachlamydia*. These emerging pathogens may represent the tip of the iceberg of a large number of as yet unknown intracellular pathogenic agents.

Keywords: Fetal loss, intracellular bacteria, miscarriage, pregnancy, preterm labour
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Introduction

There is accumulating epidemiological and experimental evidence that maternal infection is a significant risk factor for adverse pregnancy outcomes. Untreated infection may cause miscarriage, stillbirth and preterm labour by several mechanisms, including direct fetal infection, placental damage, and severe maternal illness. In many instances, however, no pathogens are identified, despite increases in the levels of inflammatory markers in the mother or histological evidence of chorioamnionitis. Intracellular bacteria, which do not grow on media that are used routinely to detect human pathogens from clinical samples, could be the aetiological agents of these obstetric complications.

The effects of several human infections with intracellular bacteria on the outcome of pregnancy have been recognized for many years. Indeed, *Listeria monocytogenes*, *Coxiella burnetti* and *Chlamydia* species are known agents of adverse pregnancy outcomes. However, owing to their fastidious growth requirements, their role probably remains largely underestimated. With the increased availability of modern diagnostics and rigorous screening, a higher proportion of agents may now be detected during pregnancy. Moreover, much of what is known about the pathogenesis of these intracellular microorganisms has emerged within the past few years as a result of improved molecular-based tools being available for their detection.

TABLE 1. Epidemiology, clinical presentation, diagnosis and treatment of intracellular bacteria that are potentially implicated in miscarriage

	Listeria monocytogenes	*Coxiella burnetti*	*Chlamydia trachomatis*	*Chlamydia pneumoniae*	*Waddlia chondrophila*	*Parachlamydia acanthamoebae*
Zoonotic potential from:	+	+	–	–	+	(+)
	Ruminants	Placenta from ruminants (pets)			Ruminants? Pets?	Ruminants? Pets?
Other mode of transmission	Foodborne	Aerosols	Sexually transmitted disease	Aerosols	Aerosols? Water? Food?	
	Soil, vegetables, milk	Between pregnant women	(vertical transmission)		Uncooked meat? Milk?	
Main clinical presentation in animals		Stillbirth and spontaneous abortion	–	–	Abortion	Abortion
Main clinical presentation in humans	Non-specific influenza-like symptoms Febrile gastroenteritis, sepsis, meningitis	Non-specific influenza-like symptoms Hepatitis or pneumonia, chronic Q-fever (endocarditis)	Urethritis, cervicitis and PID	Respiratory tract infections	?	Respiratory tract infections
Clinical presentation during pregnancy	Miscarriage, stillbirth Premature labour Granulomatosis infantiseptica	Spontaneous abortion, IUGR, stillbirth, premature labour	Miscarriage Premature labour	Pre-eclampsia, IUGR, peripartum cardiomyopathy	Miscarriage Premature labour	Miscarriage Premature labour IUGR
Diagnostic tests						
PCR	(+)	+	+	+	+	+
Serology		+	(+)	(+)	+	+
Culture	+		(+)	(+)	(+)	(+)
Treatment during pregnancy	Ampicillin	Co-trimoxazole (azithromycin, erythromycin)	Azithromycin	Azithromycin	Azithromycin	Azithromycin

IUGR, intrauterine growth restriction; PID, pelvic inflammatory disease.
+ gold standard; (+) possible diagnostic test, but not considered as gold standard.

We will first describe well-known intracellular infections during pregnancy, such as those caused by *L. monocytogenes*, *C. burnetti* and *Chlamydia* species, and then summarize current knowledge on the role of *Chlamydia*-like pathogens during pregnancy. This review will focus on the epidemiology, pathogenesis, zoonotic potential and mode of transmission of all of these intracellular bacteria (Table 1).

Listeria

L. monocytogenes is a small, facultative, intracellular, Gram-positive bacterium. The bacterium is particularly successful in causing foodborne diseases, because it can continue to slowly multiply even in properly refrigerated foods. *Listeria* infects many types of animal, especially ruminants, and is thus an important cause of zoonoses. However, soil, vegetables and milk may also be contaminated. In recent years, an increasing rate of listeriosis has been reported [1,2]. Whereas much has now been learned about outbreaks of listeriosis, sporadic listeriosis still represents the majority of cases.

The rate of infection is highest among neonates, adults >60 years of age, immunocompromised patients and pregnant women. Pregnant women account for 27% of all cases, and 60% of cases occur among 10–40-year-olds. Moreover, pregnant women have a 20-fold increased risk of developing diseases as compared with the general population [3,4], indicating that pregnancy may constitute a risk factor for the acquisition of listeriosis. In contrast to the other groups at risk cited above, pregnant women rarely present with gastroenteritis or meningitis. However, more than 30% of infections during pregnancy result in miscarriage, stillbirth or premature labour [5]. Most maternal infections occur following ingestion of the organism in food during the third trimester of pregnancy, when maternal T-cell immunity is most impaired [4]. Alkalinization of the stomach by antacids or iron supplements [6], which are frequently prescribed during pregnancy, promotes infection. *Listeria* crosses the mucosal barrier of the intestine to disseminate haematogenously to any site, with a unique tendency to infect the fetoplacental unit. Bacteraemia generally manifests clinically as non-specific influenza-like symptoms, and may remain asymptomatic. The fetus may be stillborn or die within hours of a disseminated form of listerial infection known as granulomatosis infantiseptica, which is characterized by the widespread presence of microabscesses and granulomas. The highest concentrations of bacteria are found in the lung and gut, suggesting that infection is acquired *in utero* via inhalation of infected amniotic fluid.

Smith *et al.* [5] recently reviewed 36 cases of maternofetal listeriosis. The mothers were generally only mildly affected by the infection. Twelve pregnancies ended with abortion or stillbirth. Among the 24 children born alive, 15 were diagnosed with bacteraemia/septicaemia, three with pneumonia, and three with neonatal meningitis; three were unaffected. Only one of the live-born children died from the infection, and none of the surviving children showed signs of permanent damage. In another study [7], 16.7% of all cases of listeriosis reported in the USA were pregnancy-related. Among 128 cases of maternofetal infection, four resulted in neonatal deaths and 26 (20.3%) in fetal loss. Invasive illnesses in newborns (n = 85) were mainly meningitis (32.9%) and sepsis (36.5%). Pregnant women with *Listeria* infection were more likely to belong to ethnic minorities, as reported by others [2].

Listeriosis may be diagnosed by culture of *Listeria* from maternal blood, amniotic fluid, placenta, meconium, or lochia, as well as from tracheal aspirates or ear swabs from newborns [4]. When bacteria are obtained in culture, bacterial identification at the species level may be performed with the use of a few different phenotypic analyses, which include Gram staining, catalase, motility, haemolysis, and the Christie, Atkins and Munch-Petersen (CAMP) reaction test (Fig. 1). Matrix-assisted laser desorption ionization time-of-flight mass spectrometry may also provide rapid and accurate identification. Identification at the species level is especially important when a member of the genus *Listeria* is isolated from a possible source of an outbreak, as the other, non-pathogenic, species may also contaminate the food. PCR is useful when antibiotic treatment has already been started in cases of preterm labour, as prior antibiotherapy may compromise culture. Since listeriosis during pregnancy may be fatal, blood cultures should be considered for any pregnant woman presenting with fever, influenza-like illness, or gastrointestinal symptoms [8]. As asymptomatic faecal carriers have been reported, vaginal or stool cultures are not recommended. Antimicrobial treatment started before a definite diagnosis has been made can result in the birth of a healthy infant. Thus, any fever during pregnancy in someone with a positive history of consumption of raw vegetables, uncooked meat or non-pasteurized milk raises suspicion of *Listeria* infection, and should be empirically treated with intravenous ampicillin (200 mg/kg/day, which corresponds to 12 g/day, i.e. 2 g every 4 h) for 2–3 weeks [4,9]. For women with proven β-lactam allergy, erythromycin may be considered,

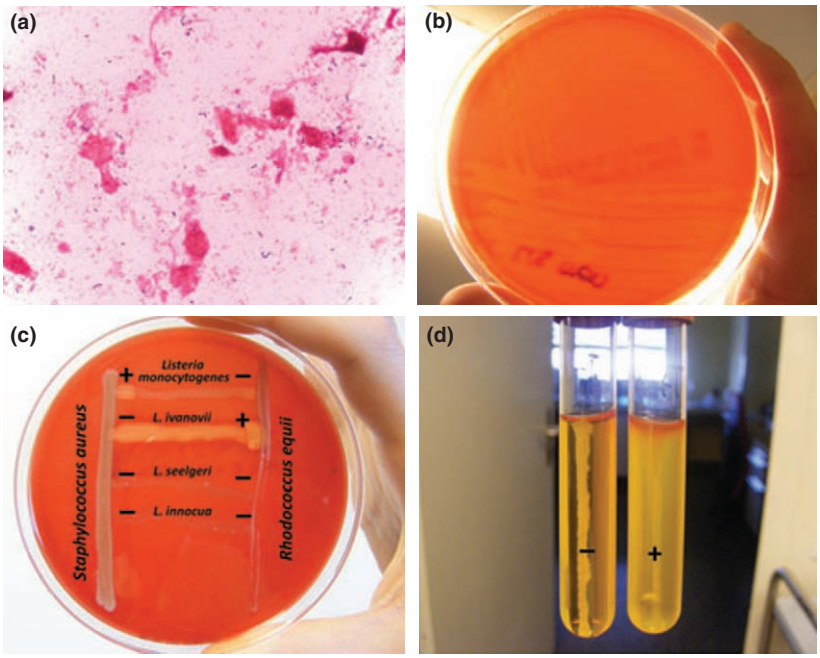

FIG. 1. Phenotypic identification of *Listeria* at the species level. *Listeria* spp. appear as short Gram-positive rods (a). *Listeria monocytogenes* is β-haemolytic (b); this haemolysis allows discrimination of *L. monocytogenes* from *Listeria innocua* (the most common non-pathogenic *Listeria* species), which is non-haemolytic (c); to differentiate *L. monocytogenes* from *Listeria seelgeri* and *Listeria ivanovii*, which are haemolytic, the Christie, Atkins and Munch-Petersen (CAMP) test is generally used (c); briefly, the haemolysis caused by *L. monocytogenes* is enhanced in the presence of *Staphylococcus aureus*, whereas the haemolysis caused by *L. ivanovii* is increased by the presence of *Rhodococcus equii*. Note that, in contrast to *Lactobacillus* spp., *Listeria* species are catalase-positive and motile; the test of motility is shown in (d).

despite its poor transplacental passage leading to subtherapeutic concentrations in both the fetal serum and amniotic cavity [1]. Concerning prevention, pregnant women should be advised to avoid consumption of raw milk, unpasteurized soft cheeses, uncooked meats, refrigerated patés, and smoked seafood [4].

Coxiella

Increased awareness and better diagnostic tests may not be sufficient to explain the rising number of recent outbreaks of Q-fever [10–12]. Q-fever is a widespread zoonosis caused by *C. burnetii*, an intracellular Gram-negative bacterium. In cattle, sheep, and goats, *C. burnetii* infection is often asymptomatic [13,14]. However, infection of pregnant animals is associated with stillbirth and spontaneous abortion [14].

Infected placentas contaminate the soil at the time of parturition, leading to the persistence of viable organisms for years [14,15]. Aerosols from infected placentas, secretions or excretions of animals are the major source of transmission to humans, and can occur many kilometres from the animals [14]. The most commonly identified sources of human infection are farm animals. Pets, especially cats, also represent an important vector of the disease in urban areas [16]. Ingestion of unpasteurized milk products is also a source of human infection by *C. burnetii* [17], which is of concern given the growing interest in 'bio' alimentation. Moreover, there is growing evidence regarding the role of air-conditioning as a potential source of Q-fever [18]. Inter-human transmission has also been described [19,20]. A case of Q-fever occurring after contact with an infected pregnant patient at the time of delivery has been published [21]. Psychiatric patients may also represent an important risk group [22]. *C. burnetii* is highly infectious, as a single bacterium may infect a human [13].

Human primary infection is asymptomatic in 50% of cases. However, acute Q-fever may also present with an influenza-like illness, hepatitis, or pneumonia, and rarely endocarditis [14,15]. Fever spontaneously resolves after 10 days, but approximately 5% of infected patients will develop chronic Q-fever months to years after the acute illness [14,15]. Endocarditis and aortic infections represent the main forms of chronic Q-fever, mainly in patients with valve lesions, arterial aneurysms, or prostheses, or in those who are immunocompromised [15]. Less common presentations include osteoarticular infections, chronic hepatitis, and lung infections [15].

The prevalence of acute Q-fever has been reported to be 1/2000 people, but epidemiological studies have demonstrated 10–36% seropositivity in blood donors [20]. In women with uneventful pregnancies, a seroprevalence rate of 6.5% has recently been reported [23]. The prevalence of Q-fever during pregnancy might be as high as 0.19% [24], which is a similar value to those for other better-studied maternal infections, such as those with cytomegalovirus [25] and hepatitis C virus [26]. However, the incidence of Q-fever during pregnancy is likely to be underestimated, and thus underinvestigated. Indeed, obstetricians' knowledge about *Coxiella* is poor [20], and acute Q-fever is significantly more asymptomatic in pregnant women than in the general population [20]. However, Q-fever may result in severe obstetric complications, including spontaneous abortion (26%), intrauterine growth retardation (5.3%) or fetal death (5.3%), and premature delivery (44.7%) [20]. No teratogenicity has been observed, although the first trimester of pregnancy is the period when there is the highest risk of adverse pregnancy outcomes and chronic Q-fever for the mother [20,27]. *Coxiella* represents a risk not only for the pregnancy in which the infection occurs, but also for future pregnancies. Seropositive women were found to be three times more likely to have a current or previous neonatal death [28]. In addition, pregnant women have been shown to exhibit a ten-fold increased risk of chronic Q-fever relative to the general population (52.8% vs. 5%) [20,27]. Moreover, endocarditis may occur even in the absence of underlying valvular lesions.

The pathophysiology of *Coxiella* in pregnancy remains uncertain. *Coxiella* will colonize and multiply in the uterus and placenta. Recent studies have shown that *C. burnetii* replicates not only within human monocytes and macrophages, but also within trophoblastic cells [29]. This may result in placental insufficiency following vasculitis and vascular thrombosis. Moreover, *Coxiella* has also been identified in fetal organs, demonstrating possible transplacental transmission [20].

The diagnosis of acute Q-fever relies on the detection of specific IgG and IgM antibodies directed to phase II *C. burnetii*, whereas chronic Q-fever is indicated by high titres of phase I IgG antibodies [14,20]. Immunofluorescence is considered to be the reference standard for *Coxiella* diagnosis [14,15,20]. However, serology can also be performed by ELISA or the complement fixation test [14,30]. Infected tissues (placenta, heart valves, etc.) may also be tested by PCR [14,20].

The treatment of choice for *C. burnetti*, doxycycline, is contraindicated during pregnancy. In a series of 53 infected pregnant patients, co-trimoxazole decreased the incidence of the adverse pregnancy outcomes cited above, although it was not curative in ten of 17 patients [27]. However, *C. burnetii* can be definitely treated with doxycycline after delivery.

Other authors have reported successful treatment (uneventful pregnancies) with azithromycin [31] or erythromycin and rifampicin [32].

Brucella

Human brucellosis remains the most common zoonotic disease worldwide, with more than 500 000 new cases annually [33,34]. Although brucellosis has been eradicated in many developed countries, it is still endemic in the Mediterranean area, Middle East, Southwest Asia, and parts of Latin America. In a recent study in the UK [23], only two of 438 pregnant women exhibited anti-*Brucella* antibodies, both patients originating from Sudan.

Brucella species are intracellular bacteria that may infect humans [34,35]. Four species may cause disease in humans—*Brucella abortus*, *Brucella melitensis*, *Brucella suis*, and *Brucella canis*—with the animal reservoirs being cattle, sheep and goats, pigs, and dogs, respectively [34–37]. Two novel species, *Brucella ceti* and *Brucella pinnipedialis*, have been isolated from marine mammals [33,36].

In animals, brucellosis manifests as a chronic infection resulting in abortion and sterility. A large bacterial load is present in milk, urine, and the products of pregnancy [33,37]. Intrauterine transmission, transmission during delivery and transmission through breast milk are among the main routes of transmission in the mammalian reservoirs [33,34].

Brucella infects humans via aerosol inhalation, ingestion, or mucosal or skin abrasions. Suspicion of patient infection is raised when a detailed history reveals ingestion of contaminated dairy products or when contact with infected animals has occurred [33,34]. Recent reports also suggest possible infection via blood transfusion and sexual transmission, and from patients to medical personnel, especially in an obstetric setting [38].

In humans, brucellosis is a multisystemic disease. Fever is the most common feature, followed by osteoarticular involvement (sacroiliitis, spondylitis, peripheral arthritis, and osteomyelitis) [33–35,39]. However, all systems might be affected, such as the nervous (neuropathies, chorea, and meningoencephalitis), gastrointestinal, hepatobiliary (hepatomegaly), genitourinary (orchiepididymitis, glomerulonephritis, and renal abscesses), musculoskeletal, cardiovascular (endocarditis, mainly with involvement of the aortic valve) and pulmonary (pleural effusions and pneumonia) systems. Approximately 10% of patients with brucellosis experience relapses, 90% of which occur within a year after discontinuation of antimicrobial drug therapy [34]. Disease reactivation has been described as long as 28 years after the initial infection [40].

Increased rates of spontaneous abortion, premature delivery and intrauterine infection with fetal death have been described among pregnant women with clinical evidence of brucellosis [41,42]. Left untreated, surviving pregnancies may lead to serious sequelae for the newborn, such as *Brucella* myocarditis [43], severe respiratory distress, and hepatosplenomegaly [44]. Women who received early diagnosis and adequate treatment had successful maternal and fetal outcomes [41]. Rifampicin 900 mg once daily for at least 6 weeks is considered to be the regimen of choice during pregnancy [37]. Tetracyclines (especially doxycycline 100 mg twice daily for 6 weeks), the most effective drugs for brucellosis, can only be initiated after delivery [37]. Although vaccines are available for animals, there is currently no licensed vaccine for brucellosis in humans [33,36].

Brucellosis is routinely misdiagnosed, or at best diagnosed incidentally; therefore, physicians in both endemic and non-endemic areas should be aware of brucellosis and consider this disease in the differential diagnosis of febrile episodes during pregnancy. Blood culture is the reference standard for the diagnosis of *Brucella* infection in humans [33,34]. The Rose Bengal test is used as a screening serological test, and positive results are confirmed by the serum agglutination test [45]. Other tests, such as ELISA and PCR, are increasingly used for the diagnosis of brucellosis.

Chlamydia

Chlamydiae are obligate intracellular bacterial pathogens. There is increasing evidence that *Chlamydia trachomatis*, *Chlamydia pneumoniae* and *Chlamydia abortus* infections may result in adverse pregnancy outcomes in humans and/or animals [46].

Chlamydia trachomatis infection is the most commonly diagnosed bacterial sexually transmitted infection worldwide [46,47] and a major cause of infertility [48,49]. Given the importance of *Chlamydia trachomatis* in miscarriage and premature delivery, *Chlamydia* screening should be considered as part of routine antenatal care [50]. This screening was well accepted among young pregnant women, who recognized the benefit of screening and strongly supported its implementation [50].

During the first trimester, many studies (reviewed in [46]) have shown a relationship between miscarriage and *Chlamydia trachomatis* infection by detection of anti-chlamydial IgG and/or IgA antibodies [51–55] and endocervical swabs [55,56]. In a recent study performed in our university

hospital, 386 women with and without miscarriage were prospectively included [57]. Most patients suffered from a first-trimester miscarriage. The anti-*Chlamydia trachomatis* IgG prevalence was higher in the miscarriage group (15.2%) than in controls (7.3%, p 0.018). The association between *Chlamydia trachomatis*-positive serology and miscarriage remained significant after adjustment for age, origin, education level, and number of sexual partners (OR 2.3, 95% CI 1.1–4.9). *Chlamydia trachomatis* DNA was more frequently amplified from products of conception or placentas taken from women who had suffered miscarriages (4%) than from controls (0.7%, p 0.026). Moreover, immunohistochemistry confirmed the presence of *Chlamydia trachomatis* in the placentas of five of seven patients with positive PCR findings, whereas immunohistochemistry gave negative findings in placentas taken from all eight negative controls tested [57].

In the second and third trimesters, several studies have suggested an association between molecular and/or serological evidence of *Chlamydia trachomatis* infection in the mother and premature uterine activity, premature birth of infants with extremely low birthweight, and perinatal death [58–60]. Other studies have identified similar correlations between preterm labour, premature preterm rupture of membrane (PPROM), or prematurity and infection with *Chlamydia trachomatis* [61–64].

The pathogenesis is still only partially understood. Chlamydial infection may increase the probability of an adverse pregnancy outcome by infecting the fetus, by stimulating a fetal inflammatory response, or by leading to excessive maternal immunogenic reaction to its fetal heat shock protein-60 antigen [46,65].

In pregnant patients, *Chlamydia trachomatis* might be identified from urine, as well as from vaginal, cervical or urethral swabs. Nucleic acid amplification offers high sensitivity (>97%) and specificity (>99%), and is thus mostly used for detection of *Chlamydia trachomatis* [47,66], largely replacing cell culture and antigen-based detection methods (immunological assays).

The benefit of widespread testing in terms of decreasing the frequency of pregnancy complications has not been studied in randomized controlled intervention trials, for ethical reasons. However, *Chlamydia trachomatis* testing should form part of pre-conceptional screening, to reduce the prevalence of adverse pregnancy outcomes [57]. Moreover, all women experiencing a miscarriage should be screened for *Chlamydia trachomatis* infection, and if found to be positive, should be adequately treated to prevent recurrent episodes of miscarriage [46,57,67]. Finally, all patients presenting with preterm uterine contractions and/or preterm premature rupture of membranes should be tested for the presence of *Chlamydia trachomatis* [46]. Azithromycin is safe during pregnancy, and is the recommended treatment.

Chlamydia pneumoniae, a closely related intracellular bacterium, which is known to cause respiratory tract infections [68], has recently been associated with adverse pregnancy outcomes, such as pre-eclampsia [69–73], intrauterine growth restriction [74], and peripartum cardiomyopathy [75]. A recent meta-analysis confirmed an association between anti-*Chlamydia pneumoniae* IgG seroprevalence and the risk of developing pre-eclampsia [69]. This is most consistent for women with a prior history of pre-eclampsia, women who will develop early-onset pre-eclampsia, and women with pre-eclampsia at the time of serological testing [69].

Moreover, Xie *et al.* [69] showed that *Chlamydia pneumoniae* DNA copy numbers were significantly higher in sera from women with pre-eclampsia than in sera from normal pregnant or non-pregnant controls. The physiopathology of *Chlamydia pneumoniae* during pregnancy is unclear, but *Chlamydia pneumoniae* is able to infect human trophoblast cells [70,74], *Chlamydia pneumoniae* DNA being more frequently identified in the trophoblast cells of pre-eclamptic patients than in controls [70]. Infection may reduce trophoblast invasion into the uterine wall, which may lead to placental dysfunction and pre-eclampsia [70].

Chlamydia abortus (formerly *Chlamydia psittaci* sensu lato), which causes fetal wastage in ruminants [76], is also abortigenic in many other species, such as swine, horses, rabbits, guinea pigs, koalas, and mice [46]. In humans, *Chlamydia abortus* has been implicated in spontaneous miscarriages or stillbirths in farm women after contact with infected animals [46,77–79]. The incubation period before miscarriage may last for weeks or months after animal contact. Indeed, human abortion generally occurs late in pregnancy and following a febrile influenza-like syndrome, with or without disseminated intravascular coagulation or impaired liver and renal function [46]. Women should therefore avoid contact with ruminants during pregnancy [46].

Although other members of the family *Chlamydiaceae* (*Chlamydia pecorum*, *Chlamydia suis*, and *Chlamydia felis*) have also been associated with urogenital infection and adverse pregnancy outcomes in many animals [80], no human zoonotic infection by these species has yet been described.

Chlamydia-like Organisms

In addition to the family *Chlamydiaceae*, new family-level lineages have been added to the order *Chlamydiales*: *Parachlamydiaceae*, *Simkaniaceae*, *Criblamydiaceae*, *Rhabdochlamydia-*

ceae, and *Waddliaceae* [80]. Members of these newly described family-level lineages contain an 80–90% identical 16S rRNA-encoding gene sequence with members of the *Chlamydiaceae*, and exhibit a *Chlamydia*-like replicative cycle. Among these families, two species have been associated with human adverse pregnancy outcomes, i.e. *Parachlamydia acanthamoebae* and *Waddlia chondrophila*. Both species are able to enter and replicate within human macrophages before inducing a cytopathic effect [81,82]. *Waddlia* was also recently shown to multiply inside endometrial cells [83] (Fig. 2). These *Chlamydia*-like organisms are susceptible to doxycycline and azithromycin, but resistant to β-lactams and fluoroquinolones [84,85].

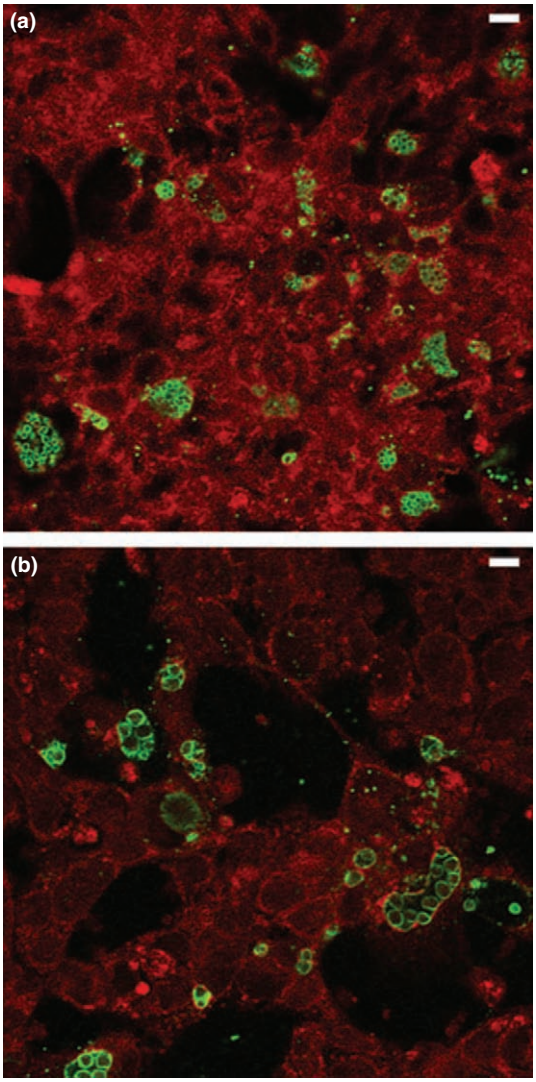

FIG. 2. *Waddlia chondrophila* within endometrial cells, as seen by confocal microscopy. Note that, 48–72 h post-infection, there are numerous bacteria (stained green with polyclonal anti-*Waddlia* antibodies) in Ishikawa endometrial cells (a). However, at 96 h post-infection, the bacteria transform into persistent enlarged aberrant bodies that may be up to 5 μm in diameter (b). These persistent forms may explain the occurrence of recurrent episodes of miscarriage.

Seroepidemiological and molecular studies provided the first evidence that *P. acanthamoebae* plays a role in human pneumonia [68,86]. However, *Parachlamydia* might also be an agent of miscarriage in humans. Indeed, in a study including 438 women, none of the 169 uneventful pregnancies had positive serological findings for *Parachlamydia*, whereas seven (2.6%; p 0.047) of 269 women experiencing a miscarriage exhibited *Parachlamydia* IgG titres of 1/64 or greater [51]. Moreover, maternofetal transmission of *Parachlamydia* has been recently described [87]: *Parachlamydia* DNA was amplified by PCR from one of 78 amniotic fluid samples taken from patients who had spontaneous premature deliveries (the patient, a 29-year-old woman, had an amniocentesis at 16 weeks of gestation for a Down syndrome risk of 1/100; at that time, the patient had cough and influenza-like symptoms; the pregnancy ended with a premature spontaneous vaginal delivery 4 weeks before her due date). Finally, 31% of respiratory samples from 29 premature babies hospitalized in neonatology units showed the presence of *Parachlamydia* DNA [86]. Although neonatal infections might have occurred after delivery, systemic infection during pregnancy is possible and may have induced the premature labour.

W. chondrophila, another *Chlamydia*-like organism, has also been implicated in human adverse pregnancy outcomes [46]. A serological study showed a strong association between miscarriage and the presence of anti-*Waddlia* antibodies [51]. The seroprevalence was higher in patients with sporadic (31.9%) and recurrent (33%) miscarriage than in patients with uneventful pregnancies (7.1%; p <0.001). *Waddlia* seropositivity was associated with animal contact (pets), suggesting that this *Chlamydia*-like organism may be zoonotically transmitted, but infection through contaminated water, uncooked meat, milk or sexual contact might also be the mode of transmission to humans. This strong serological association was confirmed in a second study (D. Baud, G. Goy, M.-C. Osterheld, N. Borel, Y. Vial, P. Hohlfeld, A. Pospischil, G. Greub, unpublished data). Moreover, a case was identified that exhibited immunohistochemical and molecular evidence of *Waddlia* infection [57].

The pathogenesis of adverse pregnancy events caused by *Parachlamydia* and *Waddlia* remains to be defined. The recent availability of the *Waddlia* and *Parachlamydia* genomes opened new research perspectives [88,89]. Because, in a recent study, we observed the presence of *Waddlia* in endometrial cells [57], we hypothethized that deciduitis

(infection of the maternal part of the placenta) may lead to uterine contraction. However, at least in animals, abortion may also occur following chorioamnionitis and fetal infection. Indeed, *P. acanthamoebae* was identified in aborted bovine placenta samples [90–94], and *W. chondrophila* was first isolated from an aborted bovine fetus [95,96]. As *Waddlia* was associated with recurrent miscarriage [46,51], and may develop into aberrant bodies (a persistent stage; see Fig. 2b), at least *in vitro*, upon infection of endometrial cells [83], such persistence, if confirmed *in vivo*, underlines the likely importance of diagnotic procedures to identify women with asymptomatic infection at risk of recurrent miscarriage and who will clearly benefit from therapy with azithromycin or doxycycline.

Relationship Between Mammals and Human Abortion

Cross-species infection is a major cause of emerging infectious diseases [97], and has been promoted by intensive animal husbandry, the presence of pets at home, and animal migration [98]. The influence of human behaviour and environmental perturbations induced by humans (deforestation and climatic changes) is also responsible for newly emerging human infections [99]. Few infectious diseases are human-specific: most human pathogens also circulate in animals or even originated in non-human hosts [97–100]. Indeed, these pathogens have evolved elaborate mechanisms to jump between species, such as domestic fowls, farm animals, and humans [99]. Influenza, SARS, AIDS and Creutzfeldt–Jakob disease are classic examples of zoonotic infections that are transmitted from animals to humans [99,101].

Much attention has been focused on these pathogens, which are of immediate global importance. Meanwhile, intracellular bacteria have been neglected, as have crucial processes such as cross-species transmission. However, several emerging intracellular bacteria pose a serious and increasing threat to human reproductive health, as pregnancy itself is their target.

C. burnetti, *B. abortus*, *Chlamydia abortus*, *W. chondrophila* and *Parachlamydia* are examples of intracellular bacteria that induce both animal abortion and human miscarriage. Either through direct contact with the host animals or through ingestion of their products (meat and milk), the same intracellular bacterium can induce a similar disease, probably through the same pathophysiological pathway [46]. It may be possible that many as yet unknown intracellular bacteria are responsible for 50% of miscarriages of unknown aetiology. The multi-host ecology of zoonoses should lead to the development of effective control policies. Human disease surveillance must clearly be associated with enhanced longitudinal veterinary and wild animal infection surveillance [101].

Recommendations and Guidelines for Pregnant Women

Although primary preventive measures mainly concern veterinary practices, agricultural areas, pet shops, and the food industry, every woman intending to become pregnant or who is already pregnant should be informed about the following three main infection sources:

1. Environment: fruit and vegetables should be well washed prior to consumption. Pregnant women should be advised to avoid contact with soil, and ideally avoid gardening. Work or contact with farm animals should be discouraged, especially with pregnant or recently delivered animals. Pregnant women should not drink/eat non-pasteurized milk or cheese.
2. Meat: handling of raw meat should be avoided. Only well-cooked meat should be eaten. Raw food should not be prepared at the same location and/or with the same ustensils as those used to handle raw meat.
3. Domestic animals: domestic cats should be fed with canned food rather than with raw meat. Contact with pet's faeces and cat litter should be strictly avoided. Hands should be washed after contact with soil, dogs, and cats, and before each meal.

Conclusions

C. burnetti has been associated with adverse pregnancy outcomes. However, because of their intracellular lifestyle, the detection of infections caused by these intracellular bacteria is challenging. In the next decade, the number of reported cases might increase as a result of improved tools for their detection and better awareness of clinicians. *C. burnetti* and *Chlamydia* species should be added to the TORCH screen in the category of 'Other etiological agents of intrauterine causes of foetal mortality and morbidity', which already includes the following: viral hepatitis, syphilis, varicella and herpes zoster. Women who are affected with any of the TORCH pathogens during pregnancy are at risk for miscarriage, stillbirth, preterm labour, or delivering a child with serious birth defects and/or illness. Thus, this test is performed before pregnancy or as soon as pregnancy is diag-

nosed to determine the mother's history of exposure to these organisms.

Concerning the different members of the order *Chlamydiales*, and especially *Chlamydia trachomatis*, pre-conceptional molecular testing should be offered to reduce the prevalence of adverse pregnancy outcomes. When this is not possible, this screening should be proposed during the first antenatal assessment (and following any adverse pregnancy outcomes).

In addition, there is increasing evidence of the human pathogenicity of some *Chlamydia*-like organisms, especially as agents of adverse pregnancy outcome. Consequently, there is an urgent need for efficient and standardized diagnostic approaches, using both molecular and serological methods, as well as immunohistochemistry protocols to confirm the presence of the bacteria in placental lesions or fetal tissues.

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Transparency Declaration

GG hold two patents for the serological diagnosis of infections due to *Chlamydia*-related bacteria.

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