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Spread of Q fever in Bulgaria and risk assessment for animal health and public health in 2023

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Summary

Q fever, first described among slaughterhouse staff in Australia in 1937, is now recognized as an endemic disease throughout the world, except New Zealand. Since this first description, knowledge of this pathogen and its associated infections has increased dramatically. From the public health point of view and economic consequences, Q fever is a zoonotic disease of high public importance, the etiological agent of which, *Coxiella burnetii*, is included in group B of the list of potential biological weapons of the World Organization for Animal Health (WOAH, founded as OIE).

The 14 positive cases of Q fever in humans in the Gabrovo Region (including 4 veterinarians and staff working on the farms) and the positive herds found in three cow farms and one sheep farm also raised concern. For this reason, it is important to recall the importance of this disease and the risks it poses to human and animal health, as well as to outline the necessary measures for surveillance and prevention of new cases. In relation with the detected positive cases of Q fever in humans (among them, farm owners and staff, veterinarians) and positive herds of large and small ruminants in the last five years in a number of regions of Bulgaria, we tracked and analyzed the spread of *Coxiella burnetii* and the possibilities for the control and eradication of the Q fever disease, as well as with the aim of establishing the trends in the prevalence of the disease and especially the relationship between the morbidity in humans and animals.

Key words : Q fever, spread, public health

Introduction

General information about the disease

Causative agent. The causative agent of Q fever, *C. burnetii*, is a strict, intracellular, pleomorphic, Gram-negative coccobacillus with an incubation period of 9-40 days. The average incubation period is 20 days (range 18-21 days). Originally classified as a species of the genus *Rickettsia* due to its obligate intracellular growth requirements, *C. burnetii* is now recognized as a bacterium in the gamma group of *Proteobacteria*. The whole genome sequencing identifies significant homology to *Legionella pneumophila*, also a member of this taxonomic group.

C. burnetii has 2 morphological variants:

- large cell variant that proliferates in the host's body in the host's monocyte and macrophage and
- small cell variant (0.2 by 0.7 microns) – a small, thick and highly resistant spore-like form that survives well in the environment due to its resistance to heat and desiccation, pressure and chemical agents. For example, pasteurizing milk at 72°C for 20 seconds does not destroy it. Disinfectants such as 5% formalin and 5% chlorinated lime destroy *C. Brunetti*, but the bases do not.

Like other Gram-negative bacteria, *C. burnetii* possesses lipopolysaccharide as a virulence factor, which is also responsible for antigenic phase variation, an important property that was first used for serological diagnosis by Bengtson in 1941. The infection has 2 phases that are analogous to the lipopolysaccharide rough and smooth phases of *Enterobacteriaceae* organisms. Phase variation is mainly associated with mutational variation in lipopolysaccharide (LPS). Phase I is the natural phase found in infected animals, arthropods, or humans. It is highly infectious and corresponds to a smooth LPS that helps protect the microorganism from the host's defense mechanisms. In contrast, phase II is not highly infectious and occurs only in laboratories after serial passages on cell cultures or in avian embryos. Corresponds to coarse LPS (Maurin M et al., 1999).

Epidemiology. Q fever is a natural outbreak disease that belongs to the group of zooanthroponosis and can affect mammals, birds, reptiles, arthropods and humans. Cattle, sheep and goats are the main reservoirs of *C. burnetii*. Infection has been found in many species of other domestic animals, including dogs, cats, rabbits, horses, pigs, camels, buffalo, rodents, and some birds, which can transmit the infection to humans without showing signs of illness. More than 30 different animal species susceptible to Q Fever have been reported in Europe. Human infection is directly related to animal morbidity. Ruminants (mainly cattle, sheep and goats) are recognized as the main source of infection for humans (Bony et al., 1998, Ebany, 2020, Kelly et al., 2019, Lyoo, 2017, Malik et al., 2022). The epidemic process is characterized by the sporadic spread of the disease, but also by the occurrence of intense epidemics.

Ticks are one of the main reservoirs of *C. burnetii* in nature and have an important role in maintaining outbreaks in farm animals. *C. burnetii* carriage has been demonstrated in 62 species of ticks from the Ixodidae *family* (Hard ticks), *Argasidae* (Soft ticks) and subclass *Gamasida*. Among the most common carriers of *C. burnetii* are *Rh. sanguineus, H. plumbeum, Rh. turanicum, I. ricinus, H. marginatum, H. punctata* and others. Ixodes ticks have been found to carry the causative agent for life.

There are **two types** of Q fever outbreaks: natural and human-caused (agricultural):

Natural outbreaks: The animal reservoir in nature is extensive – more than 60 species of mammals are susceptible. The causative agent is found in **wild animals** that play the role of a reservoir of the infection – rabbits, foxes, badgers, ground squirrels and many types of birds and ticks. In them, the disease occurs mainly as a hidden infection. Birds are involved in the spread of Coxiella over long distances, outside of natural outbreaks. Ticks contaminate the external environment with Coxiella hay, straw, boards, etc., which play the role of infection transmission factor of important epidemiological significance.

Outbreaks in productive and domestic animals (agricultural outbreaks, anthropurgic): Q fever is also found among farm and domestic animals. *C. burnetii* are excreted with the placenta, amniotic fluid, urine, feces, secretions from the nose and mouth. Ticks are also involved in the transmission mechanism of the infection. These outbreaks have a greater epidemiological significance for humans than natural ones. Reservoir of the infection in these outbreaks are various types of animals: from productive ones - small and large ruminants, ungulates and birds, and from domestic ones - cats, dogs, birds (Bony et al., 1998, Ebany, 2020, Kelly et al., 2019, Lyoo, 2017, Malik et al., 2022). **In Bulgaria, small and large ruminants are of the greatest epidemiological importance as sources of infection from the productive animals.**

Transmission. *Aerosol pathway* – air-droplet and air-dust pathway, which is of primary importance. *C. burnetii* survives very well in the environment by becoming a small, compact, spore-like form resistant to heat and desiccation, and remains viable even for years. It is so highly contagious that just one inhaled microorganism can cause clinical disease in an animal or human. Contagion occurs by inhalation of contaminated with Coxiella dust and droplet aerosol from pens, stables, contaminated straw, fodder, wool (when shearing animals, during processing), skins, from

amniotic fluid during the birth of sick animals, etc. **The bacterium can also be spread by wind (over short distances), thus appearing in patients with no apparent animal link.**

• *Alimentary route* of infection - considered less common, probably a minor factor in the transmission of *C. Brunetti* and now even controversial, but there are such reports in the scientific literature. It occurs when consuming contaminated products obtained from sick animals (especially without heat treatment) - milk, butter, meat, etc.

• *Integumentary mechanism* – by penetration of the Coxiella through healthy or injured skin and mucous membranes.

• *Transmission mechanism* – via tick bite, least relevant for human infection and arthropod transmission to humans is unlikely.

• *Vertical and sexual transmission* - such routes of infection are discussed in the scientific literature.

Human-to-human transmission is probably extremely rare (sporadic cases of transplacental transmission resulting in congenital infections, transmission during autopsies, via intradermal inoculation or by blood transfusion).

Transmission of the infection can occur horizontally from animal to animal and without the mediation of ticks. Carriage in animals is of great importance for maintaining the outbreaks during certain intervals of time. Under the influence of certain factors, the infection worsens and the infected animals begin to release the infectious agent, the period of birth being especially dangerous. *C. burnetii* is released in large quantities and for a long time in the environment mainly during birth or abortion with the amniotic fluid, placenta, uterine secretions, but it can be released from animals through the vaginal mucus, milk, feces, urine and semen (sheep emit the bacterium mostly in faeces and vaginal mucus, while goats and cattle emit mostly in milk) (Maurin et al., 1999).

Clinical manifestation in animals. The incubation period is usually 2-3 weeks, but varies from 4 days to 6 weeks. Animals may develop acute mastitis, loss of appetite and depression, nasal and eye discharge, difficulty breathing, abdominal atony. In ruminants, *C. burnetii* infection mainly results in reproductive disorders such as abortions, stillbirth, birth of weak calves, metritis and infertility, with associated negative economic impact on farms (Simeonov, 2020).

Nowadays, medical measures consist of the use of antibiotics and/or vaccines to control the spread of the bacterium in farm animals. Currently, in infected cattle herds, Q fever control measures consist of environmental measures such as - destruction of placentas or disinfection of places of birth, parenteral antibiotic treatment with oxytetracycline during the last month of pregnancy and vaccination. According to Rodolakis., vaccination will be an effective means of disease control (Rodolakis, 2006).

Antibiotics (eg, *tetracyclines*) reduce the incidence of abortions, but do not prevent *C. burnetii* shedding from animals.

Two *C. burnetii* vaccines are currently available on the world market (Coxevac, phase I, CEVA Santé Animale and Chlamyvax FQ, Phase II, MERIAL). Current scientific studies suggest that the efficacy of Q fever vaccines in controlling infection varies mainly depending on their composition, particularly the phase of *C. burnetii* (phase I, virulent phase with full lipopolysaccharide (LPS), or phase II, avirulent phase with incomplete LPS). As a general rule, vaccines prepared with phase I are recognized as more effective and protective than those prepared with phase II. Thus, to protect at-risk groups of people such as slaughterhouse workers, veterinarians or farmers, a phase I vaccine is currently administered in Australia.

Q fever in humans. Humans are mainly infected by inhalation of aerosols or dust contaminated with extracellular forms of *C. burnetii*, and the same applies to non-immune animals, especially when environmental conditions are favorable for the spread of bacteria.

Therefore, humans can become infected through contaminated milk or when they come into contact with the fetus, placenta, or amniotic fluid during the animal's birth. The bacteria can survive in dry dust for months, therefore Q fever infection can also occur through contact with contaminated agricultural products such as wool, hair/bristle, straw or hay.

The incubation period varies from 2 to 6 weeks (average 20 days). Although Q fever in humans is asymptomatic in more than 60% of the cases, in some cases it can manifest either as an acute or as a chronic disease. Acute illness occurs primarily as a flu-like syndrome with fever, headache, myalgia, vomiting, and diarrhea. Severe complications such as pneumonia or hepatitis may occur. Cardiovascular and neurologic manifestations occur in approximately 1% of the patients and include pericarditis, myocarditis, acute endocarditis, and meningoencephalitis. A very small percentage of people (less than 5 in 100), who become infected with *C. burnetii*, develop a more serious infection called chronic Q fever, which develops months or years after the initial *C. burnetii* infection. In the chronic course, the most common manifestation is endocarditis, especially in patients with previous valvulopathy and *C. burnetii* is often involved in chronic fatigue syndrome. Pregnant women are particularly susceptible to *C. burnetii infections*, which can lead to premature birth or abortions. Deaths in humans are rare less than 2%. After recovery, the body builds permanent immunity (Kerry, 2023).

Significance of Q fever for certain professional groups. Q fever is a zoonosis defined as an occupational disease of workers in slaughterhouses and meat processing, in the leather and fur industry, personnel in livestock facilities, farmers, veterinarians and laboratory personnel.

In 2020, an up-to-date study was carried out in Germany on the importance of Q fever for certain professional groups. The study aims to obtain reliable data on seroprevalence in different occupational groups in regular contact with products/materials excreted from the animals, using an assay with proven excellent sensitivity and specificity for the detection of past infections. The second objective is to obtain primary data on obstetricians and physicians, representatives of another professional group potentially at risk of Q fever, for which there is little data. Midwives can become infected during the delivery of Q fever infected women (Groten et al., 2020).

A cross-sectional study was carried out covering 77 shepherds, 14 animal breeders (cattle farm owners), 74 veterinarians, 17 employees in administration and 68 obstetricians from Thuringia, Germany. The control group included 92 blood donors.

The results of the study show that out of 250 blood samples analyzed, the highest seroprevalence (64% - 77%) was observed in persons having frequent contact with animals. There are no significant differences between shepherds, farmers and veterinarians.

Seroprevalence in administrative workers was lower but still significantly greater than the control group. No positive results were found for midwives and doctors.

Conclusions are that shepherds, livestock breeders and veterinarians are at high risk of *C. burnetii infection*. The study clearly demonstrates that there is no increased risk for the people, working in the human maternity wards. The high hygiene standards already in place in maternity wards are sufficient to control Q fever as an occupational hazard for humane doctors (Groten et al., 2020).

Spread of Q fever in the European Union (EU), according to data from the European Food Safety Authority

1. In animals

Commission Implementing Regulation (EU) 2018/1882 list Q fever as a category E disease, which means that, in accordance with Regulation (EU) 2020/2002, Member States report to the EU-ADIS system by 30 April each year data for the previous calendar year, for the detection of the disease in their territory in *Bison* ssp., *Bos* ssp., *Bubalus* ssp., *Ovis* ssp. and *Capra* ssp. Member States also provide EFSA with data from annual surveillance and monitoring activities in accordance with Directive 2003/99/EC. Q fever falls within the scope of Annex I, Part B. 4. "Other zoonoses and infectious agents causing zoonoses" of this Directive, as an agent to be monitored if the epidemiological situation in a Member State so requires.

Large and small ruminants suspected of being infected with *C. burnetii*, are sampled during clinical trials and passive surveillance (blood samples, samples from fetuses and stillborn animals, placenta, vaginal swabs and bulk milk screening). The main animal species investigated are sheep, goats and cattle.

- A total of 17 member states (15 in 2020 y) and five non- EU countries (six in 2020) have reported data for 2021 d. for *C. burnetii*.
- The proportion of positive animals in virological tests was 5.9% in sheep (8.7% in 2020 year), 16.5% in goats (11.3% in 2020) and 5.2% in cattle (3.8% in 2020 d.).
- The share of positive herds in virological tests was 4.1% in sheep (1.4% in 2020 y.), 2.0% in goats (1.2% in 2020) and 4.8% in cattle (6.7% in 2020).
- The share of seropositive animals is 10.3% in sheep (11.4% in 2020 year), 24.6% in goats (25.0% in 2020) and 12.2% in cattle (9.6% in 2020 d.).
- The share of seropositive herds is 18.9% in sheep (5.9% in 2020), 50.0% in goats (78.7% in 2020 y.) and 15.1% for cattle (14.4% in 2020) (EFSA Journal, 2022).

Results were reported from various other domestic and wild animal species and only Italy reported positive results, mainly from dogs (73.2% of 541) and water buffalo (4.7% of 43) (Bony et al., 1998, Ebany, 2020, Kelly et al., 2019, Lyoo, 2017, Malik et al., 2022). EFSA recommends that monitoring be extended to include species other than domestic ruminants, such as pets, which may be reservoirs. In 2021 Italy reported a high number (394) and proportion of positive dogs (76.8%) compared to published data for 2020, showing 8.2% positive dogs. Pets are now considered to be reservoirs for *C. burnetii* in Australia, Asia and Africa and can be taken as sentinel (indicator) for humans and farm animals (Bony et al. 1998, Kelly et al. 2019, Lyoo, 2017, Toze et al. 2014). In the coming years, it is critical to collect more data on ongoing environmental contamination (animal waste, dust) to better assess risk factors (Carrie et al, 2019) in order to reduce human exposure to this zoonosis.

2. In humans

In Europe, the majority of clinical cases are sporadic. However, a few outbreaks in humans have been reported. Until 2016, France and Germany have reported the majority of confirmed cases. In 2017, two outbreaks were reported from Bulgaria in Gabrovo and Blagoevgrad regions (Genova et al. 2019). Since 2017, Spain has reported the highest number of cases per year. In 2021, the number of human Q fever cases in the EU is the lowest recorded in the last 5 years. Compared to 2020, there has been a decrease in reported cases from Bulgaria and Spain. Spain accounts for about a third of the total number of cases, while Germany has reported an increase in Q fever in humans. A few cases of Q fever reported in Germany and Spain were associated with occupational (waste treatment workstations or laboratory personnel) or recreational activities, and exposure was from animal and environmental contamination. A significant downward trend has been observed in the last 5 years (2017 – 2021). In 2021, the number of deaths decreased (1.48%) compared to 2020 (2.13%).

In 2021, the number of confirmed **cases of Q fever was 460**, which corresponds to a notification rate in the EU of 0.11 per 100,000 populations. This is a decrease of 12.0% compared to 2020 (0.12 per 100,000 population). **Bulgaria has the highest notification rate - 0.45 cases per 100,000 people**, followed by Hungary and Spain - 0.42 and 0.31 cases per 100,000 people, respectively. Q fever cases occurred from April to September in accordance with the spring/summer seasonal pattern. Occurrence is the highest for the 50-55 age group. Regional differences across Europe highlight the importance of understanding risk factors that may operate at a local level and may be difficult to detect (Georgiev et al. 2013).

Spread of Q fever in Bulgaria in the period 2017-2021.

1. In humans

The first case in the country was described in 1949 by A. Mitov in Plovdiv region **(Mitov, 1959)**. Q fever is an endemic zooanthroponosis for Bulgaria. For more than 50 years, numerous sporadic cases, small and large epidemics have been observed in the country, covering from several tens to hundreds of sick persons. In Bulgaria, the disease has no clear seasonality. In agricultural areas, an increase in morbidity is observed in the spring, around and after the lambing season and in the fall. Serological studies among at-risk groups in Bulgaria (with atypical pneumonia and cardiovascular diseases) show a prevalence of 15 to 18% for *C. burnetii*. The incidence (per 100,000 populations in Bulgaria) of Q fever in the periods 1961 – 1980, 1981 – 2000 and 2001 – 2015 was respectively from 0 to 1.5%, from 0.01 to 5, 64% for the second period and between 0.16% to 3.40% for the latter years. During the period 2014 - 2018, an increasing trend was observed in Bulgaria in the number of confirmed cases of Q fever in humans. In 2014 and 2015, their number was 15, for 2016 - 17. For the same period, for neighboring Romania, the number of confirmed cases of Q fever in humans is variable: for 2014 - 21, for 2015 -3, for 2016 - 32, for 2017 - 46 and for 2018 year - 22. In Greece, their number remains almost at the same level with small fluctuations as follows: for 2014 - 15, for 2015 -10, for 2016 - 9, for 2017 - 4 and for 2018 - 13.

Since 2017, sporadic epidemic outbreaks of a local nature have been observed in Bulgaria in different parts of the country. In 2017, the Q fever infection appeared again on the "epidemic scene" of Bulgaria with a self-limited epidemic outbreak in the region of the village of Tserovo, municipality of Blagoevgrad affecting 32 people, in 6 of whom the infection was confirmed serologically and with molecular biology testing in National Reference Laboratory (NRL) " Rickettsia and Cell Cultures", National Center for Infectious and Parasitic Diseases (NCIPD). At the same time in National Diagnostic Research Veterinary Medical Institute (NDRVMI) Coxiellosis infection was proven among goats and sheep from the same village.

In 2017, in NRL “Ricketsia and cell cultures” NCIPD, 6 positives for *C. burnetii* serum samples were diagnosed from a total of 29 received for testing in the laboratory. Serum samples were from three villages in the Gabrovo region (4 positive serum samples from the village of Velkovtsi and 2 samples from the village of Zdravkovets).

In 2020, in the Gabrovo region, an epidemic outbreak was registered among livestock breeders, agricultural workers and veterinary specialists, and 47 cases were reported (the villages of Kramolin, Lovni dol and Sabotkovtsi). In the considered 5-year period 264 cases of sick people were registered in 22 districts of the country, constantly in about 10 districts (Map 1). Cases are mostly sporadic. 92% of all 264 cases were laboratory confirmed, and the rest were determined as probable due to the presence of an epidemiological link. The patients are of both sexes, with male sex being predominantly affected (165 cases). Incidence in the period 2017–2021 was between 0.42 and 1.54 per 100,000 populations, no deaths were recorded (Table 1 and Figure 1).

Table 1. Registered cases of people suffering from Q fever in Bulgaria in the period 2017 – 2021 *(according to data from the National Center for Infectious and Parasitic Diseases)*[i]

Year	Number of human cases	Number of areas affected	Incidence per 100,000 population	Number of deaths	Mortality per 100,000 population
2017	**30**	**10**	**0.42**	**0**	**0**
2018	**47**	**15**	**0.67**	**0**	**0**
2019	**44**	**13**	**0.63**	**0**	**0**
2020	**107**	**16**	**1.54**	**0**	**0**
2021	**36**	**11**	**0.52**	**0**	**0**

Total	**264**	**permanently in about 10 districts**	**between 0.42 and 1.54**

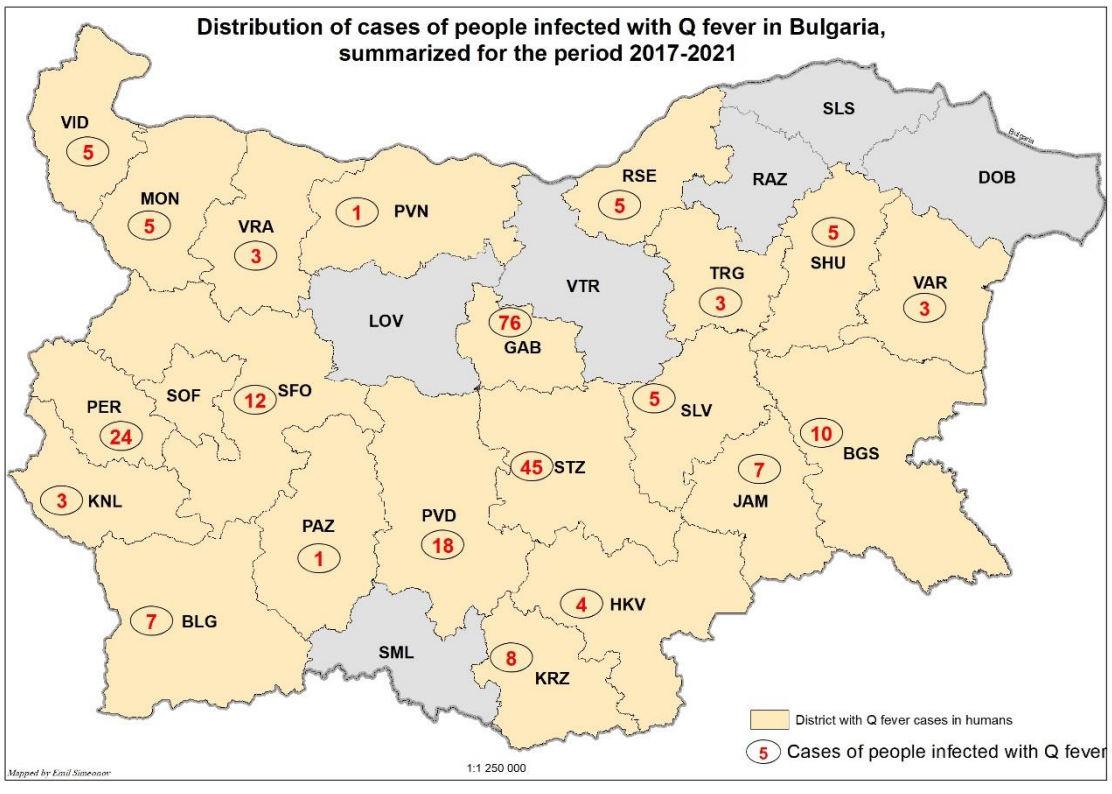

Figure 1. Distribution of cases of people infected with Q fever in Bulgaria, summarized for the period 2017-2021 *(according to data from the National Center for Infectious and Parasitic Diseases; map and charts by Emil Simeonov, RACFC)*

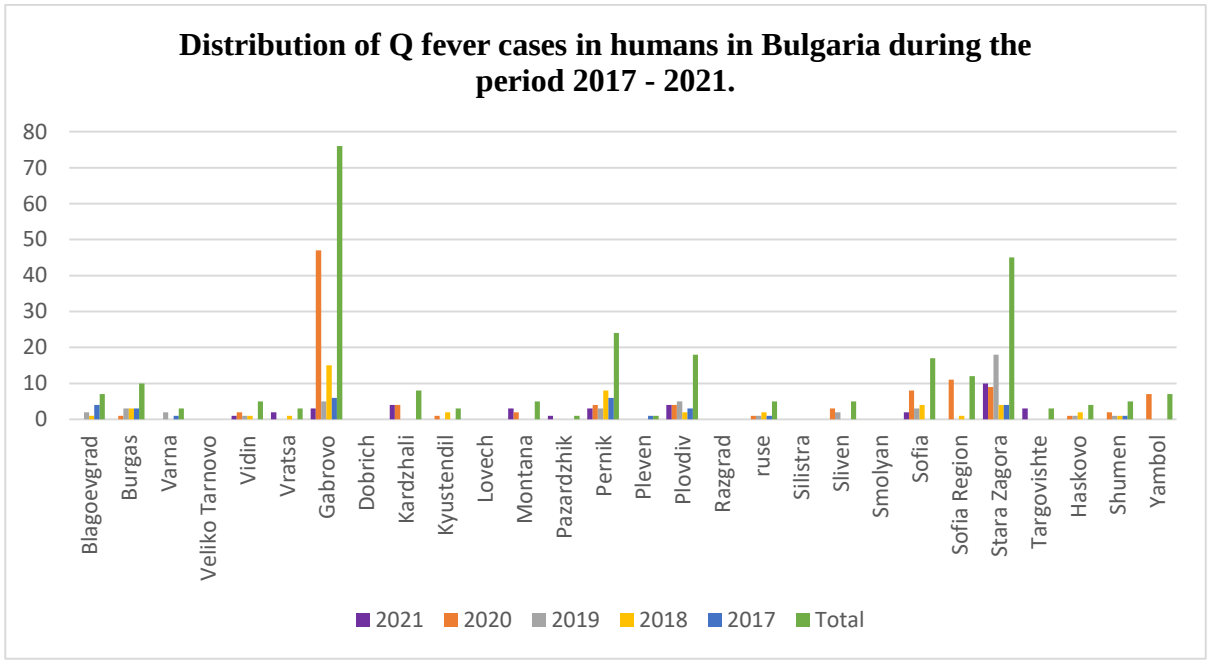

Figure 2: Q fever in humans (total) and distribution by region in Bulgaria for the period 2017 – 2021 *(chart: Emil Simeonov, RACFC)*

In Bulgaria, the disease **in humans has no clear seasonality**. In agricultural areas, an increase in morbidity is observed in the spring, which is most likely related to the conduct of the lambing campaigns in December - February. The seasonal distribution for the considered period is in the spring and summer (March - July), and the age-related involvement is in persons over 25 years of age.

The disease in humans in Bulgaria is subject to mandatory registration at the national level, according to the current Ordinance No. 21 of 2005 on the order of registration, reporting and reporting of infectious diseases (promulgated SG No. 62/2005) and subject to mandatory notification at EU level, with cases being reported through the European Surveillance System TESSy.

2. In animals

Historically in Bulgaria, surveillance of the disease through exploratory serological surveys has been carried out in the country since 1950, and in the different periods until 2004, the seroprevalence in general for the ruminant population varied from 5% to over 18%.

The Bulgarian Food Safety Agency has developed and implemented **a Program for Surveillance and Control of Q Fever Disease since 2016.** It includes an exploratory laboratory test for the presence of antibodies against *C. burnetii* in blood samples from large and small ruminants from disease-endemic and newly affected regions of the country.

The program envisages a serological examination of ruminants when cases of the disease are detected in humans, an annual two-time serological examination of all male breeding bulls bred in the regional directorates for selection and reproduction of the Executive Agency for Selection and Reproduction of Animals (EASRA). Also providing a laboratory examination of blood samples from animals originating from herds in which an increased rate of abortions was found when the etiological involvement of *C. burnetii* was established by PCR test. (BABH)

Vaccination with registered vaccines available on the market can be used to prevent the disease. Animal vaccination is not part of the National Program for the Prevention, Supervision, Control and Eradication of Animal Diseases, including Zoonoses for the period 2019-2021, and it is not planned to be financed from the budget.

Due to the outbreak of Q fever in humans and animals in our country in 2020, changes have been made to the new National Program for Prevention, Supervision, Control and Eradication of Animal Diseases, including Zoonoses in the Republic of Bulgaria 2022-2024. The

measures for the supervision and control of the Q fever disease are carried out according to **the Program for the supervision and control of the Q fever in the Republic of Bulgaria in 2022 - 2024.** (Annex 10 to the National Program). The program is implemented in all 28 regions of Bulgaria.

Program measures include:

1. annual laboratory testing for the presence of *C. burnetii* in blood samples and/or pooled milk samples from large and small ruminants (sheep and goats) throughout the country;
2. serological and etiological testing of ruminants when detecting cases of the disease in humans. Samples are taken according to instructions, SOP or others approved by the executive director of the BFSA;
3. annual examination of seminal fluid with polymerase chain reaction (PCR) of all male breeding bulls bred in the regional directorates for selection and reproduction at the EASRA;
4. mandatory laboratory examination of placenta and/or aborted fetuses originating from ruminant herds in which an increased percentage of abortions is found;
5. laboratory examination of blood samples from animals originating from herds in which an increased percentage of abortions was found, in which the etiological involvement of *C. burnetii* was established by means of PCR;
6. Vaccination of all susceptible species of animals reared in all livestock facilities located in endemic areas, following the directions and recommendations for application specified by the vaccine manufacturer for at least 3 years.

The number of positive samples (serological) for *C. burnetii* from the tested species of animals - cattle, sheep and goats, in clinical studies and official supervision in Bulgaria for the period 2017 - 2021, are presented in Table 2, on **Figure 3** (summarized for 5 years) and **on Figure 4** (according to data from the National Diagnostic Research Veterinary Medical Institute (NDNIVMI).

Of the buffalo samples tested, no positive for *C. burnetii* were found.

In Annex 2, detailed maps and charts are presented, separately by year and by region of Q fever in animals in Bulgaria.

Table 2: Number of positive (serological tests) animals - cattle, sheep and goats (in total) - in Bulgaria for the period 2017 - 2021 according to data from NDNIVMI

Year	Number of pos. cattle	Number of pos. sheep	Number of pos. goats	Total number of pos. animals for the year	Prevalence
2017	52	19	39	110	28.09%
2018	54	57	8	119	13.28%
2019	27	25	2	54	9.72%
2020	79	76	74	229	8.37% (until 18.05.)
2021	35	12	65	112	–
TOTAL for a 5 year period	247	189	188	624	

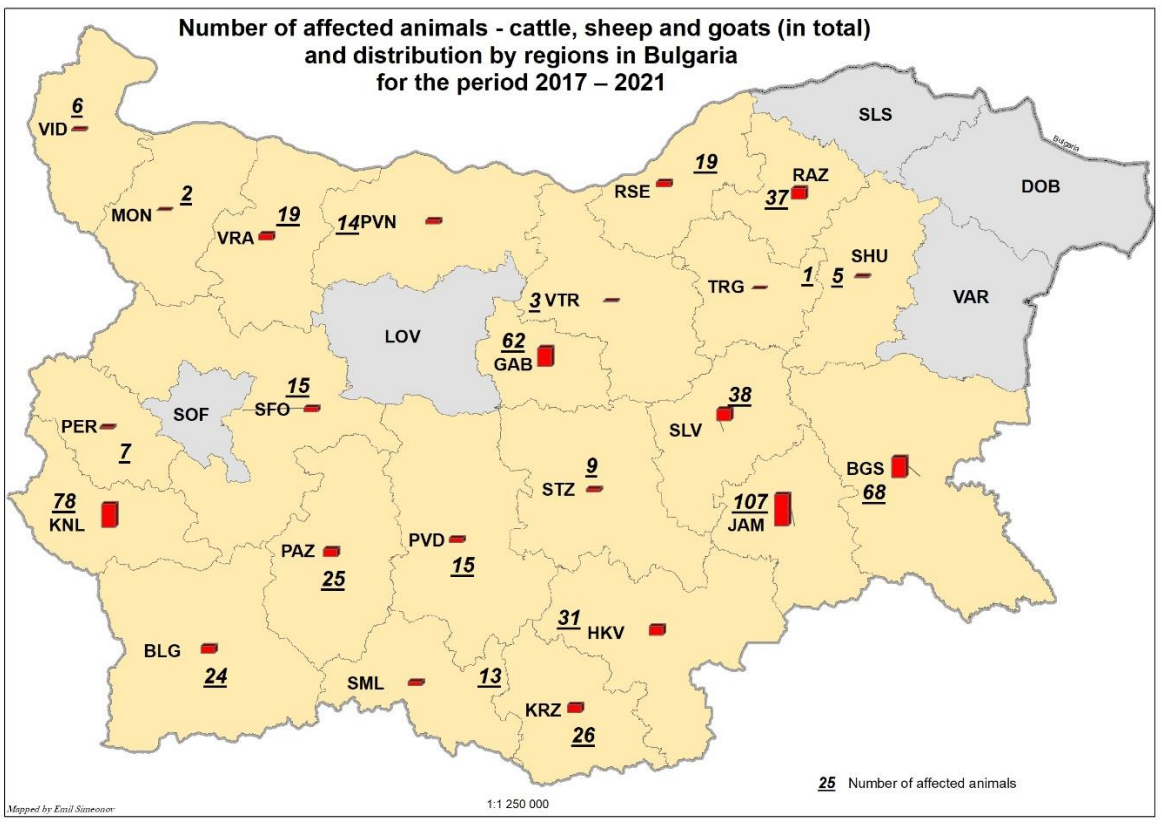

Figure 3. Number of affected animals - cattle, sheep and goats (in total) and distribution by regions in Bulgaria for the period 2017 – 2021 *(map: Emil Simeonov, RACFC); in the gray areas no positive animals were found;*

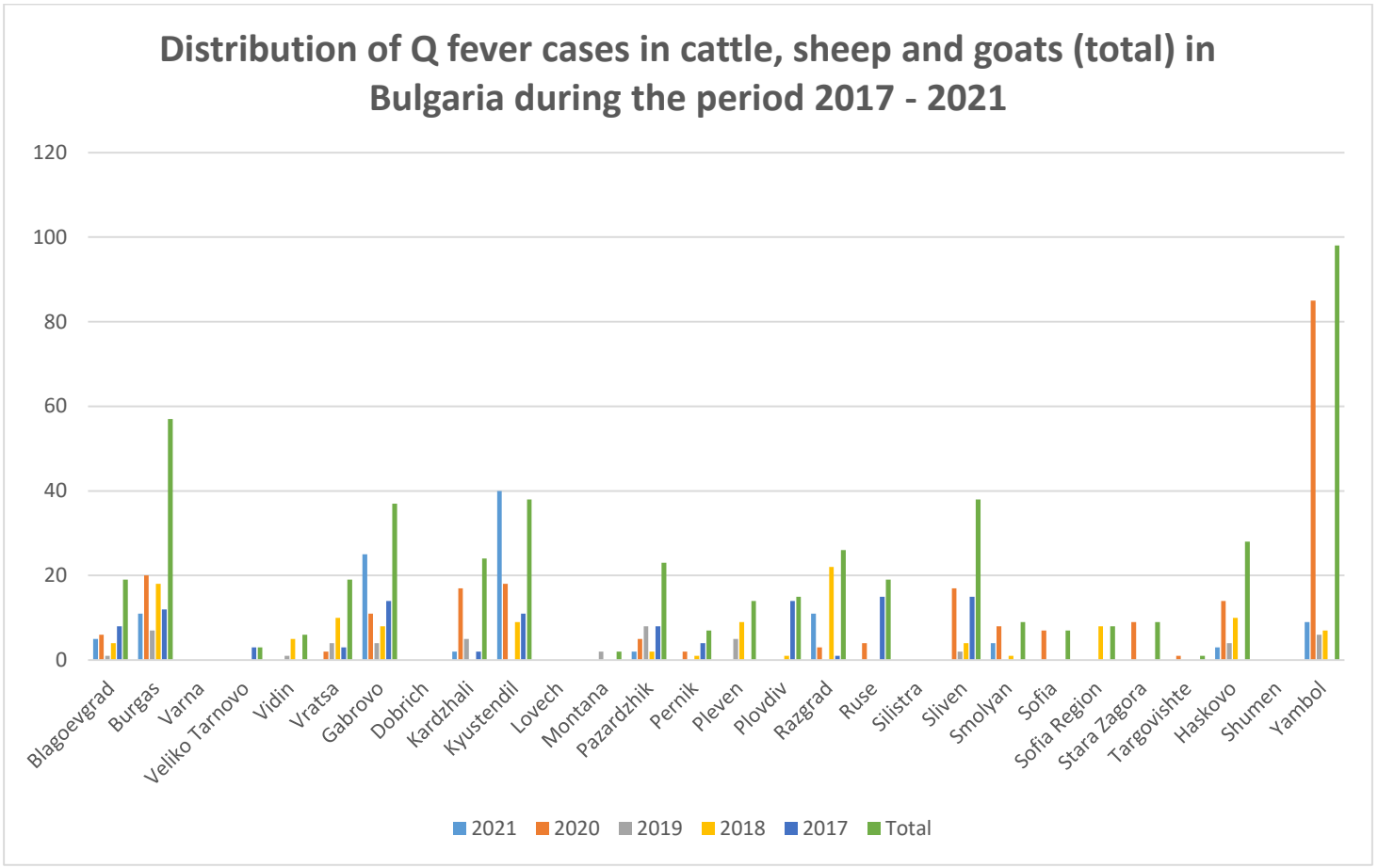

Figure 4.: Number of affected animals - cattle, sheep and goats (in total) and distribution by regions in Bulgaria for the period 2017 – 2021
(chart: Emil Simeonov, RACFC)

As is visible from the table and the attached map and chart, **in 2020, an epidemic outbreak of Q fever among animals** was reported - **229 (cattle, sheep and goats)**, with the largest number of cases **in Gabrovo region**. At that time**, the largest number of human cases** were observed in Gabrovo region too - 47 cases in animal breeders, agricultural workers and veterinary specialists.

Joint activity between the veterinary authorities and public health authorities *(according to data from the Bulgarian Veterinary Union,* Letter with Ex. No. 026/09.05.2023 d)

The Bulgarian Veterinary Union (BVU) has made it its cause to change the attitude of the health authorities towards the disease Q fever. On 10.10.2020, at its National Council, the Bulgarian Veterinary Union, which also includes veterinarians practicing with productive animals, decided that 10 doctors per region throughout the country should be examined. For this purpose, on 18.01.2021, an agreement was signed between the BVU and the National Center for Infectious and Parasitic Diseases. The subject of this agreement is for the two parties to carry out a joint scientific study on the seroprevalence of Q fever in Bulgaria among freelance veterinarians. Sampling was organized and financed by the BVU and the study for the presence of specific *anti-C. Burnetii phase* II IgG and IgM antibodies and confirmation by PCR was carried out at the NRL "Cell Cultures, Rickettsia and Oncogenic Viruses" at the Department "Virology" at the National Center for Infectious and Parasitic Diseases, under the leadership of Associate Professor Petya Genova (Genova, 2019).

Summarizing the data from the survey. In the period from August, 2020 to June, 2021, a total of 481 blood samples from veterinarians were examined in the laboratory, of which 238 were freelancers and 243 from the state veterinary system (25 Regional Food Safety Directorates (RFSD) of BFSA). Of the total number of persons tested, 363 were men and 118 were women from different regions of the country. Diagnostic of *C. burnetii* was performed by indirect ELISA (*anti-Coxiella burnetii ph. II IgG/IgM)* analysis and by means of conventional PCR (for detection of the *sodB* gene of *C. burnetii*) (Letter with Ex. No. 026/09.05.2023 d).

Results. In the period from August, 2020 to June, 2021, a total of 481 blood samples from veterinarians were examined in the laboratory, of which 238 were freelancers and 243 from the state veterinary system (25 Regional Food Safety Directorates (RFSD) of BFSA). Of the total number of persons tested, 363 were men and 118 were women from different regions of the country. Diagnostic of *C. burnetii* was performed by indirect ELISA (*anti-Coxiella burnetii ph. II IgG/IgM)* analysis and by means of conventional PCR (for detection of the *sodB* gene of *C. burnetii*) (Letter with Ex. No. 026/09.05.2023 d).

Through indirect ELISA analysis of the tested 481 clinical samples, anti- *C. burnetii* positive ph.II IgM antibodies were proven in 19% of the tested samples. A relatively high percentage of those affected are in the active age of 40 - 50 years, with a predominance among men (about 90%). In the examined serum samples positive anti- *C. burnetii* ph. A II IgG result was demonstrated in 60%. A positive PCR signal for *C. burnetii*-DNA was obtained in 48/481 (10%) and follows the trend reported above for acute infection in persons of active age. The percentage of positive seroreagents among veterinarians at the RFSD of the BFSA varies from region to region. (on average 18%). The largest number of sick full-time and freelance veterinarians were found in the regions of **Kardzhali, Vratsa, Silistra, Plovdiv, Shumen, Targovishte, Stara Zagora and Lovech**. After the study, 6 new risk areas for the spread of the disease in the country were identified - **Varna, Montana, Pleven, Silistra, Stara Zagora and Shumen**. (Summary provided by Prof. Genova). Table 3 presents the results of the conducted survey among freelance veterinarians (Genova, 2019).

Table 3. The results of the study on Q fever in freelance veterinarians in the period August 2020 - June 2021:
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Regional BVU colleges	№ of tested veterinarians	IgG - over 1.1 /got sick/	IgM - over 1.1 /sick/
Blagoevgrad	10	9	2
Burgas	0		
Varna	8	8	0
Veliko tarnovo	6	5	0
Vidin	9	7	1 suspicious
Vratsa	10	9	2
Gabrovo	11	3	2
Dobrich	10	4	1
Kardzhali	11	8	5+3 suspicious
Kyustendil	9	6	0

Lovech	6	5	2+2 suspicious
Montana	9	6	3
Pazardzhik	10	9	0
Pernik	10	6	3
Pleven	8	3	1
Plovdiv	10	10	3
Razgrad	9	7	0
Ruse	4	3	2
Silistra	11	10	1
Sliven	7	6	2
Smolyan	0		
Sofia	5	4	0
Sofia region	6	2	0
Stara zagora	16	15	3
Targovishte	12	1	2+2 suspicious
Haskovo	8	2	2
Shumen	10	8	5+2 suspicious
Yambol	10	10	3

The Bulgarian Food Safety Agency also participated in a joint study with the National Center for Infectious and Parasitic Diseases on the spread of Q fever among persons at risk (official veterinarians) in the Republic of Bulgaria.

In the combined study with a conventional PCR method and IgM ELISA, 42 cases with an acute phase of the infection were proven in 19 regions. The percentage of positive seroreagents varies in different areas, with an average of 18%.

Most of the active cases do not have a severe course of the disease (pneumonia, hepatitis and endocarditis). The most commonly reported clinical symptoms are muscle and joint pain, headache and fatigue. Affected veterinarians are referred for consultation with infectious disease specialists and initiation of appropriate antibiotic treatment.

A total of 49% of the official veterinarians surveyed had contracted and recovered from Q fever. A higher percentage of people who got sick compared to the national average was found **in 11 districts: Blagoevgrad, Burgas, Vratsa, Kyustendil, Montana, Pleven, Razgrad, Ruse, Sliven, Stara Zagora and Yambol**.

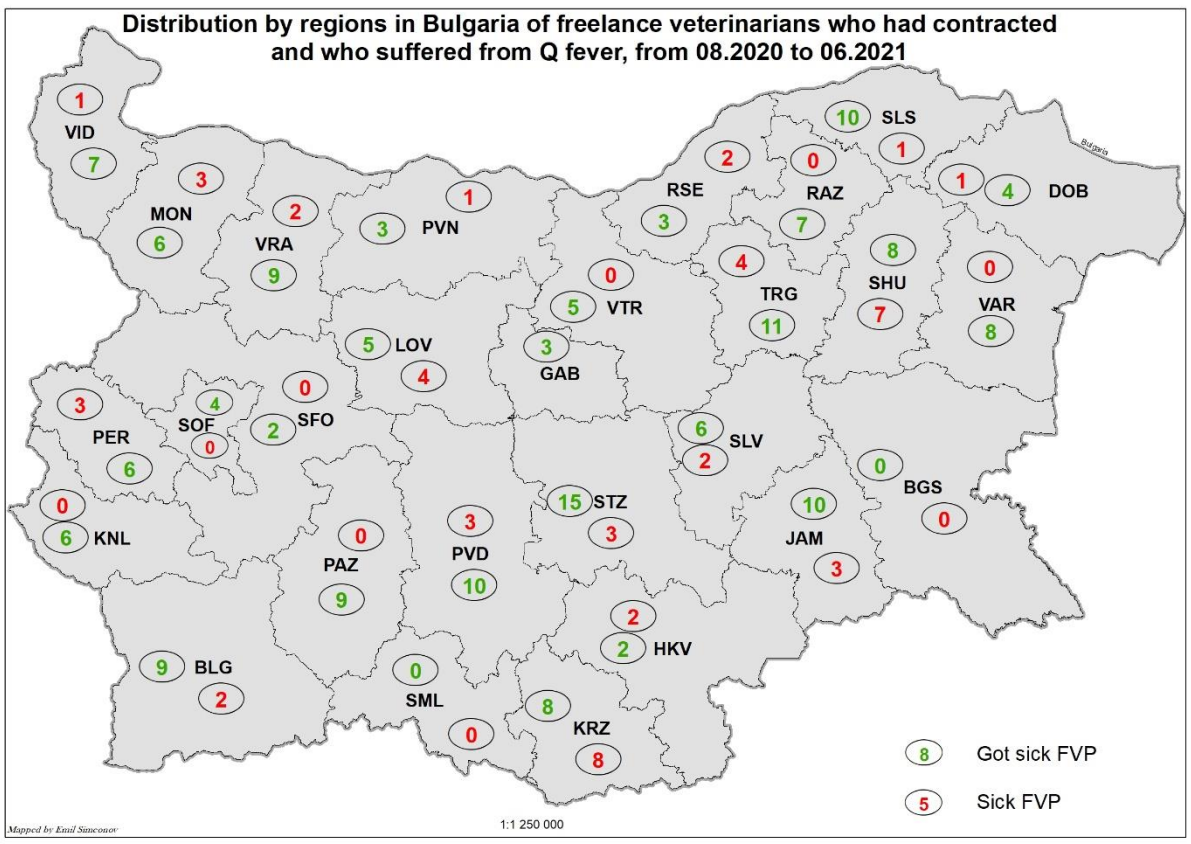

***Figure 5. Distribution by regions in Bulgaria of freelance veterinarians who had contracted and who suffered from Q fever, established in the joint study of the BVU and the National Center for Disease Control and Prevention (map**: Emil Simeonov, RACFC)*

After the study, 6 new risk areas for the spread of the disease were identified - **Varna, Montana, Pleven, Silistra, Stara Zagora and Shumen**, which are included in the 3-year Program for the control and surveillance of Q fever, for the period 2022-2024.

Discussion.

Cases of Q fever in humans are always associated with a previous or current disease in animals. This direct relationship is also reflected in **Figure 6 and 7** - in the areas where only infected people were reported without infected animals - Sofia-city, Varna, most likely the cases are either from an infection acquired outside the area or an infection, acquired without direct contact with animals, or the infected animals in the area remained undetected.

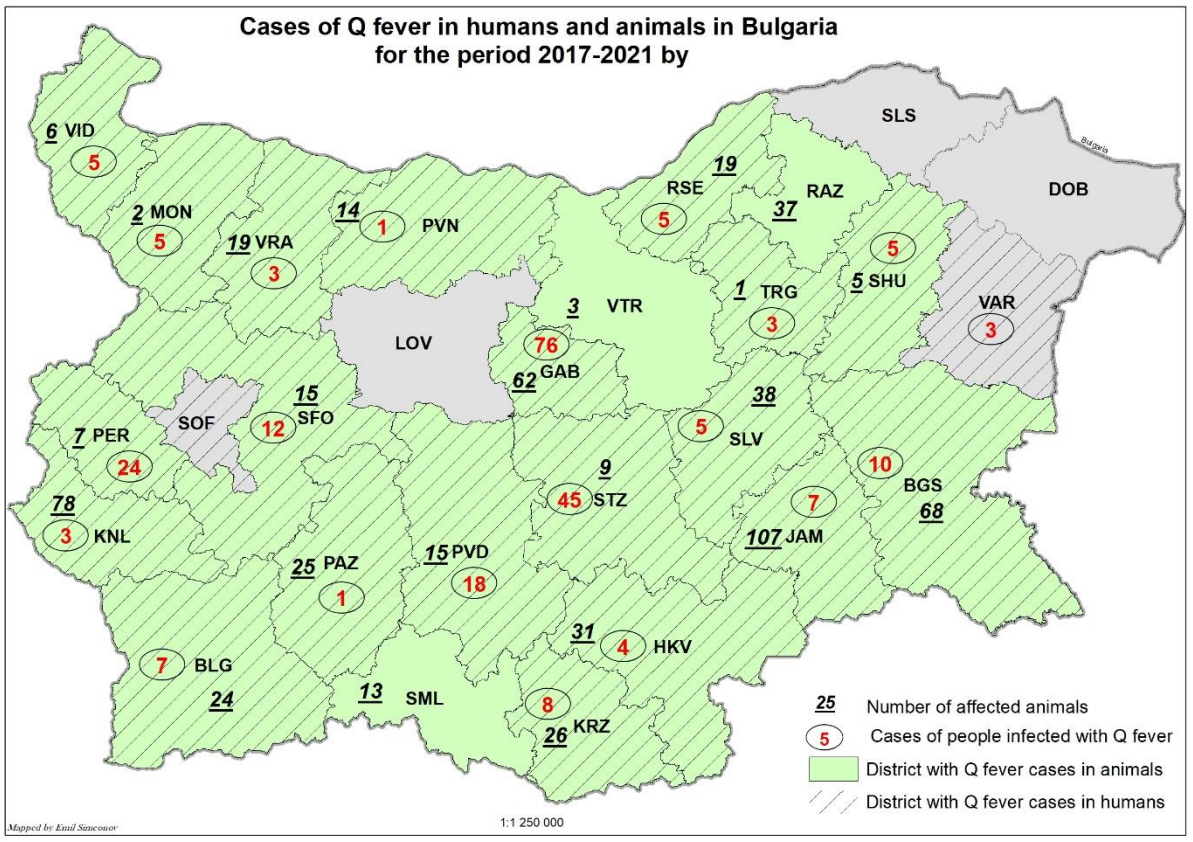

Figure 6: Cases of Q fever in humans and animals in Bulgaria for the period 2017-2021 by region *(map: Emil Simeonov, RACFC)*

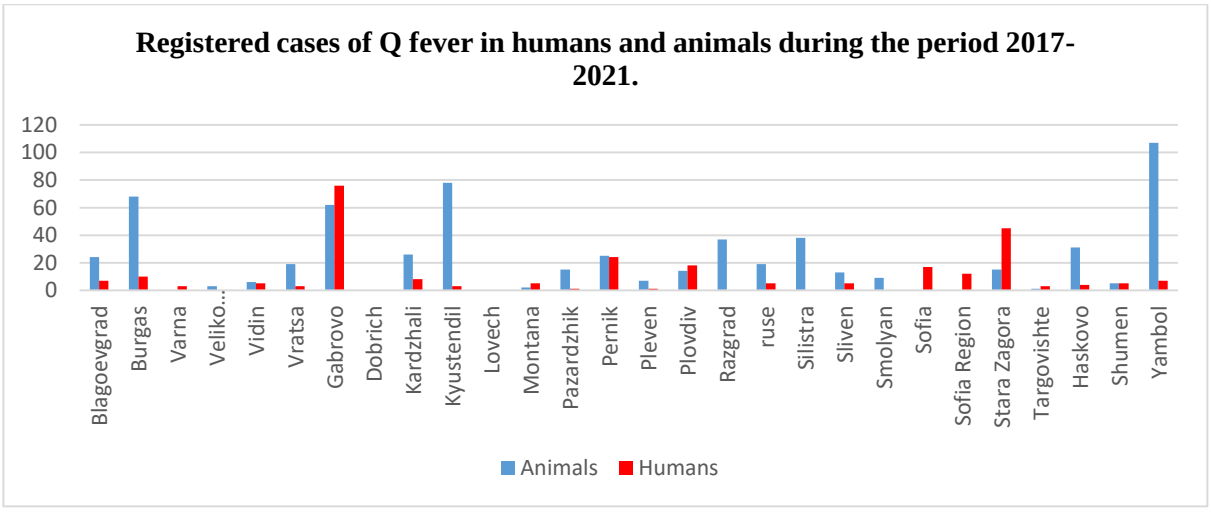

Figure 7: Cases of Q fever in humans and animals in Bulgaria for the period 2017-2021 by region *(chart: Emil Simeonov, RACFC)*

Risk Assessment analysis. Analyzing the information, we can say that the spread of Q fever in Bulgaria in humans is better studied than in animals. On the other hand, the sources of infection and the serological spread among the different target regions and the specific occupational risk groups have not yet been revealed, and hence the possibility to understand the epidemiology of the disease, its driving mechanisms of spread among animal and human populations. It is notable that disease monitoring occurs separately in humans and animals, and a link between human and animal disease cases is not always sought.

The high prevalence of *C. burnetii* infection in herds of small and large ruminants, as well as the long period of survival and persistence of this bacterium in the environment, require the implementation of control measures aimed at reducing the level of exposure at the herd level. The control measures applied are based on antibiotic treatment and the use of vaccinations. They relate mainly to the reduction of risks to human health, but not to the production and economic benefit of the affected herds. Therefore, the selection of a strategy for the control and eradication of Q fever, taking into account the economic benefits and losses on a case-by-case basis, is of crucial importance for the further management of the epidemic process at a farm level.

Outbreaks of Q fever can occur when major risk factors combine and create conditions for penetration and spread of the agent in susceptible animals. Main groups of risk factors being – lack of awareness and unfamiliarity with the disease, commercial practices and technological coordination, land use/dusting/, anthropogenic, physical environment and ecology, spread of the causative agent and animal hosts.

Conclusions

- Q fever is a zoonosis that is distributed globally and, in addition to being of great economic importance, is also of great importance to human and animal health.
- Because of the long-term survival of *C. burnetii* in the environment, Q fever can be considered an airborne infection from the areas of circulation to another area, livestock facilities and many unexpected places, with infectious dust as a source of human infection and for animals (Parker et al. 2006).
- Observations in recent years in our country show that in Bulgaria the ruminant population remains a significant risk factor **with unclear etiological status** in relation to *C. burnetii* and with a very high probability of shedding the pathogen.[ii]
- There are no strictly prescribed requirements of a mandatory nature in European legislation, and therefore each country applies its own control strategy, tailored to its specific characteristics and the prevalence of *C. burnetii infections* in ruminant herds.
- In Bulgaria, Q fever is a highly underestimated disease, insufficiently diagnosed as quality and accuracy, the control and surveillance approach being ineffective and poorly organized, there is a lack of adequate and motivated funding of the "Program for surveillance and control of Q fever in Bulgaria". As a competent authority for the control of animal diseases in Bulgaria, BFSA, has within its obligations and duties to develop new higher standard and effectiveness program. (*conclusions of the Bulgarian Veterinary Union*).

- The control and supervision of the disease in large and small ruminants in the Republic of Bulgaria is carried out by the Bulgarian Food Safety Agency (BFSA) and is part of the National Program for the Prevention, Supervision, Control and Eradication of Animal Diseases and Zoonoses in Bulgaria 2022- 2024. The objective of the program is to minimize the risk of human infection with the disease and achieve economic benefits by reducing the abortion rate in ruminants and ensuring compliance with the requirements of the OIE standards regarding this disease in trade with live animals. The latest data from the 2020-2021 survey were taken into account when preparing the Program.
- Data from a study of Q fever show that in more than 80% of cases, small ruminants, especially goats, are "responsible" for the infection (Simeonov, 2020). In previous years, due to limited screening (1000 samples were set annually) for the country, it was not possible to cover all farms and ruminant species from the known endemic regions and recently discovered areas. For this reason, the number of incoming samples for research was limited (according to Simeonov, 2020) and half of the samples that were received in the NRL "Chlamydia and Rickettsia" of NDNVMI - Sofia, (BFSA, 2022-2024) were from cattle, which are not the target animal species to reveal the prevalence of Coxiellosis caused by *C. burnetii* in our country. To address this issue, the new national program 2022-2024 has changed the sampling strategy, targeting herds with high abortion rates or other reproductive problems and bulk milk samples.

Recommendation

1. The effectiveness of measures taken to detect and control Q fever is determined by three main factors:

1.1. Detection to the maximum extent of the potential sources of infection (infected herds or individual animals);

a. For this purpose, it is necessary to carry out a national serological survey of Q fever infection in herds of large and small ruminants and among people from the specific occupational risk groups indicated above, from the target regions of Bulgaria (regions in which there are established most positive cases in humans and animals). The number of small ruminants (goats) tested should be drastically increased, which will significantly increase the detection rate of *C. burnetii infections* at herd level. This will help to reveal the sources of infection and seroprevalence among different target regions (mapping) and specific risk groups, as well as to understand the epidemiology of the disease, the driving mechanisms of the spread of the disease in animal and human populations, and from here to identify people who are at higher risk of infection (Simeonov, 2020).

b. Surveillance in ruminants should prioritize small ruminants to better understand the risk of human exposure. A necessary preventive measure is to monitor the number of abortions (abortion storm) and premature births in small ruminants, the nature and characteristics of the abortion, the premature isolation of the aborting animals, as well as primary diagnostics of these animals - pathoanatomical findings, impression preparations, microscopic and culture testing of fetus and placenta.

c. Due to the limited opportunities for broad screening, the solution to this problem may be sought in a change in the sampling strategy, in which animals from herds with abortions or other reproductive problems are targeted, to increase the number of small ruminants examined, and in particular goats, at the expense of cattle and introducing the examination of pooled milk samples, which will significantly increase the detection rate at the herd level (Simeonov, 2020).

d. In order to establish the pattern of spread of the disease on farms, the reservoirs and routes of transmission of the infection in humans and animals should be revealed, as well as the epidemiological relationship between individual cases and outbreaks. This will provide clarity on the geographical distribution of infection and the main risk factors involved.

e. The need for cooperation/interaction between veterinary and human medicine doctors based on the "One Health" principle and multi-sectoral cooperation to reduce cases. Sharing of data/information generated through monitoring studies and laboratory results between competent authorities in veterinary and human medicine.

1.2.Adequate diagnostic and assessment of the degree of shedding of the pathogen in the environment;

a. It is necessary to strengthen the diagnostic capabilities for the detection of Q fever among the specific occupational risk groups (livestock breeders, official and private veterinarians and personnel working in the meat chain) in Bulgaria by strengthening the laboratory diagnostic capacity and assisting in the development of a national strategy for disease prevention, monitoring and control to improve the epidemiological situation in the country. This includes:

   i. Upgrading and re-equipment of the existing diagnostic laboratories for Q fever at the Ministry of Health and BFSA, including the introduction of new methodology of isolation and molecular detection; rapid and definitive detection in samples and environment; display and analysis of Q fever data with ArcGIS; isolation and molecular detection of an infectious agent in animal and human samples.

   ii. Exchange of experience on methodic for molecular detection and diagnosis of Q fever;

   iii. Assessment of the ability of existing diagnostic methods to detect antibodies, antigens and nucleic acids for the purposes of disease surveillance and control;

   iv. Exchange of experience for the development and validation of molecular technologies for the diagnosis of Q fever;

   v. Establishing a scientific network and dissemination of knowledge by conducting national seminars and scientific visits.

The improved diagnostic capabilities and the results obtained from a national serological survey of Q fever infection can serve as a basis for introducing more appropriate and effective Q fever control measures and for determining the scale, prevalence, impact and significance of the infection and the disease in domestic ruminants and humans, and to develop and refine the national strategy for the prevention, prophylaxis and control of the disease and to improve the epidemiological situation in the country (Simeonov, 2020).

1.3.Taking measures to control infection and limit the zoonotic risk.

The control of Coxiellosis in animals is a complex problem, the solution of which requires the application of a complex approach. The discovery of clusters of infection and infected animals must necessarily be followed by sanitary and medical measures aimed at limiting the spread of the infection and its transmission to humans, as well as limiting the economic consequences arising from its clinical manifestation. In general terms, these measures are aimed at limiting the possibilities of the spread of infection between animals within the herd, its transfer from one herd to another and its transmission from animals to humans. They can be of a long-term nature or taken urgently in the case of an epizootic (most often manifested by abortions in animals) or an epidemic outbreak. The applied measures are very diverse, with a sanitary (biosecurity), therapeutic and prophylactic nature, and their effectiveness increases significantly when they are applied in combination (Simeonov, 2020).

a) From the first group, these are: restricting access to the infected farm, setting aside isolators for birth or lambing, collection and destruction of puerperal products (aborted fetuses, placentas, amniotic fluid) with subsequent disinfection, not allowing the removal of manure before it is decontaminated, removal of the positive reagents and/or pregnant animals, temporary prohibition on reproduction until the farm recovers, movement control and restriction of common grazing with other herds, etc. (Simeonov, 2020).

b) To the first group should be added the additional measures taken to minimize the risk of human infection, including the personnel related to animal caretaking and processing of raw materials of animal origin, as well the consumers of animal products. These measures include notifying the local

health authorities (LHAs) about the presence of an outbreak of infection, limiting the possibility of people with immune diseases, valvular heart disorders and endocarditis to work in livestock facilities and those for processing animal products; use of personal protective equipment when handling animals; directing the attention of the medical professionals treating the staff of such farms to the likelihood of Q fever developing signs consistent with that disease (fever, influenza-like illness); limiting processing of vaccination?

c) from such farms the products with a short maturation period are directed to mandatory extended pasteurization at 72^{o} C for 15 min, as well as other measures subject to veterinary-sanitary expertise (Simeonov, 2020).

d) Methods for specific disease prevention with biologics (vaccines) based on *C. burnetii phase 1* immunization can be extended and used. BFSA can encourage farmers to administer registered vaccine products against *C. burnetii* in our country, by stimulating their additional participation in the Rural Development Program and together with improving biosecurity measures to ensure better herd health (Simeonov, 2020).

e) Designation and sanitation of animal movement routes in the city and their assembly points, prohibition of grazing within the settlements,

f) Regular mowing of lawns in residential areas and periodic treatment of lawns with a suitable insecticide - to destroy the vectors (ticks).

g) In animals, the vaccines considered most effective are those made from inactivated whole bacterial cells. These vaccines effectively prevent abortion and have been shown to reduce bacterial shedding in utero-vaginal discharge, faeces and especially in milk. It should be noted that vaccination is more effective in nulliparous animals than in parous animals.

2. Recommendations of the European Food Safety Authority:

a) Monitoring should be expanded to include species other than domestic ruminants, such as pets, which can be reservoirs for Q fever and human infection.

b) In the coming years, it is extremely important to collect more data on ongoing environmental pollution (animal waste, dust) to better assess risk factors in order to reduce human exposure to this zoonosis.

3. Recommendations of the European Center for Disease Prevention and Control (ECDC)

According to ECDC experts, it is necessary to consider the following:

a) Adherence to good hygiene practices in animal handling facilities, especially sheep and goats, will help prevent the transmission of this disease.

b) Since the disease could also be transmitted to humans through contaminated milk, pasteurization of milk and milk products is an important factor in preventing infection.

c) In addition, transmission of the disease can occur with a therapy known as "fresh cell therapy". According to the ECDC, countries may consider regulating such practices and establishing national xenotransplantation surveillance systems.

d) In rare cases, Q fever can also be transmitted through the bite of an infected tick. Exposure to infected ticks should be avoided or minimized by using tick repellents, wearing protective clothing, and prompt and proper removal of ticks.

The data thus presented show that the control of Coxiellosis in animals is a complex problem, the solution of which requires the application of a complex approach. The detection of anthropurgic foci of infection and infected animals through monitoring programs is only the first step, which must necessarily be followed by taking sanitary measures aimed at limiting the spread of the infection and its transmission to humans, as well as limiting the economic consequences arising in its clinical manifestation. Global experience, the expertise of leading scientists in this field and experimental data show that among these measures the most effective and with a long-term effect is phase I immunization *C. burnetii* vaccine. In combination with the above measures, this would lead to minimizing the human health consequences and economic losses caused by this zoonotic infection (Simeonov, 2020).

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