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The Swiss national reference centre for tick-borne infections

Due to climate changes, the geographical surfaces suitable for ticks has increased in ten years from 16% to 25%, as shown by Rochat et al [1]. Moreover, due to societal changes (partially boostered by the SARS-CoV-2 pandemics), the Swiss population appears to be increasingly exposed to ticks due to more diversified and more regular outdoors activities. Thus, in 2021, according to a recent SUVA press communicate, as many as 14'000 human tick bites have been recorded in Switzerland as compared to about 10'000 human tick bites per year a few years earlier. This trend may have a significant impact on public health given the different tick-borne pathogens observed in Switzerland, such as Borrelia spp. and the tick-borne encephalitis virus (TBE virus).

Due to globalisation and increased travel rates in exotic areas (slowed down by the SARS-CoV-2 pandemics but rapidly regrowing in 2022), we also have to deal in Switzerland with a number of patients returning home suffering from spotted fever due to various Rickettsia species [2]. In addition, there are a number of tick-borne infections that remain likely underdiagnosed (infections with Anaplasma phagocytophilum and Candidatus Neoehrlichia mikurensis, for example). Moreover, several novel agents related to Chlamydia have been documented in ticks [3], emerging as new pathogens or having yet an unknown pathogenic role [4, 5].

This leaves to the Swiss national reference centre for tick-borne diseases (CNRT) a number of challenges that will be described in the present article. We will also summarize key aspects about some of these microbes studied at the CNRT.

The Swiss national reference centre for tick-borne diseases (CNRT)

Since 1st January 2022, the Swiss National Reference Center for tick-borne infection is directed by Professor Gilbert Greub. With his team at the Institute of Microbiology of the University of Lausanne (V. Cagno, A. Coste, and O. Opota), he is actively collaborating with ADMED Microbiologie at La Chaux-de-Fonds (R. Ackermann, A Croxatto and R. Lienhard). G. Greub, R Lienhard and R. Ackermann were already actively driving the CNRT since more than a decade [6].

The Federal Office of Public Health (FOPH) defined Q fever and Lyme disease as the two main priorities of the CNRT, despite the fact that Coxiella burnetii is a zoonotic disease mainly transmitted by exposure to goats and sheep, and which was undetected in Swiss ticks in a vast survey [7]. However, the tasks and perimeter of the CNRT also includes a number of other infections and pathogens, as summarized in Figure 1 & Table 1.

Lyme disease

Lyme Disease is endemic in Switzerland. The first clinical feature described in Europe was already in 1883 as «acrodermatitis chronica atrophicans». Erythema migrans was described medically in 1909 by the Swedish dermatologist A. Afzelius. However, the agent named Borrelia burgdorferi was only described in 1982.

Often considered the new great imitator, this bacterium can be responsible for skin, articular, neurological, cardiac and optical manifestations [8]. Its main clinical sign is the pathognomonic «erythema migrans», a localized acute stadium usually recognized by physician and some patients, even when it reaches as much as 1 meter in diameter. Less frequently, this skin manifestation can disseminate in multi-erythema all over the body. As disseminated acute disease, Lyme neuroborreliosis is the second most frequent clinical feature. Reported as «tick paralysis» in 1922, Garin and Boujadoux described the poly-meningo-radiculoneuritis after a

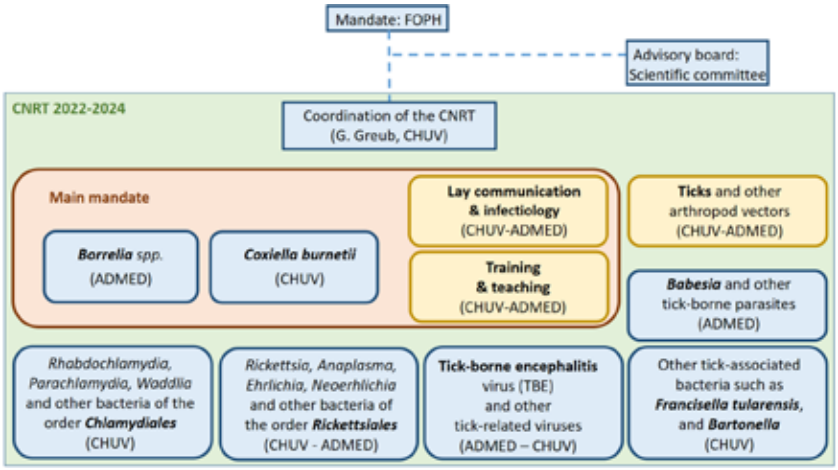

Figure 1. General organization of the Swiss National Reference Center for Tick-Borne Infections (CNRT). This chart highlights the diversity of pathogens that are part of the tasks and perimeter of the CNRT, as revised in 2021 by the FOPH. It also shows that the CNRT is active at the epidemiological level but also represents a reference resource in diagnostic microbiology and infectious diseases.

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Provision of expertise (control) of positive C. burnetii serologies for all positive cases detected in diagnostic laboratories in Switzerland (including individual cases) using complementary serologies available in the CNRT laboratories.
Evaluation and verification of commercial tests for PCRs and serologies for Lyme borreliosis and Q fever, as well as some other tick-borne agents. Publicly disseminate evaluation reports.
Creating and maintaining a homepage.
Evaluate a novel test for serology for Lyme borreliosis
Implement PCRs for Lyme borreliosis, Q fever and tick-borne encephalitis in the two CNRT laboratories taking into account the different target genes so that two independent analyses can be offered for confirmatory diagnosis and to ensure method redundancy
Mapping of laboratories performing diagnostic methods for Lyme borreliosis, Q fever and tick-borne encephalitis.
Publication of recommendations in the diagnosis of Lyme borreliosis and Q fever for physicians (RMS -SMW) and laboratories (SSM CCCM).
Designing immunofluorescence tests for C. burnetii serology (home-made)
Creation of an EQA for the control of C. burnetii serologies in collaboration with the CSCQ (provision of samples, technical expertise, analyses, reporting).

Table 1. Some of the projects that will be tackled by the CNRT during the next 3 years. These tasks are largely focused on Q fever, Lyme disease and tick-borne encephalitis, since so far these three infectious diseases represent the main identified public health zoonotic threats in Switzerland.

tick bite. This aseptic meningitis is typical enough to be recognized as Lyme disease when occurring during the tick season, especially when following erythema migrans, which occurs weeks before. However it becomes more difficult out of this context and when more aspecific neurological manifestations appear. Concerning the last stage of the disease, late disseminated borreliosis is mainly featured by the arthritis described first at Lyme (Connecticut, USA) as an emergent disease in 1975, and Acrodermatitis Chronica Atrophicans (ACA) associated sometime to peripheral neurological disorder.

Since Borrelia burgdorferi was identified as the etiologic agent of Lyme disease, diagnostic tools have been rapidly implemented. By the end of the 80's, the «Diagnostic Parasitaire Laboratory» (DPL) from the Zoology Institute of the University of Neuchâtel, set up the first test in Switzerland. It was a home-brew indirect immunofluorescence assay (IF) using as antigen a culture of the B31 Borrelia burgdorferi sensu stricto strain. This first tool has then rapidly been complemented by an ELISA with a whole antigenic extract of the B31 bacterial strain. This 96 wells plate Enzyme Immunoassay (EIA) enabled to handle more analyses at once, as the diagnostic requests extended and epidemiological studies became more frequent. The serology sat as the main diagnostic tool. However, the use of native antigens rapidly questioned the specificity of the assays, with cross-reactions with syphilis markers (TPHA) and with rhumatoid factors (RF).

In the early 90's, a more specific capture test was validated to eliminate RF negative impact for IgM diagnostics. This new assay uses whole purified flagella antigen as described in Hansen and Lebech [4]. This was especially meant to enhance and to facilitate neuroborreliosis diagnostic.

Specificity problems were partially resolved by using «lab-made» western-blot as confirmatory test [10]. These western-blot assays included two Swiss B. burgdorferi sensu lato isolates from ticks. The use of other strains was then motivated by the identification of the new etiological species present in Europe. Thus, a US B. burgdorferi strain (B31), a B. garinii strain (NE84) and a B. afzelii strain (NE17) were added to the blot panel used to confirm Lyme borreliosis. This confirmation procedure was shown to be useful, allowing a specificity of 95%.

However, the high sero-prevalence in the Swiss population remain a challenge for the diagnostic, particularly in high risk groups such as orienteer runner, old rural population or hunters since positive serology may only reflect a past infection without any causal relationship with the current symptoms that triggered the serology. Borrelia culture and PCR were then introduced as complementary tools, since these assays are highly specific. However, their systematic routine diagnostic use is not possible as (i) it needs more invasive sampling such as biopsies or lumbar punctures and (ii) they both lack sensitivity.

Beginning of this century, the use of recombinant antigens in EIA for Lyme borreliosis diagnostic launched a new era [9]. Proteins as outer surface protein C (OspC) or Variable Lipoprotein Surface-Exposed protein (VlsE) showed their importance and utility [reviewed in 10]. Moreover, with such recombinant proteins, we can achieve a higher quality and an improved stability in the production of diagnostic assays. These two antigens are now used in instruments-based EIA (such as chemiluminescence immunoassays or enzyme-linked fluorescence assays) and in immunoblot assays on different matrices. They enhance both sensitivity and specificity of serological assays, but are still not able to differentiate passed contact or infection with active disease. Today, serology remains the main tool to diagnose Lyme borreliosis. In Switzerland as in many country in Europe, it is recommended to use an EIA as first test and if reactive, to confirm by an immunoblot.

The CNRT has the mission to lead the microbiological diagnostic of borreliosis. By evaluation, validation and verification of new commercial methods, we are able to help the companies to introduce the appropriate testing methods. This is only possible with an established and qualified collection of sera for the different disease stages. We will continue to bridge the communication between the actors of diagnostics in Europe grouped in ESGBOR, test companies and diagnostic laboratories to help introduce new methods that are of interest. The goal of the CNRT is to help the population, the physician and the authorities to find answers and solutions to problems.

As a service, we aim to rapidly (=same day results) diagnose acute disseminated stage such as neuroborreliosis. For this purpose, we set up rapid ELISA tests to exclude Borrelia as the aetiolog-

ical agent (if negative) and we confirm diagnosis within 48h (if ELISA was positive). Parameters such as CSF IgG and IgM antibody levels, specific intrathecal production and the new CXCL13 marker detection have been now tested routinely for more than 5 years for our and other hospitals' patients. Culture and PCR are systematically tested when enough CSF is available, in order to complete diagnosis with very high positive predictive value and to also study the epidemiological profile of Lyme Borreliosis in Switzerland. This is a needed step in the prospective vision to develop a Lyme vaccine.

The role of the CNRT extends of course beyond diagnostics and epidemiological surveys and we are highly active by providing advices on patients care and in lay communication, providing for instance in collaboration with the «tick league» a comprehensive set of answers to the most frequently asked questions (https://www.bag.admin.ch/dam/bag/de/dokumente/mt/infektionskrankheiten/zecken/FAQ-Zecken-Zeckenstiche.pdf.download.pdf/FAQ%20Zecken%20DE.pdf).

Q fever

Q fever is a disease caused by a bacterium called Coxiella burnetii. The natural reservoir of this bacterium is domestic animals, pets, some wild animals and more rarely ticks. Infection in humans usually occurs through inhalation of dust containing the infectious agent excreted by carrier animals, also by ingestion of contaminated food, and more rarely by tick bites. Noteworthy, the main ticks associated with Coxiella transmission seems to be Rhipicephalus evertsi and Amblyomma variegatum, according to a recent meta-analysis, which are tick species uncommonly seen in Switzerland contrarily to Ixodes ricinus.

Symptoms in the acute phase are generally a prolonged fever (> 10 days) with normal white blood cell count, thrombocytopenia and elevated liver enzymes [12]. Subjects with predisposing factors, such as heart valve disease, may develop chronic disease. Endocarditis and infections of aneurysms or vascular prostheses are the most common forms of chronic Q fever [13] and are usually fatal if left untreated. Underdiagnosis may especially occur in subjects with an initially pauci-symptomatic endocarditis that may then get superinfected by S. aureus or other Gram positive cocci [14] and Coxiella should thus be considered systematically. Chronic Coxiella hepatitis are also commonly seen [12]. While Q fever is rarely reported in children, the chronic picture is very different from adults with osteomyelitis being the most common.

In Switzerland, Q fever has only been notifiable since November 2012, following an epidemic in the Lavaux region linked to the presence of infected sheep flocks that caused a dozen human cases [15]. The introduction of this mandatory notification was done despite the small outbreak in humans since the epidemic potential of C. burnetti is much higher, as demonstrated by the epidemics in Bagnes in 1982 and in the Netherlands in 2009, where more than 1000 human cases have been documented [16, 17]. Today in Switzerland, each year, between 40 to 60 cases are reported. In addition to sporadic cases, outbreaks occurs also occasionally, but is generally limited thank to active preventive measures.

Diagnosis is based on PCR or serology. PCR can be performed on whole blood or serum during acute phase [18], and helps diagnose acute Q fever in the first 2 weeks of infection. Pcr may also then be done on infected tissue (heart valve samples, liver biopsy, bone biopsy, …). In Lausanne, we are using a Coxiella burnetii specific TaqMan PCR [18], that exhibits x% sensitivity on valve samples and which was also proven sensitive enough for the diagnosis of hepatitis, uveitis, aortic prosthetic infection and spondylodiscitis. Serology, on the other hand, can be done by using a highly sensitive screening test followed by an immunofluorescence test for confirmation. A quadrupling of the phase II IgG antibody titre by immunofluorescence between matched acute and convalescent specimens is the diagnostic gold standard for confirming the diagnosis of acute Q fever. However, a negative serology in the acute phase does not exclude Q fever. Indeed, the immunofluorescence is negative during the early stages of acute illness, when PCR is generally still positive. Most patients seroconvert by the third week of illness. A single high 'convalescent' serum sampled after the acute phase of the disease is sufficient for the diagnosis (positive if > 1/256); however, a fourfold increase between acute and convalescent samples exhibits a much higher sensitivity (may be considered positive with titers > 1/64) and specificity than a single high convalescent titre. The diagnosis of chronic Q fever requires demonstration of a high titer of phase I IgG antibody (≥ 1:800) and an identifiable focal infection (e.g. endocarditis, hepatitis or spondylodiscitis).

PCR, immunohistochemistry, or culture of affected tissues can provide definitive confirmation of C. burnetii infection. However, those approaches have a low sensitivity and are somehow difficult to implement, since samples are not systematically available. Thus, the diagnosis of Q fever relies heavily on serological monitoring of the patient. It should be noted that immunofluorescence requires considerable expertise. The implementation of specific and relatively sensitive PCR is also a challenge and few laboratories offer it.

Tick-borne encephalitis (TBE)

Tick-borne encephalitis (TBE), the most important tick-borne viral disease of humans in Eurasia, is caused by tick-borne encephalitis virus (TBEV), a member of the genus Flavivirus of the family Flaviviridae. TBEV is mainly transmitted to humans via tick bites. The distribution of TBEV correlates with the occurrence of its vector ticks and ranges from Western Europe to Russia, Siberia, and Far-Eastern countries. Three viral subtypes, i.e. European, Siberian, and Far Eastern, have been described; in addition, two new subtypes (Himalayan and Baikalian) have been proposed. When transmitted to humans, TBEV may cause disease of variable severity, ranging from subclinical infections to severe courses with neurological involvement and potentially fatal outcome. While the infection is asymptomatic in 70–95 % of cases, symptomatic disease may occur as meningeal, encephalitic, poliomyelitic, and myelo-radiculitic forms. [19]. In

Switzerland, neurological disease most frequently manifests as meningoencephalitis (55%), followed by meningitis (22%) and encephalomyelitis (3%) [20]. Overall, the FOPH recorded 454 cases in 2020 and 285 in 2021, with an incidence of 5.11 and 3.27 per 100,000 inhabitants, respectively [21]. The case fatality rate during the acute phase is 0.9% and 45% of symptomatic patients present neurological sequelae [22].

Currently, no specific treatment against TBEV is available. Besides preventing tick bites, active immunization is the most important protective measure against infections with TBEV. Six licensed vaccines exist, all of which use inactivated whole virus strains [23] (8). In Europe, two vaccines based on European subtype isolates are available, which can be used interchangeably: FSME-IMMUN® (Pfizer) and ENCEPUR® (Bavarian Nordic). The vaccination schedules comprise three doses for primary immunization. Thereafter, vaccine manufacturers prescribe booster vaccinations for maintaining protection (first booster dose three years after primary immunization, subsequent booster doses every five years [<50 years] or every three years [≥50 years]). Deviating from this schedule, Switzerland has extended the booster intervals to ten years [24].

Virus-neutralizing antibody titers are believed to be responsible for long-term immunity after natural infection and vaccination [25]. While after a 3-dose primary series seropositivity persist for more than 10 years in >90% of younger subjects, it drops to 37.5% in those 60 years or older. However, a systematic review found field effectiveness to remain high in irregularly vaccinated subjects and thus not to correlate well with the percentage of subjects achieving an arbitrarily defined threshold of persisting antibodies [26]. Since the decision for the prolongation of vaccination intervals by the FOPH Switzerland in 2006, no indication was found that extended booster intervals resulted in an increased rate of breakthrough infection. On the other hand, there was a marked public health benefit with respect to increased acceptability of TBE immunization in the general population [27]. Various information campaign have been done to increase awareness about TBEV, vaccine and tick-bite prevention, including the development of a game called Krobs, which preventive messages are provided on a corresponding dedicated website (www.krobs.ch).

The diagnosis of TBE must be established in the laboratory because of the non-specific clinical presentation. The detection of specific nucleic acid (RNA) in the blood by RT-PCR is only successful during the first viremic phase of the disease before seroconversion. The method of choice is thus the demonstration of specific IgM- and IgG-serum antibodies, since these antibodies are detectable in practically every case at time of hospitalization. Most often Enzyme-linked Immunosorbent Assays (ELISA) are used. However, in cases of contacts with other flavivirus (e.g. vaccinations against yellow fever or Japanese encephalitis; dengue virus infections), a neutralization assay should be performed to assess immunity due to the interference of flavivirus cross-reactive antibodies in ELISA test [29]. Seroneutralization assays can be performed in biosafety level (BSL)-3 conditions with the wild-type virus. Alternatively pseudovirions and recombinant viral particles exposing structural proteins of TBEV (and other flaviviruses) can be produced [30, 31]. These systems allow to assess the presence of specific antibodies for TBEV in a BSL-2 condition and in parallel for different flaviviridae.

For the differentiation of vaccination-induced antibodies from those induced by natural infection, assays detecting antibodies against the non-structural protein 1 (NS1) of the virus have been introduced. As available vaccines are highly purified and inactive, without substantial amounts of NS1, there is no TBEV replication and therefore no formation of NS1 protein and/or NS1-specific antibodies [32].

Conclusions

The ticks are able to transmit a number of significant pathogens and in addition to Borrelia, Coxiella and to the tick-borne encephalitis virus (TBEV), there are a number of additional major human pathogens transmitted by ticks such as Anaplasma, Ehrlichia and all the agents of spotted fever. The latter, due to various rickettsial species, are not yet recognized as significant public health problems in Switzerland, likely due to underdiagnosis of Rickettsia slovacae and Rickettsia helveticae infections. The same is true for Anaplasma phagocytophilum, which has been largely documented in Ixodes ticks collected in various Swiss region [7], but remain often undiagnosed, when causing leucopenia or pancytopenia considered of unexplained etiology.

Presence of various chlamydia-related bacteria have also been documented in Swiss ticks [3, 33], with especially several documentation in Ixodes ricinus ticks of very high bacterial load of Rhabdochlamydia bacteria, that allowed direct full genome sequencing [34]. Thus, in addition to tackle the problems raised by the circulation in Switzerland of Borrelia, Coxiella and to the tick-borne encephalitis virus, by providing (i) prevention advices, (ii) up-to-date diagnosis and (iii) expert opinions on clinical aspects, the national center for tick-borne infections is also in charge of improving knowledge about novel emerging pathogens such as Rhabdochlamydia and Parachlamydia [35].

Moreover, since a number of tick-borne infections are acquired during travel abroad, surveillance of imported tick-borne infections and advices to travelers are also important, especially for (i) African-tick bite fever, that represents the more common spotted fever observed in Swiss travelers, (ii) Mediterranean spotted fever, which exhibits the highest mortality of European tick-borne rickettsial infections, and (iii) Rocky Mountain spotted fever, which exhibits a very high mortality of up to 30%.

Finally, the CNRT has also the task to better understand ticks biology and to determine the regions where current climatic conditions are favorable for ticks & tick-borne pathogens and we recently demonstrated an increased proportion of surfaces suitable for ticks in Switzerland, [1], leading to changes in the FOPH recommendations regarding the vaccine against the tick-borne encephalitis virus [24].

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