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Emerging Aspects of *Coxiella burnetii* and Molecular Identification along with Treatment Approaches

Arslan Habib[1*], Zeeshan Ashraf[2], Muhammad Nabeel[3], Dominic Kwesi Quainoo[4], Umutumwa Eric Principe[5], Naeem Ullah Khalil[6], Muhammad Rizwan Ullah[7] and Bilal Khalid[8]

[1]Laboratory of Molecular Immunology, School of Life Sciences, Fudan University, Shanghai, China.
[2]Department of Fisheries & Aquaculture, University of Veterinary and Animals Sciences, Lahore, Pakistan.
[3]Knowledge Unit of Science and Technology, Faculty of Sciences, University of Management and Technology, Pakistan.
[4]Department of Biotechnology, Faculty of Biosciences, University for Development Studies, Ghana.
[5]School of Biotechnology, East China University of Science & Technology, Shanghai, China.
[6]Centre of Biotechnology and Microbiology, Faculty of Life Sciences, University of Peshawar, Pakistan.
[7]Department of Zoology, University of Lakki Marwat, Pakistan.
[8]Institute of Zoology, University of the Punjab, Lahore, Pakistan.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Q fever is an infectious disease caused by *Coxiella burnetii* which is an obligatory intracellular parasite. Globally Q fever is a widespread zoonosis. It is characterized by headaches, sudden fever and atypical pneumonia. In the rural environment, the main reservoir includes goats, sheep, cows, dogs, cats and rabbits. The main reservoir of this bacteria is considered domestic animals. They produce in a large number in amniotic fluid and placenta during childbirth. The main route of

**Corresponding author: E-mail: arslanhabib09@gmail.com;*

infection is inhalation. Q fever can cause both acute and chronic infection, mostly asymptomatic in humans and animals. Inactivated whole-cell bacteria vaccination strategy has been performed which provides effective outcomes in humans and animals but many side effects have been observed. The recombinant vaccine has been developed and provides many effective results in experimental conditions. One of the major challenges is the lack of accurate diagnosis facilities if it becomes possible, the prognosis of disease development can be reduced. Direct detection of bacteria is the accurate test for the diagnosis. Different procedures are involved in this method such as immunodetection, PCR amplification and shell vial cell culture. Due to the severe infectivity of *C. burnetii* all these procedures require a biosafety level 3 lab and qualified staff. Q fever is a challenging disease for scientists to reduce its burden globally. The review discloses the *Coxiella burnetii* genome, the clinical manifestation of Q fever as well as emerging issues, diagnosis, treatment, prevention and future directions.

Keywords: Coxiella burnetii; diagnosis; Q fever; prevention; treatment.

1. INTRODUCTION

Globally Q fever (*Latin:* debris *Q, coxiellosis)* infected different animal species along with humans. The causative agent of Q fever is a unique phylogenetic and taxonomical bacterium (C. burnetii), which produces spores intracellularly that are impervious to different environmental factors. In 1935 the disease was first time recognized and in 1937 Derrick explained this disease during his research among abattoir workers in Australia with unknown disease outbreaks [1]. Q fever occurs globally, most cases of such disease victims are reported in Australia and southern France. Different kinds of hosts get infected with *C. burnetii* which includes pets, ruminants, humans and a few ticks, reptiles and birds. The discharge of bacterium is found in the birth products, urine, feces and milk. Such products consist of many bacteria that become aerosolized after being deceased. A few organisms are responsible for the prevalence of *C. burnetii* infections. *C. burnetii* can persist viable and toxic for months due to its spore-like-life cycle. Transmission of infection occurred through inhalation or skin exposure and direct contact with a ruminant is not a risk factor for infection. Transmission of infection from human to human is rare except for some exceptional cases such as infected blood transfusion and placenta of infected female [2]. Sexual transmission is also a risk of infection. In humans, asymptomatic infection of *C. burnetii* is common or some influenza-like illness or atypical pneumonia are observed. *C. burnetii* infection in few cases (<5%) becomes chronic with noxious outcomes especially in patients with pre-existing valvular heart disease [3]. *C. burnetii* is identified as a latent agent of bioterrorism because of its major infectious nature and acquiring inhalational mechanism of transmission [4]. The bacterium is highly viable and toxic because of its virulence to cause infection. Different routes are possible risk factors of infection transmission including exposure to contaminated milk, wool, meat and inhalation of contaminated dust and importantly birth products. Ticks are also involved in the transmission of pathogenic agents to other animals [5-8].

1.1 *C. burnetii* Entry and Survival in the Host Cells

Different kinds of cells are infected by the *C. burnetii* which includes macrophages, monocytes, in vivo, in vitro and diversity of transformed cells such as VERO cells, L929, or HELA [9]. The possible factor of the virulence of *C. burnetii* is its magnitude to occupy and thereafter to mature within such eukaryotic cells which permits its multiplication in different niches of the host [10]. The acidified environment is the suitable environment for its survival and multiplication which they complete in the phagolysosome. Different kinds of reviews explained the *C. burnetii* bacterial factors used for the intracellular survival and biphasic developmental cycle in the host cell [11,12]. The small and large appearance possess different genes that allow the bacterium to survive in the particular severe niche of each form. For instance, during the stationary period in major bacteria RpoS (sigma S) is a sigma subunit that negotiates promoter-specific transcriptional induction by RNA polymerase to genes. RpoS causes different physiological and morphological modifications along with multi-stress resistance. LCV exhibits a large number of RpoS while SCV does not possess notable RpoS [13], expressed that LCV and SCV multiplications adaptations may not be the functional parallel types of logarithmic and stationary period bacteria as

suggested initially [14]. During the surviving pressure in metabolically active LCV, this sigma factor may synchronize genes [15]. Therefore, the small types are proficient to detain phagolysosomal fusion, possibly to promote the adaptation from SCV to LCV that present at pH 5.5 in the endosome [16]. Larger duplicative vacuoles possessing *Coxiella* are obtained from fusion with late endosomal-lysosomal organelles by the use of cellular markers such as rab7, LAMP-1 and EEA.1 [17,18]. The secretion system should be associated with the development of phagosome containing *Coxiella* suggested by the sequence homologies between the type IV secretion system proteins of *Coxiella* and *Legionella* [19]. In fact, during in vivo host cell infection, *Coxiella* encodes functional constituents of the type IV secretion system [20]. While the *C. burnetii* secretion system is mechanistically associated with the *Legionella* Dot/Icm system even, *C. burnetii* IcmQ protein and the *Legionella* IcmR protein do not interrelate with each other [20,21]. For producing exclusive vacuole that assists *C. burnetii* replication this secretion mechanism could play an important role. The assimilation of virulent *C. burnetii* depends on phase I LPS on TLR4 and $\alpha_V\beta_3$ integrin [22]. Early occasions of *C. burnetii* infection controls by TLR4 which includes cytokine production, granuloma formation and macrophage phagocytosis [23]. This receptor is not limited to humans but also associated with insects [24,25]. Therefore, *C. burnetii* has adapted a cell utilization method that permits it to enter an exclusive host range and has established a specific tactic to replicate in the adverse phagolysosome environment of the cells. In fact, from different hosts, *C. burnetii* has been isolated such as mammals, amoeba, birds and ticks which adapted them to survive in the vast environment for long-term periods and to increase its prevalence.

2. CLINICAL MANIFESTATION

During the acute infection Q fever mostly express subclinical or highly mild symptoms. Approximately 20 days (range 14 to 39 days) incubation period has been observed. There is no particular type of Q fever and the clinical symptoms of infection differentiate from patient to patient. The epidemiological circumstance is the most principle diagnostic indicator. Some of the major descriptions are as follows:

(i) The self-confined flu-like syndrome is the maximum prominent manifestation of q fever. In Spain, 21% of the occurrence of this shape of q fever has been illustrated which lasting for multiple weeks and less than 3 weeks. The most possible clinical signs encompass excessive-grade fever (104◦ for 40 ◦C), myalgias, unexpected onset, fatigue and headache. Growing age reasons an increase within the duration of fever.

(ii) One of the maximums regularly diagnosed styles of acute q fever is abnormal pneumonia. Most inflamed individuals expressed clinically asymptomatic or moderate, recognized via an ineffectual cough, fever and negligible auscultatory abnormalities, but a few individuals expressed acute breathing misery. Pleural effusion also can be determined. Detection on the chest radiograph is negligent. Findings on the chest radiograph are nonspecific. Marrie et al. [26] expressed that 3.7% of all infected individuals with community attained pneumonia confessed to a tertiary-care teaching health center in nova scotia over five years have been due to c. Burnetii, which is correlated to the results of Lieberman et al. Israel (5.8%) [27].

(iii) Hepatitis (inflammation of the liver) is the main type of acute q fever and it reveals particularly granulomatous hepatitis. Variable length of clinical symptoms turned into discovered from 10 to ninety days. The mortality rate relies upon attention ranging from 0.5 to 1.5% [28]. 3 fundamental forms of hepatitis may be involved together with clinically asymptomatic hepatitis, an infectious shape of hepatitis with hepatomegaly however every so often jaundice and unknown foundation persevered fever with particular granulomas on liver biopsy [29]

(iv) Many different exclusive varieties of acute q fever scientific manifestations are feasible together with pericarditis, myocarditis, headache, maculopapular or purpuric exanthema in 10% of patients [28]. Aseptic meningitis and encephalitis with q fever which accounts for 0.2-1.3% of infected individuals are occasionally attended with the aid of seizures and coma [30]. Hemolytic anemia, gastroenteritis, erythema nodosum, inappropriate secretion of antidiuretic hormone and splenic rupture are unfamiliar manifestations of acute q fever.

2.1 Emerging and Re-Emerging Aspect

Different published research findings on the Q fever outbreak and reflective studies of isolated cases or unfamiliar clinical expressions have been flourishing since 1999 (Table 1). Similarly, this rise about the outbreak may influence the awareness status among the community rather than its emergence Seroprevalence of animal Q-fever obtained in the main recent worldwide surveys mentioned in Table 2. Distribution of confirmed Q fever cases and rates per 100 000 population by country and year, EU/EEA, 2014–2018 mentioned in Table 3 and Fig 1. Clinical signs in humans may fluctuate according to the region as well as the age and gender of the infected individuals. Most commonly asymptomatic infection was observed among the children and non-pregnant women. Among the older patient's atypical pneumonia is the major possible Q fever manifestation [31] and was observed in the different countries of a reported outbreak among the infected individuals such as (Nova Scotia Canada [32], Greek Island of Crete [33], Italy [34], Japan [35], Switzerland [36] and the United Kingdom). In some other countries like France, California and Australia [37], prolonged fever and hepatitis are the most familiar manifestations. Atypical pneumonia was observed in Spain and specifically in Catalonia [38] and the Basque country [39], while hepatitis and prolonged fever-like symptoms were observed in Andalusia [40]. The description of such variability is still under observation and could be due to the genetic variations among the strains. *C. burnetii* strains isolated from ticks are more virulent as compared to those isolated from bovine milk experimented in a guinea pig model [41]. Furthermore, it would be provocative to understand if virulence factors of the bacteria could be the cause of intensity of the disease in some caprine flocks where pregnant females aborted nearly 30% while were asymptomatic but eliminate the bacteria for several months. Currently, still, there are missing genomic markers and epidemiological antigenic or under the developmental stage. However, recent research has utilized different approaches to study the prevalence of Q fever [42].

2.2 *Coxiella burnetii* Genome

The genome of *C. burnetii* Nine Mile phase I consists of a 1.9 Mb chromosome and plasmid QpH1 (37,393 bp) [43]. In the National Centre for Biotechnology Information (NCBI) database, there are 6 available genomes of *C. burnetii*, among which 4 are fully sequenced and 2 are still being analyzed (Table 4). All strains of *C. burnetii* possess 1 autonomous plasmid with a size ranging from 37–55 kb, and in some cases, plasmid sequences can be integrated with bacterial chromosomes (Table 5) [44]. High homology among plasmid sequences has led to the hypothesis that they are essential for the survival of the pathogen [45]. This hypothesis was confirmed with the PCR-RFLP (Polymerase Chain Reaction – Restriction Fragment Length Polymorphism) method, and as a result, 6 different genomic groups (genomic groups I-VI) have been characterized [44]. To ensure that the PCR-RFLP is a sufficient method, MLVA (Multilocus Variable Number Tandem Repeats Analysis) analysis [46] and the multispacer sequence typing (MST) method [47] were also performed. The results suggest that all of the above phylogenetic methods can be successfully used in the molecular analysis of *C. burnetii*. A novel approach in molecular testing was an application of the microarray method in the molecular characterization of isolated strains. This method revealed 2 new genomic groups of *C. burnetii* (VII and VIII) [48]. The molecular characteristics of *C. burnetii* strains also included sequencing and the PCR-RFLP of specific genes: *icd* (isocitrate dehydrogenase) [49], *com1* (outer membrane protein) and *mucZ* (mucoid protein) [50].

2.3 Antibiotic Susceptibility and the Role of pH

For years, because of the obligate intracellular behavior of *C. burnetii* the antibiotic susceptibility trying out (AST) was arduous. Distinctive strategies had been used for the assessment of antibiotic activity as firstly observed in animal fashions than in embryonated egg models and the long run-in cell culture systems. At some stage in the early research inoculation in guinea pigs turned into implemented for susceptibility evaluation of streptomycin [51]. The potential of the examined antibiotic improved the survival duration in eggs infected with *C. Burnetii* was located throughout the embryonated-egg approach. To evaluate the ability of aureomycin, streptomycin, oxytetracycline and chloramphenicol against *C. Burnetii* this technique changed into carried out inside the older researches [52]. After that cell culture system was then performed and persist the reference technique for *C. burnetii* AST. In 1987, *C. burnetii* infected L929 fibroblast cells have been used by Yeaman et al. and differentiated the ratio of infected cells in antibiotic-treated cultures to that during drug-free controls [53].

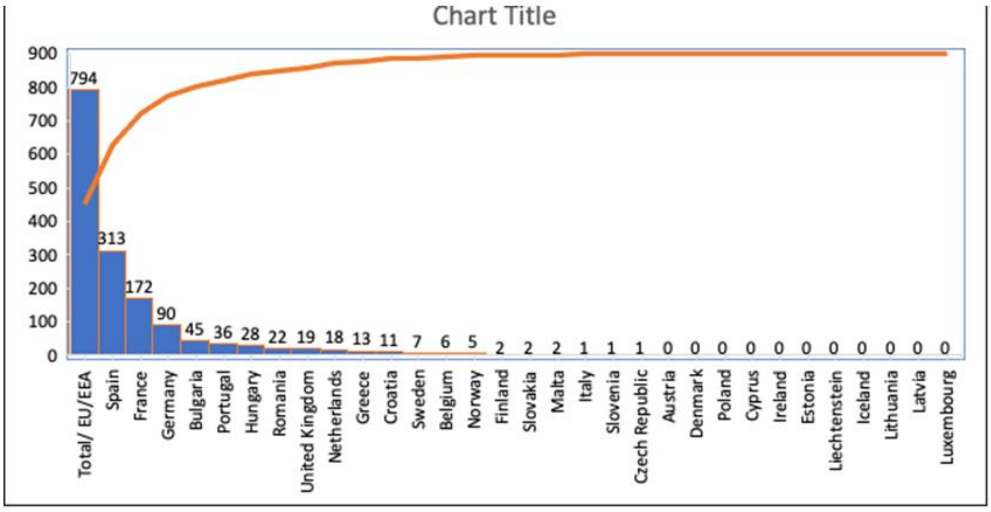

Fig. 1. Distribution of confirmed Q fever cases EU/EEA, 2014–2018

Table. 1. Main outbreaks of Q fever described since 1999

Country	Outbreak	Source	No. of cases	Diagnosis	Ref.
Italy	1987-1998	Ovine	235	?	[118]
France	1990-1995	Ovine	289	IF	[119]
Kenya	?	Goat	4	IF	[120]
Germany	1999	Ovine, dung	?	?	[121]
Netherland	?	Vacation in France	4	Serology	[122]
Newfoundland	1999	Goat	60	IF	[123]
French Guiana	1996-2000	Wild reservoir	132	IF	[124]
Australia	1998	Ovine	33	PCR	[125]
Israel	1999	?	16	ELISA	[126]
Japan	?	Travel in Australia	3	IF & PCR	[127]
France	1996	Ovine	29	IFI	[128]
Japan	?	Dogs & Cats	2	IF & PCR	[129]
Bosnia	1997	Ovine	26	Serology	[130]
Australia	2000	?	16	CFT[†]	[131]
France	2000	Ovine manure	5	IF	[132]
France	2000	Goat manure	10	IF	[132]
France	2002	Ovine	88	IF	[132]
Italy	2003	Ovine	133	IF	[133]

With the help of particular antibodies and the immunofluorescence assay, the more appropriate shell vial assay was developed for the detection of intracellular *C. burnetii* [54]. Brennan and Samuel in 2003 by using quantitative PCR (qPCR) developed a modified shell vial assay for the identification of *C. burnetii* intracellular computation. This approach was more sensitive and observable as compared to the indirect immunofluorescence assay (IFA) [55]. One more experiment confirmed its magnificent reliability for MIC confirmation [56]. In recent years, among the different eukaryotic cell lines, both the IFA and qPCR experiments targeting *com1* or *apoB* have been used for *C. burnetii* AST [57]. Currently, with the usage of particular immunofluorescent probes and flow cytometry, a new method developed. This approach with its particular morphological characteristics permits a very susceptible counting of *C. burnetii* cells [58].

2.4 Main Susceptibility Features

Many studies reported that doxycycline is the most potent drug against *C. burnetii* with MICs of 2mg/liter [58]. Although, doxycycline resistant strains have been reported and express a

Table. 2. Seroprevalence of animal Q-fever obtained in the main recent worldwide surveys

Country	Year	No. of Herds	No. of Animals	Seroprevalence of animals (% of herds	Diagnosis	Ref.
Goat						
Germany	1999	1	100	57	ELISA	[111]
Chad	2000	28	142	11	ELISA	[112]
Italy	2000	104	2155	47	ELISA	[113]
Sheep						
Germany	1999	1	100	57	ELISA	[111]
Chad	2000	28	142	43	ELISA	[112]
Italy	2000	675	7194	38	ELISA	[113]
Cattle						
Italy	1998	21	544	13	IF	[114]
Germany	1998	ND	21 191	8	ELISA	[111]
Turkey	1998	48	416	6	IF	[115]
Chad	2000	19	195	37	ELISA	[112]
Other						
Indonesia	1999	2	327 rats	0	IF	[116]
Chad	2000	14	142 camels	100	ELISA	[112]
Korea	2000	ND	116 pet cats	9	IF	[117]
Japan	2003	ND	316 pet cats	14	IF	[117]

Table. 3. Distribution of confirmed Q fever cases and rates per 100 000 population by country and year, EU/EEA, 2014–2018

Country	2014		2015		2016		2017		2018			
	Number	Rate	Number	Rate	Number	Rate	Number	Rate	Reported cases	Rate	ASR	Confirmed cases
Malta	0	0.0	0	0.0	0	0.0	0	0.0	2	0.4	0.4	2
Norway	1	0.0	1	0.0	2	0.0	4	0.1	5	0.1	0.1	5
Iceland	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0.0	0
Czech Republic	0	0.0	1	0.0	2	0.0	0	0.0	1	0.0	0.0	1
Liechtenstein	.	.	.	.	.	.	.	.	.	.	.	.
Slovenia	3	0.1	1	0.0	1	0.0	3	0.1	1	0.0	0.0	1
Bulgaria	15	0.2	15	0.2	17	0.2	28	0.4	47	0.6	0.6	45
Spain	77	-	97	-	330	0.7	379	0.8	418	0.7	0.7	313
Austria	.	.	.	.	.	.	.	.	.	.	.	.
Finland	0	0.0	3	0.1	2	0.0	4	0.1	2	0.0	0.0	2
United Kingdom	60	0.1	21	0.0	34	0.1	21	0.0	19	0.0	0.0	19

Country	2014		2015		2016		2017		2018			
	Number	Rate	Number	Rate	Number	Rate	Number	Rate	Reported cases	Rate	ASR	Confirmed cases
Croatia	21	0.5	14	0.3	8	0.2	23	0.6	16	0.3	0.3	11
Germany	238	0.3	310	0.4	270	0.3	107	0.1	93	0.1	0.1	90
Romania	21	0.1	3	0.0	32	0.2	46	0.2	22	0.1	0.1	22
Denmark	.	-	0	0.0	0	0.0	0	0.0	0	0.0	0.0	0
Italy	.	-	-	-	3	0.0	7	0.0	1	0.0	0.0	1
Sweden	2	0.0	4	0.0	3	0.0	1	0.0	7	0.1	0.1	7
Belgium	3	0.0	8	0.1	16	0.1	7	0.1	18	0.1	0.1	6
France	209	0.3	250	0.4	251	0.4	194	0.3	172	0.3	0.3	172
Poland	1	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0.0	0
Cyprus	1	0.1	4	0.5	2	0.2	3	0.4	1	0.0	0.0	0
Ireland	0	0.0	4	0.1	6	0.1	2	0.0	0	0.0	0.0	0
Slovakia	1	0.0	0	0.0	0	0.0	0	0.0	2	0.0	0.0	2
Estonia	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0.0	0
Portugal	25	0.2	20	0.2	17	0.2	48	0.5	36	0.3	0.3	36
Greece	15	0.1	10	0.1	9	0.1	4	0.0	13	0.1	0.1	13
Netherlands	26	0.2	20	0.1	14	0.1	22	0.1	18	0.1	0.1	18
Hungary	59	0.6	35	0.4	39	0.4	29	0.3	28	0.3	0.3	28
Lithuania	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0.0	0
Latvia	3	0.1	1	0.1	0	0.0	0	0.0	0	0.0	0.0	0
Luxembourg	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0	0.0	0
EU/EEA	781	0.2	823	0.2	1058	0.2	932	0.2	922	0.2	0.2	794

Table. 4. Comparison of *C. burnetii* genomes

Properties	*C. burnetii* Strain			
	Dugway	G	K	Nine Mile
Size of chromosome (bp)	2,158,758	2,008,870	2,063,100	1,995,281
Coding regions (%)	90.7	28.7	90.3	90.7
GC content (%)	42.4	42.6	42.7	42.7
Total ORFs	2,265	2,300	2,325	2,227
Known function	1,391	1,403	1,441	1,348
Unknown function	874	897	884	879
Pseudogenes	265	484	476	413
Transposases	32	40	59	31

C. burnetii strain Dugway, C. burnetii strain G, C. burnetii strain K, C. burnetii Nine Mile phase I [134]

Table. 5. Comparison of *C. burnetii* plasmids [134]

Properties	Plasmid		
	QpDG	QpRS	QpH1
Size of plasmid (bp)	54,179	39,280	37,393
Coding regions (%)	84.9	79.6	81
GC content (%)	39.8	39.7	39.3
Total ORFs	66	48	50
Known function	26	20	19
Unknown function	40	28	32
Pseudogenes	13 (7)	10 (6)	15 (10)
Transposases	1	0	0

harassing condition. Firstly, a patient died from *C. burnetii* endocarditis recognized with a resistant strain. By using the shell vial assay and qPCR the doxycycline minimum inhibitory concentration (MIC) of 8 mg/liter was reported [59]. Meanwhile, Rolain et al. during the same study observed an association between the percentage of serum concentration to MIC for doxycycline and the tendency of reduced anti-*C. burnetii* antibody titers in patients infected with *C. burnetii* endocarditis. For 16 *C. burnetii* strains isolated from cardiac valves extract from endocarditis patients, a percentage of serum concentration to MIC of 1 associated with a prompt reduction in particular antibody titers. The percentage between 0.5 and 1 was correlated with a steady minimization in antibody titers [61]. The entire genome of the *C. burnetii* strain infecting that patient (Cb109) was observed, but no particular sequence could be associated with doxycycline resistance [60]. Similarly, two other doxycycline resistant strains have been observed such as human isolate associated with acute Q fever and another from goat isolate [61].

During the early studies, for the elimination of *C. burnetii* from L929 cells fluoroquinolones were reported to be one of the most potential agents [62]. For that purpose, in 1989 to treat resistant isolates of C. *burnetii* infection it was suggested to incorporate doxycycline with fluoroquinolone drugs [63]. Fluoroquinolones due to their effective cerebrospinal fluid insertion are also suggested for the treatment of acute meningitis caused by *C. burnetii* [64]. Spyridaki et al. and Musso et al., have determined in vitro pefloxacin or ciprofloxacin-resistant strains of *C. Burnetii* with MICs as much as sixty-four mg/liter [65]. For the PCR- restriction fragment length polymorphism (PCR-RFLP) identifications of those resistant isolates, these authors detected factor mutations in the *gyrA* gene [66]. However, thus far, clinical strains of *C. burnetii* persist susceptible to moxifloxacin, levofloxacin and to a minimal with ciprofloxacin [67].

For the treatment of *C. burnetii* pneumonia, erythromycin was suggested as a sustainable approach. Meanwhile, Raoult et al. in 1991, observed resistance to this antibiotic in almost 6 out of 13 clinical isolates of *C. burnetii* [68] and in Cayenne, French Guiana such resistance was more recently recognized in 6 isolates [69]. Therefore, clarithromycin with MICs between 2 and 4 mg/liter was observed to be active [70]. A higher MICs up to 8 mg/liter has been reported with azithromycin [68]. Telithromycin with MICs between 0.5 and 2 mg/liter was observed active against *C. burnetii* for 13 clinical isolates [61]. But recently a resistant strain to this antibiotic was reported from French Guiana [58].

Thus far, no resistance to sulfamethoxazole-trimethoprim has been determined, thinking about that this antibiotic is powerful all through being pregnant. Anecdotal reported susceptibility to tigecycline and linezolid and cautioned them as alternative dealers [70]. Unsworth et al. recently described the susceptibility of *C. Burnetii* to antimicrobial peptides [71]. In vitro movement in opposition to *C. burnetii* changed into located with different forms of nonantibiotics. During in vitro interest, it changed into located that lovastatin and pentamidine can retard the *C. burnetii* growth [72]. For minimizing the dimensions of *C. burnetii* intracellular vacuoles omeprazole is taken into consideration as effective [73].

2.4.1 Role of pH in persistent infection

For decades, the antibiotic treatment of steady *C. burnetii* infection is difficult because no antibiotic has uncovered a bactericidal impact. In 1990, antibiotic activity inhibited the multiplication of *C. Burnetii* within the phagolysosome- like vacuole for the duration of the acidic environment. At some stage in the in vitro persistent cell infection

model, it became determined that acidification of the *C. burnetii* multiplication vacuole remained regular over time for 3 strains [74]. For that goal, Raoult et al. located a reconditioning of the bactericidal action of doxycycline after homogenization of alkalinizing retailers with doxycycline [75]. Chloroquine and amantadine had been used among alkalinizing marketers and healing bactericidal impact changed into accelerated with doxycycline. These results had been eventually validated clinically via differentiating the outcome of patients with *C. Burnetii* endocarditis medicated with the association of either doxycycline at the side of fluoroquinolone or doxycycline with hydroxychloroquine. A shorter period of treatment and much less periodic relapses were found in many of the patients who benefited from later combinations [76].

3. LABORATORY DIAGNOSIS TOOLS

General routine laboratory way of life strategies is not suitable for the growth of *C. Burnetii*, which may be a motive for the diagnosis-specific indirect diagnostic tools are used. In addition, for trying out, c. burnetii infection serology is still the most familiar technique. Presently, qPCR is used for the detection of *C. burnetii* DNA from one-of-a-kind medical samples. Before seroconversion in patients with primary infection, it has the gain of detecting *C. burnetii*. A biosafety degree 3 (BSL3) laboratory is recommended for culturing identical scientific samples. Finally, after immunohistochemistry staining, a pathological exam of infected tissue samples is a provocative mechanism for prognosis when these samples are on hand. In recent years improvements in the sensitivity of the principle, diagnostic techniques have been the top target.

3.1 Serological Methods

Q fever is difficult to diagnose due to an infectious disease. Clinical symptoms, serological outcomes and direct exposure with animals are the initial diagnosis approaches [77]. Serological methods such as IFA (Indirect immunofluorescence assay) and ELISA (Immunoenzymatic assay) are used for the detection of two forms of *C. burnetii*. The first form occupies smooth LPS detected the virulent phase also known as phase I. The second form occupies rough LPS also known as phase II [78]. Describing the normality of phase I and phase II antibodies permit determine the acute type from the chronic form of Q fever and is a forecast measuring the evolution of the acute type to the chronic type [79]. The necessity for clinical inspection, using, for example, 2-D echocardiography, and serological evaluation of recovering who expressed the acute form of the disease, for many years has been determined as vital in the phase of the probability to evolve the chronic form in these patients [80]. Currently, ECDC recognized this postulate as a standard method [81].

IFA and ELISA are commonly used to define the normality of antibodies against *C. burnetii*. Antigens of phase I and phase II are adapted in the IFA procedure, which is induced in murine epithelial placenta cells and fibroblasts of infected mice. This procedure permits acquiring large quantities of greatly specific antigens which can be used for the identification of IgG, IgM, and IgA antibodies [82]. Peter *et al.* [83] and Cowley *et al.* [84] expressed that ELISA and IFA can be used in routine serodiagnosis of Q fever, while ELISA is more strive and laborious and needed highly certified staff [82]. Antibody feedback to *C. burnetii* phase II antigen is prevalent and is greater than phase I antibody feedback during acute infection. An elevated phase I IgG normality was examined which is frequently excessive as compared to phase II IgG during the chronic infection.

Indirect immunofluorescence assay in association to *C. burnetii* antigen is the standard serological test for the detection of Q fever, which is carried out on paired serum samples to illustrate a significant (4- fold) increase in antibody normality. In many cases of Q fever observed that the first IgG IFA normality is usually low or negative and the second usually express a significant (4-fold) rise in IgG antibody levels. Antibodies concentration is usually considered positive for phase II above 1:64 for IgM and above 1:256 for IgG [85]. The serological diagnosis of humans for many years depends on 2 extremely specific tests: complement fixation test (CSF) and microagglutination test in phases I and II, which carried out simultaneously [86]. Different kinds of other methods have also been practiced in the diagnosis of Q fever such as radioimmunological assays, complement fixation tests, microagglutination tests and western-blotting [82].

3.2 Molecular Detection

For the identification of *C. Burnetii* in medical samples, various PCR-based total analyses have

been developed. The first perfect PCR systems targeted sequences of various varieties of plasmids [87], the 16s-23s RNA, the superoxide dismutase gene, the com1 gene or the IS*1111* repetitive factors in human or animal samples [88]. Such different techniques ranged detection limits from 10 to 10^2 bacteria. Nested PCR strategies had been additionally encouraged however these systems lack accuracy [89]. Real-time PCR or quantitative PCR (qPCR) has the benefit of comparing the volume of bacteria in clinical samples and is a much less enormous approach than PCR. As a result, for the analysis, this method has emerged as the maximum generally used than others. The qPCR technique focused on IS*1111* (a repetitive detail that is found in about 20 copies in the *C. burnetii* 9-mile genome) is the maximum sympathetic [90]. The modern-day qPCR technique inside the first 2 weeks of infection can stumble on the bacterium in the sera while the serological assay is not still high quality. Additionally, continual *C. burnetii* infection permits the identity of *C. burnetii* DNA in the blood of patients [91]. Schneeberger et al. within the Netherlands, found patients with signs of preliminary infection diagnosed with *C. burnetii* DNA in 10% of seronegative samples, verifying the effectiveness of this technique within the first weeks of infection [92]. One extra trial from the Netherlands at some point of the outbreak expressed sensitivity, specificity, PPV, and negative predictive value (NPV) of 92.2%, 98.9%, 99.2%, and 89.8%, respectively [92]. In that experiment, during the primary infection, a higher DNA load was correlated with advancement to persistent infection. Tilburg et al. used the DNA extraction technique among seven laboratories beyond the Netherlands and evaluated the interlaboratory compliance of IS*1111* qPCR according to the method [93]. They observed that different associations of DNA extraction kits and qPCR analysis performed similar outcomes for Q fever detection. In Switzerland, for the diagnosis of *C. burnetii* in clinical samples a qPCR approach concentrated on the *ompA* gene has been carried out for 7 years [94]. The sensitivity became 50% for urine samples, 69% for blood samples and 88% for valvular samples. Another qPCR technique focused on IS*30A* repetitive factors expressed a decreased sensitivity than IS*1111* qPCR [95].

Recently, multiplied the sensitivity of the qPCR test concentrated on the IS*1111* gene by concentrating DNA extracted from medical samples by way of lyophilization [96]. The diagnostic extent of *C. Burnetii* DNA changed into the 100-fold decrease in lyophilized sera (1 bacterium/ml) compared to nonlyophilized sera (102 microorganism/ml). Almost seventy-three patients acquired this approach which was normally infected with *C. burnetii* and 10 samples from endocarditis patients in whom the IS*1111* qPCR carried out under the normal conditions remained negative.

3.3 Culture

A huge variety of clinical samples can offer the possibility to isolate the *C. Burnetii*, along with old samples before cultivation which are stored at -80°C. The maximum normally used method is the shell vial technique [97]. On HEL cell monolayers in a shell vial, a pattern of one ml of the clinical specimen is inoculated. Centrifugation became achieved for shell vials (seven-hundred g at 20°C) for 1 h. Centrifugation permits more extension and perforation of *C. burnetii* inside cells. For 5-7 days inflamed cells are then incubated at 37°c in a 5% CO_2-enriched ecosystem. For the diagnosis of the bacterium internal cells, Gimenez or immunofluorescence staining turned into performed. Lockhart et al. making use of one-of-a-kind isolates, the Heinzerling and Arrandale lines in affiliation to differentiate four different cell lines for the isolation of *C. Burnetii* [98]. For the Arandale isolate, Vero cells expressed excessive sensitivity at the same time as, for the Heinzerling strain, DH82 cells have been the most sensitive. For the culturing of *C. burnetii* L929 and XTC, cell lines have been much less appropriate.

Recently, in axenic medium from clinical samples, the first isolation of *C. burnetii* become observed using ACCM2 [99]. An affected person sample of the coronary heart valve inflamed with *C. Burnetii* endocarditis become incubated in 20 ml of ACCM2 and after 6-8 days of incubation, the increase was examined. ACCM2 produces numerous colonies at day 5 after incubation of an ACCM2 agar plate with a sample of the culture-positive [99]. Such specific possibilities could substantially promote the regular cultivation of *C. Burnetii* from scientific samples.

3.4 Pathology and Immunohistochemistry

The immune reaction produced in different organs by *C. burnetii* can be observed by pathological examination of tissue samples fixation and paraffin embedding. After hepatic biopsy specimen's analysis, a typical fibrin-ring

granuloma with a “doughnut” feature can be detected during the primary infection [100]. In the bone marrow, these granulomas have also been identified. Pathological examination of vascular tissue and cardiac valves can also be descriptive during the persistent infection. Histological examination among the patients with *C. burnetii* endocarditis can disclose significant calcifications, fibrosis, slight inflammation and vascularization and less or no vegetation [101]. Such characteristics represent a passive “degenerative-like” infectious process. For the identification of *C. burnetii* in tissues, Immunohistochemical (IHC) detection is a more specific approach. An immunoperoxidase-based procedure along with a monoclonal antibody was used in this process. A procedure called “autoimmunochemistry” was developed by Lepidi et al. using antibodies from the patient’s serum. Samples from hepatic and valvular biopsy specimens and aortic grafts used the IHC procedure for the detection of *C. burnetii* [102].

3.5 Immuno-PCR

Immuno-PCR is a provocative process, incorporating the amplification potential of PCR with the precision and flexibility of ELISA, which permits enhancement insensitivity. This procedure has also been applied to different samples from infected individuals [103]. Immuno-PCR sensitivity is better as compared to ELISA and IFA (90% versus 35% and 25%, respectively) in sera assembled during the first two weeks after the emergence of symptoms [103]. Its specificity was assessed at 92%.

3.6 Skin Test

A skin check technique became suggested to look at the cellular feedback and to enhance the diagnosis of inflamed cows on the herd degree [104]. Intradermal injection of incredibly diluted inactivated vaccine (Coxevac, *CEVA-Sant´e Animale*, Libourne, France) became applied for a skin test. Antigenic reactions induce with such diluted vaccine. A nodule of irregular size will develop on the site of injection if the animal has previously been infected by using q fever. Rural practitioners can without difficulty observe this test.

3.7 Prevention

In some situations, q fever is an occupational ailment. This has been verified by the primary rationalization of the sickness, which takes vicinity in a populace of slaughterhouse employees. In 1930, additionally, a member of a laboratory organization cultivating *C. Burnetii* turned into infected [105]. The primary type of individuals which inflamed with such ailment includes people receives uncovered to animals and laboratory workers who cultivating the bacterium. Instances that interact scientific workforce include folks that participate in autopsies of patients with q fever [105] or an obstetrician who regulated parturient ladies with q fever [106]. Instances among military officials have additionally been found.

3.8 Vaccination

Since 1989, Australia has the facility of vaccination (Q-Vax; CSL Biotherapies, Parkville, Victoria, Australia) [107]. It is licensed in Australia with whole-cell formalin-inactivated vaccine. A randomized control trial was performed among the 200 slaughterhouse workers to determine its efficacy. Seven cases were reported in the control group during the 15 months of follow up and not no case was reported in the vaccinated group [108]. However, this vaccine can provoke local reactions and before vaccination for Q fever patients should be assessed with a cutaneous test (Q-Vax skin test) to avoid severe side effects. This vaccine is recommended by the Australian Veterinary Association (AVA) for all veterinarians, veterinary nurses and veterinary students. A recent survey performed in Australia confirmed the vaccination of 74% of veterinarians and 29% of veterinary nurses [107]. In 2002, also a nationally sponsor vaccination program was started in the country [108]. Program fidelity was 100% among slaughterhouse workers and 43% among farmers. After this massive campaign, Q fever cases decreased by 50%, and the number of hospitalizations also decreased [108]. During the epidemic in the southeast of the Netherlands in 2011, a vaccination movement started targeted people at risk for advancement to endocarditis and vascular infections [109]. To date, no other country has started a massive vaccination program among occupationally exposed persons. Awareness campaigns about the risk for *C. burnetii* infection in these areas will be helpful in the reduction of infection and early diagnosis of the infection.

3.9 Isolation

All manipulations must be performed in a biosafety level 3 (BSL3) laboratory with

appropriate personal protective equipment (PPE) concerning the prevention of transmission of contamination to some of the laboratory workers dealing with *C. burnetii* cultures. For health care workers to convey out autopsies on patients remarkable to have died from q fever, have to be used N95 respiration protection masks is supported. Similar guidelines should be carried out for obstetrical personnel who are in exposure with parturient girls detected with *C. burnetii* infection. Furthermore, contaminated surfaces with *C. burnetii* must be disinfecting with a dual quaternary ammonium-detergent compound [110]. Family bleach dilution is likewise an effective solution for disinfection.

3.10 Perspective and Future Challenges

A more precise evaluation of the risk factors in the progression to *C. burnetii* endocarditis will require specific studies. The consideration of individual susceptibility factors will permit stronger directions through active treatment to prevent progression to this still-severe disease. By multiplex PCR techniques the systematic diagnosis of *C. burnetii* in syndromes such as hepatitis, fever, endocarditis and pneumonia during pregnancy should permit identification and treatment of more cases in the world. Finally, for endocarditis and vascular infections unique therapeutic techniques with routes and better sensitivity should be developed. New techniques such as unique antibiotic combinations, in vitro observation and randomized experiments, should be compared with the therapeutics methods that have been used for the last 20 years are compulsory and will be a major tool of future research.

4. CONCLUSION

The current review explained the molecular mechanism and characteristics of *C. burnetii* pathogenesis. Current approaches used for the diagnosis of disease, treatment and vaccine strategies were also described. Since the 1930s Q fever has been presented but not described as an emerging zoonosis. By the advancement of diagnostic approaches such as PCR and serological assays allow exact identification of the infected group or increased surveillance of professionals with flu-like symptoms or unexplained fever could be helped in the re-emergence of Q fever. To date still, Q fever remains poorly understood. Lack of awareness among the public is the one of major concerns related to the underdiagnosing and distribution of Q fever cases. The prevalence of Q fever can have a significant economic impact on animal trade, reproduction and the commercialization of different animal products. To prevent from burden of Q fever prevalence there should be introduced some novel diagnostic techniques and to call a massive campaign about the awareness of such disease.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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