Prevention of tick-borne diseases: challenge to recent medicine

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Received: 16 June 2021 / Accepted: 10 November 2021 / Published online: 9 March 2022
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Abstract
Ticks represent important vectors and reservoirs of pathogens, causing a number of diseases in humans and animals, and significant damage to livestock every year. Modern research into protection against ticks and tick-borne diseases focuses mainly on the feeding stage, i.e. the period when ticks take their blood meal from their hosts during which pathogens are transmitted. Physiological functions in ticks, such as food intake, saliva production, reproduction, development, and others are under control of neuropeptides and peptide hormones which may be involved in pathogen transmission that cause Lyme borreliosis or tick-borne encephalitis. According to current knowledge, ticks are not reservoirs or vectors for the spread of COVID-19 disease. The search for new vaccination methods to protect against ticks and their transmissible pathogens is a challenge for current science in view of global changes, including the increasing migration of the human population.

Highlights
• Tick-borne diseases have an increasing incidence due to climate change and increased human migration
• To date, there is no evidence of transmission of coronavirus COVID-19 by tick as a vector
• To date, there are only a few modern, effective, and actively-used vaccines against ticks or tick-borne diseases
• Neuropeptides and their receptors expressed in ticks may be potentially used for vaccine design

Keywords Tick-borne disease · Vector · Vaccine · Neuropeptides · COVID-19

Abbreviations
TBEV Tick-borne encephalitis virus
LB Lyme borreliosis
TBE Tick-borne encephalitis
TBP Tick-borne pathogens
OspA Outer surface protein
TROSPA Tick receptor for outer surface protein A

TSLPI Tick mannose binding lectin inhibitor
tHRF Tick histamine release factor
Salp15 Salivary gland protein of 15 kDa
CCHF Crimean-Congo haemorrhagic fever
SUB Subolesin
TBF Tick-borne fever
ompA Outer membrane protein A
AipA Infection protein A
dsRNA Double stranded RNA
MIP Myoinhibitory peptide
SIFa SIFamide
TK Tachykinin
AT Allatotropin
AST-A Allatostatin A
ILP Insulin-like peptide
PDF Pigment dispersing factor
Elev Elevenin
Introduction

In recent years, modern medicine and biology have focused their attention on tick-borne diseases, the incidence of which is increasing due to factors such as climate change, but also increased human and animal migration. We classify ticks among obligate ectoparasitic hematophagous arthropods capable of transmitting various viruses, bacteria, or parasites (Hoogstraal 1981). They usually use the blood of reptiles, birds and mammals as a source of food. Ticks belong to the Arthropoda phylum, subphylum Chelicerata comprising the Arachnida class with several suborders; while the subclass Acarina includes ticks (Anderson and Magnarelli 2008). Most Ixodidae prefer mammals and birds; however, for example *Ixodes ricinus* (Linnaeus, 1758) and *Amblyomma* sp. also feed on reptiles (Dantas-Torres et al. 2008; Rizzoli et al. 2014). Due to climate change, some tick species, e.g. *I. ricinus* are moving to higher geographical locations, up to altitudes above 1000 m (Medlock et al. 2013). *Ixodes* spp. and *Dermacentor* spp. are questing, i.e. passively waiting on the vegetation until their host approaches. On the other hand, *Amblyomma* spp. and *Hyalomma* spp. are actively looking for their host.

Ticks are ectoparasites with high reproductive potential and a wide host range. They transmit the widest range of pathogens, including viruses, bacteria and protozoa, collectively called tick-borne pathogens (TBP) to humans and animals, often endangering human life, but also the lives of livestock (Parola and Raoult 2001; Anderson and Magnarelli 2008; de la Fuente et al. 2008a, b; Dantas-Torres et al. 2012; Brites-Neto et al. 2015). Transmission of the pathogen to the host is enabled through the tick salivary glands and gut (Šimo et al. 2017; Pospisilová et al. 2018). TBPs are thus taken up by ticks during their feeding on infected hosts. From the midgut, TBPs pass through the gut epithelium to the hemocoel, from where they can further penetrate into the SG epithelium, where they multiply and are transmitted to a new host through saliva (Šimo et al. 2017; de la Fuente et al. 2017a, b; Kurokawa et al. 2020).

The global number of cases of tick-borne diseases is increasing every year (Silatsa et al. 2019; Muhanguzi et al. 2020). While tick-borne diseases are a serious problem, especially for the human population in Europe and Northern America, they also pose a significant threat to livestock production in sub-Saharan Africa, Latin America, and Asia. The European Parliament has expressed concern about the spread of Lyme borreliosis (LB) as a major risk to public health (Sprong et al. 2018). Based on the spread models for LB and tick-borne encephalitis (TBE), an increase in infection risk is expected (Paules et al. 2018). Other tick-borne diseases, such as anaplasmosis, rickettsiosis, babesiosis, theileriosis and others, have also emerged and spread in new regions (Vayssier-Taussat et al. 2015; de la Fuente et al. 2017a, b; Gharbi et al. 2020).

Recently, a huge threat to the economy and agriculture are ticks of the genus *Ornithodoros*, which transmit African swine fever with a mortality of almost 100% (Gaudreault et al. 2020).

Ticks induce skin damage in vertebrates and counteract haemostasis and inflammation (Chmelaf et al. 2016). Without external suppression, these processes would cause tick rejection, disrupt food intake, and arrest development. For this reason, ticks have evolutionary-developed sophisticated defence strategies. For example, tick saliva contains proteins that inhibit haemocoagulation (Prevot et al. 2016; Decrem et al. 2008), modulate angiogenesis (Chmelaf et al. 2016) and affect normal defensive functions of B-cells, T-cells and dendritic cells (Anguita et al. 2002; Hannier et al. 2004; Hovius et al. 2008a, b).

These are all reasons why we need to pay attention to the issue of ticks, the diseases they transmit, treatment and also prevention in the form of vaccines. The aim of the present review is to summarize current knowledge on vaccines against ticks with emphasis on novel approaches in vaccine design based on disrupting the regulation of the tick digestive mechanisms.

Vaccination against ticks and tick-borne diseases

Tick antigen-based vaccines

Acaricides are a commonly-used and effective measure to combat ticks. As significant resistance of ticks to these substances has recently been reported (Rodríguez 2016; Rodriguez-Vivas et al. 2018), including the accumulation of acaricides in the environment and animal products (Abbas et al. 2014), the scientific community has focused on searching for new control measures against these ectoparasites. An anti-tick vaccine is based on the presumption that the host develops immunity to the tick and thus prevents it from feeding on the host blood. The first proposal was published in 1939 (Trager 1939). After many decades, the first anti-tick vaccine targeting the gut protein Bm86 of the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini, 1888) was developed (Kemp et al. 1989; de la Fuente et al. 1999).

One option in identifying potential anti-tick vaccines is to analyse antigens using the RNA interference (RNAi) method. Although RNAi is a method used primarily to study genes that affect important physiological functions, these genes may not be immunogenic (Aljamali et al. 2002). Antigens are screened by this method for sequence identity,
directly in the transcriptome or cell proteome (Antunes et al. 2019). It is possible to use a so-called cocktail vaccine against several tick antigens at the same time—the advantage of which is usually higher efficacy (Sherrard-Smith et al. 2018). This type of broad-spectrum vaccine also makes it possible to establish protection against several tick species (Ndawula and Tabor 2020). However, in the case of the use of cocktail vaccines, there may be an undesirable reduction in antibodies to related types of antigens, which may significantly reduce the effectiveness of vaccination (Ndawula and Tabor 2020).

The study of the role of proteins found in tick saliva has become a powerful tool in vaccine design in the prevention of tick-borne diseases, because saliva creates the pathway of pathogen transmission into the host blood (Labuda et al. 2006). Tick saliva is a carrier medium for the transmission of pathogens (Nuttall and Labuda 2008). Anti-tick vaccines have been designed based on the observation that some mammals were able to develop immunity against tick-feeding after being bitten (Anguita et al. 2002). At the same time, it has been found that the highest probability of pathogen transmission by ticks is during their contact with a naïve host, i.e. a host that has never had ticks before (Leboulle et al. 2002). The reason why vaccination against ticks is crucial is due to the fact that it is easier to vaccinate against ticks than against every possible pathogen they transmit.

One of the most widely used vaccines is the Gavac™ vaccine, which was developed against the cattle tick in Cuba. The vaccine reduces tick infestation by reducing the ability to feed and by preventing females from reproducing (De la Fuente et al. 1999). It is a recombinant vaccine based on the gut protein Bm86 of B. (R.) microplus (Willadsen et al. 1995). The antibodies recognize the Bm86 protein present in the tick gut cells to which they bind and form irreversible lesions that damage the gut wall. A secondary consequence in tick females is reduction in oviposition leading to infertility (Rand et al. 1989; Rodríguez et al. 1994; Rodríguez et al. 1995). The antibodies recognize the Bm86 protein present in the gut protein Bm86 of B. (R.) microplus (Willadsen et al. 1995). The antibodies recognize the Bm86 protein present in the tick gut cells to which they bind and form irreversible lesions that damage the gut wall. A secondary consequence in tick females is reduction in oviposition leading to infertility (Rand et al. 1989; Rodríguez et al. 1994; Rodríguez et al. 1995). The antibodies recognize the Bm86 protein present in the gut protein Bm86 of B. (R.) microplus (Willadsen et al. 1995). The antibodies recognize the Bm86 protein present in the gut protein Bm86 of B. (R.) microplus (Willadsen et al. 1995). The antibodies recognize the Bm86 protein present in the gut protein Bm86 of B. (R.) microplus (Willadsen et al. 1995). The antibodies recognize the Bm86 protein present in the gut protein Bm86 of B. (R.) microplus (Willadsen et al. 1995). The antibodies recognize the Bm86 protein present in the gut protein Bm86 of B. (R.) microplus (Willadsen et al. 1995). The antibodies recognize the Bm86 protein present in the gut protein Bm86 of B. (R.) microplus (Willadsen et al. 1995). The antibodies recognize the Bm86 protein present in the gut protein Bm86 of B. (R.) microplus (Willadsen et al. 1995). The antibodies recognize the Bm86 protein present in the gut protein Bm86 of B. (R.) microplus (Willadsen et al. 1995).

In this paper, we deal with tick-borne diseases as follows: viral, bacterial, protozoan, and focus mainly on zoonotic pathogens and the current state of vaccine availability against them. The work is mainly focused on pathogens that cause diseases in humans. Theileriosis and babesiosis were also added because they are very important from the economic point of view, causing significant economic damage.

**Viral diseases**

**Crimean-Congo haemorrhagic fever**

Crimean-Congo haemorrhagic fever (CCHF) is a severe viral disease transmitted by ticks with an incubation period of less than seven days and a mortality of up to 50% (Ergonul 2006). It occurs in Asia, Africa, and Europe. The name is derived from a combination of the names „Crimea“, the location where the disease was first described, and „Congo“, where the virus was isolated from a febrile patient (Chumakov 1949). The disease is caused by a ssRNA nairovirus, CCHF virus. The disease is transmitted by ticks of the genus *Hyalomma* (family Ixodidae), in particular *H. marginatum* (Koch, 1844). The main targets of the virus are mononuclear phagocytes, mucous membranes, and hepatocytes (Burt et al. 1997).

The disease begins suddenly with fever, headache, shoulder and back pain, vomiting, and diarrhea may also occur (Hoogstraal 1979a, b). The affected person has a reddened face, congested conjunctiva and pharynx, sometimes with minor bleeding. Bleeding from the gums, nose, lungs, bladder, and intestines occurs in severe forms of the disease (Hoogstraal 1979a, b). However, these symptoms affect only a small percentage of patients. Nevertheless, the prognosis is uncertain in all cases. It is possible to predict the fatal development of the disease on the fifth day of infection. The cut-off blood parameters for this assay are: platelets < 20 × 109 / L, aspartate aminotransferase > 680 U/L, fibrinogen < 110 mg/dl or less (Ergonul et al. 2006). On the seventh day of infection, a haemorrhagic phase occurs. Death occurs on the tenth day. The disease can be confirmed by PCR, similar to other diseases (Mazzola and Kelly-Cirino 2019).

There is no vaccination against the disease (Table 1), and there has been no significant progress in the treatment of the disease in the last ten years. Fortunately, hope for a mRNA vaccine was described in 2020 (Tipih and Burt 2020).

**Tick-borne encephalitis**

TBE is a viral inflammation of the brain. It is a seasonal disease for which no effective treatment is known. The first case was described by the Austrian, H. Schneider, in 1931.
as "meningitis serosa epidemica" of unknown etiology (Schneider 1931).

We now know that the disease is caused by the tick-borne encephalitis virus (TBEV) of the genus Flavivirus with three known subtypes, which most often enter the body after the attachment of an infected tick or the consumption of raw milk from infected sheep, cows, and goats. The virus subtypes are named after the geographical origin and include the European subtype (TBEV-Eu), the Siberian subtype (TBEV-Sib), and the Far East subtype (TBEV-Fe) (Süss 2011). The vector of TBEV-Eu is *Ixodes ricinus*, while the remaining two subtypes are transmitted mainly by *I. persulcatus* (Schulze, 1930) and *I. ovatus* (Neumann, 1899) (Süss 2011). These species of ticks play a key role in the spread and epidemiology of the disease (Gresikova and Kaluzova 1997; Labuda and Randolph 1999).

The reservoir hosts of the virus are mainly rodents. There is no human-to-human transmission. The individual subtypes differ in terms of symptoms at an early stage. TBEV-Fe causes various chronic diseases, with the frequency of meningeal and focal forms not exceeding 26% and 64%, respectively (Pogodina et al. 2004). Complete recovery from the disease occurs in 25% of all cases (Ternovoi et al. 2007). Mortality reaches up to 30% (Dumpis et al. 1999). In the case of TBEV-Sib, focal encephalitis accounts for 5% of cases, while meningeal forms up to 47% of cases. Complete recovery occurs in more than 75% of infected patients and mortality is less than 2% (Pogodina et al. 2004). TBEV-Eu, with an incubation period of 7 to 28 days, causes an atypical influenza-like disease lasting approximately 4 days. Symptoms include fever, joint pain, headache, leukocytopenia, thrombocytopenia, and elevated liver enzymes as common symptoms. This is followed by an asymptomatic phase which lasts one week, and 70% of patients do not develop further symptoms. However, meningitis, meningoencephalitis, meningoencephalomyelitis, or meningoencephaloradiculitis occur in 30% of patients. Specific antibodies are found in cerebrospinal fluid. Mortality in adults is less than 2%; however, only a small proportion of patients recover completely from the disease without chronic neurological sequellae (Mickiene et al. 2002; Lindquist and Vapalahti 2008).

There is, however, an effective, three-dose, antigenic vaccine (Table 1) (FSME-Immun) that was developed in Austria against TBEV-Eu, which was approved as early as 1976 (Kunz

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**Table 1 Overview of tick-borne diseases**

| Disease                              | Pathogen                       | Principal vectors                                                                 | Vaccine                  |
|--------------------------------------|--------------------------------|-----------------------------------------------------------------------------------|-------------------------|
| Crimean-Congo haemorrhagic fever     | ssRNA nairovirus, CCHF virus   | *Ixodes ricinus, I. persulcatus*                                                  | no vaccination          |
| Tick-borne encephalitis              | Tick-borne encephalitis virus  | *Ixodes ricinus, I. persulcatus*                                                  | FSME-IMMUN, Encepur, EnceVir |
| Lyme borreliosis                     | *Borrelia burgdorferi, B. afzelii,* | *Ixodes ricinus, I. persulcatus*                                                  | no vaccination          |
|                                     | *B. garinii*                   |                                                                                    |                         |
| Anaplasmosis                         | *Anaplasma phagocytophilum*    | *Ixodes ricinus, I. persulcatus*                                                  | no vaccination          |
| Tick-borne rickettsiosis             | *Rickettsia rickettsii,*       | *Ixodes ricinus, I. persulcatus*                                                  | no vaccination          |
|                                     | *R. peacockii,*                |                                                                                    |                         |
|                                     | *R. montana,*                  |                                                                                    |                         |
|                                     | *R. rhipicephali*              |                                                                                    |                         |
| Babesiosis                           | *Babesia divergens,*           | *Ixodes ricinus, I. scapularis,*                                                 | Gavac™ (*R. microplus*) |
|                                     | *B. microti,*                  |                                                                                    |                         |
|                                     | *B. bovis*                     |                                                                                    |                         |
| Theileriosis                         | *Theileria annulata,*          | *Ixodes ricinus, I. scapularis,*                                                 | Mugaga (*T. parva*)    |
|                                     | *T. parva,*                    |                                                                                    |                         |
|                                     | *T. equi*                      |                                                                                    |                         |
|                                     | *Rhipicephalus sanguineus*     | *(T. parva)*                                                                      |                         |
|                                     | *(T. equi)*                    |                                                                                    |                         |
|                                     | *Amblyomma americanum*         | *(T. rickettsi)*                                                                 |                         |
|                                     | *(R. rickettsi)*               |                                                                                    |                         |
|                                     | *(R. peacockii)*               |                                                                                    |                         |
|                                     | *(R. loxicephali)*             |                                                                                    |                         |
|                                     | *(R. rhipicephali)*            |                                                                                    |                         |
|                                     | *(R. microplus)*               |                                                                                    |                         |

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et al. 1976). The reason why Austria was the first to initiate vaccine development is the fact that this country had the highest mortality from TBE of all European countries (Kunz 2003). To date, the vaccine has undergone several modifications (Zent and Bröker 2005), using human albumin (source) to stabilize it. Its efficacy in adults (Loew-Baseli et al. 2006) and children under 15 years of age is close to 100% during the first year. Antibodies in the body of both adults and children remain active after three years and provide protection up to 94%—98% (Loew-Baseli et al. 2011).

A newer alternative (approved in 1994) to FSME-Immun is the German vaccine Encepur, which is intended for protection against TBEV-Eu. It does not currently contain any stabilizers (Amicizia et al. 2013). Its efficacy is almost 100% during the first year (Schöndorf et al. 2007), but has improved long-term protection, representing up to 98% after 5 years of vaccination (Schöndorf et al. 2007). No pharmacokinetic interaction between FSME-Immun and Encepur vaccines has been demonstrated, and therefore, both vaccines are routinely administered concomitantly (Prymula et al. 2012) with recommended revaccination after 5 years (Plentz et al. 2009).

Effective vaccines against TBEV-Sib and its subfamily are produced in Russia, including TBE Moscow Vaccine® against the Sofjin strain (TBEV-Fe), as well as the strain-based EnceVir (TBEV-Fe 205). However, to date, no randomized studies have been performed on these vaccines and therefore, their licenses are valid only in Russia. Efficacy is 84%—92% one year after vaccination (Krasilnikov et al. 2004).

State-of-the-art vaccines against TBE focus on the processes that take place immediately after the tick has been attached to the host. One possibility is to focus on the formation of the cement cone, which fixes the tick in the skin of the host. The 64P cement protein identified in Rhipicephalus appendiculatus (Neumann, 1901) is a suitable candidate for the preparation of broad-spectrum anti-tick vaccines (Rego et al. 2019) because its homologues are highly conserved in various ticks. The recombinant 64TRP vaccine based on the 64P cement protein significantly reduced the transmission of TBEV by I. ricinus in the laboratory mouse model. Immunized animals developed inflammatory and immune reactions at the tick-bite site, which counteracted tick feeding. The protective effect of this vaccine was comparable to FSME-Immun (Labuda et al. 2006). In addition to restricting tick feeding, there is a cross-immune reaction with antigenic epitopes of the tick midgut. A cross-immune response leads to gut rupture and subsequent tick death (Trimnell et al. 2002).

**Bacterial diseases**

**Lyme borreliosis**

LB is caused by spirochetes of the *Borrelia burgdorferi* sensu lato (s.l.) (Burgdorfer et al. 1982) complex, predominantly in the Northern hemisphere (Rosa et al. 2005; Rizzoli et al. 2011; Steere et al. 2016). It is the most common disease transmitted by ticks. The name “Lyme disease” historically comes from the epicentre of infected children in the American city—Old Lyme (USA, CT), where the disease was first described as a separate entity, despite the fact that scientific evidence has been found since the early twentieth century (Heyman et al. 2010). The incidence of the disease is dominant in northern and temperate climates. Dominant vectors are ticks of the *Ixodes* genus. In Europe, it is *I. ricinus* (Hofhuis et al. 2017) and in Asia, *I. persulcatus* (Rumer et al. 2011), while in the USA, *I. scapularis* (Say, 1821) and *I. pacificus* (Cooley and Kohls, 1943) (Hahn et al. 2016).

A typical manifestation of the disease is a migratory and gradually increasing inflammatory spot—erythema migrans in the skin around the tick bite area. However, 30% of people with LB do not develop erythema (Marques 2015). Therefore, the diagnosis and subsequent treatment are more difficult. Up to 20% of people have symptoms that persist for up to a year after overcoming the disease (Marques 2008). Multisystemic inflammatory symptoms of the disease begin 2 to 30 days after infection.

From a diagnostic point of view, serological determination is not possible at an early stage. The only indicators are clinical manifestations of the disease and a positive tick-bite history. However, the later stages can be determined from intrathecal fluids and blood. In the case of early treatment, treatment with doxycycline, penicillin antibiotics, or azithromycin is possible (Steere 2001) If left untreated, severe heart, CNS, and bone-joint problems develop within a few years (Steere 2001; Steere et al. 2016). If the nervous system is affected, intravenous treatment with Ceftriaxone for 2.5 weeks is recommended. Geographically, symptoms are different in Europe from the US due to the occurrence of different *Borrelia* genotypes on different continents (Steere 2001; Fingerle et al. 2008). In Europe, reservoir hosts of *B. burgdorferi* s.l. include hares, rodents (especially for *B. afzelii*), and insectivores, as well as several species of birds (especially for *B. garinii*) (Gern 2008). Spirochetes are inactive in the tick during the entire fasting period; however, after feeding, they multiply in the midgut, from where they migrate within 48 h from the start of feeding to salivary glands (Piesman and Dolan 2002; Wormser et al. 2007).

With early removal of the tick, no significant disease transmission has been demonstrated. Preventive treatment is only used for patients with ticks removed more than 24 h after attachment. Standard anti-*Borrelia* vaccines are based on an immunogenic protein of the bacterium and are therefore geographically linked due to the diversity of *Borrelia* genotypes and cannot be used anywhere in the world.

After infection of *I. scapularis* larvae, the spirochetes migrate to the midgut. The transfer of *Borrelia* and its establishment in the gut is enabled by the interaction of the
outer-surface protein A (OspA) of *Borrelia* with the tick receptor for outer surface protein A (TROSPA) (Pal et al. 2004). From the midgut, spirochetes are transported to the salivary glands and subsequently to the host (Dunham-Ems et al. 2009). The transmission of the genospecies *B. afzelii* in *I. ricinus* has not yet been clearly elucidated (Pospisilova et al. 2018, 2019). Expression of Salp15, Ospa, TSLPI, and tHRF proteins in the tick ensure spirochete transmission and survival in the host (Wagemakers et al. 2016). A candidate for vaccine production is the salivary gland protein Salp15, which binds to the outer surface protein C (OspC) found on the surface of *B. burgdorferi* (Ramamoorthi et al. 2005). Salp15 has been shown to promote *B. burgdorferi* infection (Dai et al. 2009). Salp15 homologues identified in *I. ricinus* do not have the same function to increase the infectivity of *B. afzelii* and *B. garinii* (Hovius et al. 2008a, b). Administration of Salp15 antiserum has been shown to provide significant protection against *Borrelia* in mice. The combination of Salp15 and Ospa increases the effectiveness of immunization (Dai et al. 2009). Salp15 inhibits T cell activation by binding to the CD4 co-receptor on host T cells. Subsequently, calcium flux induced by IL-2 and TCR production is repressed (Garg et al. 2006; Boulanger 2018; Wen et al. 2020).

An OspA-based vaccine was developed by LYMErix TM (Fikrig et al. 1990), but was later withdrawn from the market due to its epitope with lymphocyte function-associated antigen 1 (LFA1). Subsequently, vaccines lacking the described epitope have been developed (Comstedt et al. 2017).

The most modern approach in vaccination against LB is a six-component vaccine based on OspA-ferritin nanoparticles, which was developed by Czech scientists in 2020 and partially prevents the transmission of *B. afzelii* and *B. burgdorferi* sensu stricto. Its efficacy has been tested and demonstrated in mice (Kamp et al. 2020).

At the same time, the possibilities of limiting the transmission of the pathogen by influencing the metabolic pathways of the tick are being investigated. A tick mannose-binding lectin inhibitor (TSLPI) has been identified in the salivary glands of *I. scapularis* (Schuijt et al. 2011). A TSLPI homologue in *I. ricinus* has also been identified (Wagemakers et al. 2016). TSLPI inhibits mannose-binding lectin (MBL) activity. Decreased TSLPI expression or inhibition has also been identified A TSLPI homologue in *I. ricinus* has been demonstrated in mice when vaccinated with their recombinant forms (Trentelman et al. 2020).

### Anaplasmosis

Granulocytic ehrlichiosis, later called human granulocytic anaplasmosis (HGA), is caused by the bacterium *Anaplasma phagocytophilum* (Foggie, 1949) (Rickettsiales). There are very few cases of anaplasmosis in humans in Europe, and more in the United States. In Europe, the bacterium is important especially from a veterinary point of view, as it causes the so-called tick-borne fever in cattle. (Stuen et al. 2013).

HGA is manifested by vomiting, fever, urinary truss, but also by neurological disorders and heart rhythm disorders as well. The disease occurs 10 to 12 days after infection. The bacterium attacks neutrophils and causes thrombocytopenia, leukopenia, and increases in liver enzymes GOT and GPT (Bakken and Dumler 2000, 2015). Death has been observed mainly in immunodeficient patients (Bakken and Dumler 2000; Dumler et al. 2001; de la Fuente et al. 2016).

*Ixodes ricinus* is the vector for *A. phagocytophilum* in Europe (Heyman et al. 2010), *I. scapularis* in America (Turck et al. 2019) and *I. persulcatus* and other *Ixodes* spp. in Asia (Dugat et al. 2015). In most cases, the nymph stage is responsible for the transmission of this disease (Gray 2002). The characteristic transmission of *A. phagocytophilum* is horizontal, i.e. from the infected host to the tick, where it passes from the gut to the salivary glands and subsequently infects a new host (Telford et al. 1996). There are many strains of the bacterium in Europe, having many different animal species as their reservoirs, however, the competence of rodents as reservoirs is questionable. It has been proven that reservoirs in an urban environment can be, e.g., domestic dogs and birds (Silaghi et al. 2008; Massung et al. 2006; Paulauskas et al. 2009). *Anaplasma phagocytophilum* can infect domesticated and wild animals and was detected in cattle and deer (de la Fuente et al. 2005a, b; Naranjo et al. 2006). Some strains are pathogenic to carnivores, others.
to cattle or sheep, but most are not pathogenic to humans. That is why the number of cases of human anaplasmosis in Europe is so low compared to the USA (Stuen et al. 2013). In the United States, the Peromyscus leucopus (Rafinesque, 1818) mouse is the reservoir for the pathogenic strain of the bacterium and the deer Odocoileus virginianus (Zimmermann, 1780) is the reservoir for the non-human pathogenic bacterial strain (Massung et al. 2005).

In the USA, the infection has a mortality rate of 9%, while no deaths have been documented in Europe (Bakken and Dumler 2000, 2015). The reason for this difference is still unclear, despite frequent serological evidence of the disease in European residents (van Dobbenburgh et al. 1999; von Loewenich et al. 2003; Amiel et al. 2004; Heyman et al. 2010). Interestingly, in acute European cases, the presence of morulae in granulocytes (a hallmark of patients in the USA) has been reported in only 30% of patients (Heyman et al. 2010). The infection may be persistent in patients (Dumler and Bakken 1996). Symptomatic patients suspected of having HGA should receive immediate antibiotic therapy with Doxycycline (Wormser et al. 2007).

There is currently no vaccine (Table 1) against diseases caused by A. phagocytophilum (Stuen et al. 2015). However, an outer membrane protein (OmpA) of the bacterium has been detected. Antigen therapy against OmpA has shown reduced ability of A. phagocytophilum to infect host cells (Ojogun et al. 2012). The invasive proteins, AipA and Asp14, which provide permeability of the host cell, were also detected. The use of antisera against these proteins has significantly reduced the infectivity of the bacterial culture (Seidman et al. 2014).

The peptides, Salp16, P11, and lipocalins, which were detected in the salivary glands and gut of I. scapularis and are involved in the tick-pathogen interaction ensuring the survival of A. phagocytophilum, have also been described relatively well and could serve as perspective vaccine targets. Expression of Salp16 is induced in the salivary glands of the tick after interaction with A. phagocytophilum. The bacterium migrates from the infected host to the gut of the tick and subsequently to the salivary glands, from where it can infect the new host. By suppressing Salp16 expression, bacteria are unable to infect the salivary glands of I. scapularis and subsequently the host (Sultana et al. 2010). The peptide P11 has been shown to improve the uptake of A. phagocytophilum by I. scapularis haemocytes. Infected haemocytes are subsequently transported to the salivary glands (Liu et al. 2011). Lipocalins, which include inhibitors of the lectin pathway of the complement, have also been discovered in recent years in saliva of I. scapularis and a homologue was discovered in I. ricinus (Beaufays et al. 2008; Schwarz et al. 2013). Lipocalins are a family of proteins that play an important role in tick feeding. They are excreted in saliva. By binding to histamine or serotonin, they protect ticks from host immune and inflammatory reactions (Paesen et al. 2000; Valdés 2014). The expression of lipocalins is associated with tick infectivity, yet the exact mechanism of action is still unknown (Contreras et al. 2017).

**Tick-borne rickettsioses**

*Rickettsia* are intracellular alpha-proteobacteria transmitted to humans by arthropod vectors, predominantly fleas and hard ticks (Portillo et al. 2015; Ereemeva and Dasch 2015). The most known disease caused by *Rickettsia* is endemic typhus (*R. typhi*) (Wolbach and Todd, 1920), which is transmitted to humans from rodents by fleas (Robinson et al. 2003). In addition to typhus, pathogenic *Rickettsia* transmitted by ticks cause spotted fever in humans. Many other *Rickettsia* and *Rickettsia*-like species have been identified in tick organs, but their pathogenicity is still unknown (Parola et al. 2005).

Depending on the locality in which the spotted fever was identified, an adjective was added to the disease, e.g., Mediterranean fever, African fever, Rocky mountain fever, and so on. The vectors of these pathogens are, e.g., *Dermacentor variabilis* (Say, 1821), *Dermacentor andersoni* Stiles, 1908, *Rhipicephalus sanguineus* (Laterell, 1806) and *Amblyomma americanum* (Linnaeus, 1758), in which transovarian transmission of the pathogens occurs (de la Fuente et al. 2017a, b). Due to the significant difference in genotypes across geographical locations, it is necessary to specify the type of disease in patients according to their travel history before further treatment (De Sousa et al. 2003).

Spotted fever is a disease accompanied by fever, lymphadenopathy, and numerous rashes all over the body. Symptoms start on the tenth day after the tick bites. Mortality from the disease has previously been studied in Portugal in a study of 105 patients and accounted for up to 32% (De Sousa et al. 2003). Serological examination can determine the presence of the disease up to two weeks after infection. The diagnosis is therefore based on clinical signs at an early stage.

In the case of *Rickettsia*, the tick is not only a vector, but also a permanent reservoir. Therefore, since there is no effective vaccine against the disease, prevention and the use of antibiotics are the most important options (Botelho-Nevers et al. 2012; Sahni et al. 2013). Standard treatment involves high doses of doxycycline or tetracycline (Dantas-Torres 2007). Mortality increases with delay in treatment and is caused by multiorgan failure. It has been shown that it is possible to acquire infection with several species of *Rickettsia* from one vector; however, one species of *Rickettsia* can block another one in the host during the disease (Macaluso et al. 2002).

Attempts to develop a vaccine against *R. rickettsii*, the causative agent of Rocky Mountain spotted fever, based on killed bacteria did not reduce indicators of mortality from
this disease (Walker 2009). *Rickettsia rickettsii* infect cells by endocytosis (Petchampai et al. 2015) through actin-based motility (Ireton 2013). Potential new vaccination options are being studied on the actin-related protein 2/3 complex (Arp2/3), which is part of the actin cytoskeleton of eukaryotic cells (Petchampai et al. 2015). It is this complex that is involved in the interaction and infection with various *Rickettsia* species (Petchampai et al. 2014).

**Protozoan diseases**

**Babesiosis**

Babesiosis is a very common disease in livestock, but disease in humans in Europe is rare (Hunfeld et al. 2008). *Babesia divergens* (M’Fadyean and Stockman, 1911) is transmitted from natural reservoirs to humans mainly by *I. ricinus* (Zintl et al. 2003; Bock et al. 2004); however, humans are not their natural hosts (Yabsley and Shock 2012). In contrast, in the USA human babesiosis is common and is caused mainly by *Babesia microti* (Franca, 1910), with *I. scapularis* as its main vector (Yang et al. 2021). In subtropical and tropical regions, *Babesia bovis* V. Babes, 1888 and *B. bigemina* (Smith and Kilborne, 1893) Wenyon, 1926 causing disease in cattle, are mainly transmitted by *Rhipicephalus* spp. (Wise et al. 2014).

The causative agents of the disease are single-cell blood parasites—babesiae (Apicomplexa, Sporozoza, Piroplas- 
midae, Babesidae), which mainly attack the red blood cells of the host, as well as other cells of the reticuloendothelial system. *Babesia bovis*, for example, accumulates and multiplies in the lumen of the tick gut (Piesman et al. 1986). In the case of penetration into erythrocytes of the vertebrate host, they multiply asexually (Mehlhorn and Shein 1984).

Since babesiosis occurs in both humans and cattle, it is considered a medical threat and an economic danger to the agricultural industry (Bock et al. 2004). *Babesia bovis* causes the greatest loss to meat and milk production (Gohil et al. 2013).

The severity of the disease in humans varies significantly, depending on the type of babesia. The disease can be asymptomatic to fatal, depending on the patient’s immunity and comorbidities (Zintl et al. 2005; Gray 2006). A severe form with concomitant fever, jaundice, and haemolytic anaemia is symptomatic of malaria. Despite being a rare disease in Europe, it has gained scientific interest through high mortality (Vannier et al. 2015).

The onset of infection is manifested by fatigue, headache, fever, and gastrointestinal problems (Hunfeld et al. 2008). With the progressive destruction of erythrocytes, haemoglobinuria to hepatitis can also occur (Gray 2006). In the more severe forms, renal dysfunction, myocardial infarction, disseminated intravascular coagulation, or acute respiratory failure may occur as well. Transmission of the parasite is also possible within the tick from a fertilized female to eggs (transovarian transmission) (Zintl et al. 2003).

At present, there is no vaccination against babesia (Table 1). The only currently-available method of pro-
tection against babesiosis in cattle is the GavacTMC vaccine (see above) against the tick, *R. microplus*, which reduces tick infestations by reducing the ability to feed, as well as preventing females from reproducing (de la Fuente et al. 1999). However, the general efficacy of a vaccine based on Bm86 varies and depends on the heterogeneity of the Bm86 gene between strains of *R. microplus* (Kaewmongkol et al. 2015). Subsequent experiments have shown that the Bm86 homologue in *I. ricina* is not a suitable target for vaccine design as it did not provide any adequate immune response (Coumou et al. 2015). This is explained by the fact that *R. microplus* and *I. ricinus* ticks have different life cycles and susceptibility to gut damage (de la Fuente et al. 2007).

**Theileriosis**

Although theileriosis is not a disease of medical concern, it is a very important veterinary problem. Tropical theileriosis is a disease caused by the pathogens *Theileria annulata* (Dchunsky and Luhs, 1904) and *Theileria parva* (Theiler, 1904) (Bettencourt, Franca & Borges, 1907) and the vectors of these pathogens are ticks of the genera *Rhipicephalus* and *Hyalomma* (Ouelli and Pandey 1982; Mustafa et al. 1983; Ouelli 1985). In ticks of the genus *Hyalomma*, *Theileria* pass transtadial transmission, from the larva to the adult tick (Bhattacharyulu et al. 1975). Theileriosis causes serious economic problems not only in African but also in Asian countries (Minjauw and Mcleod 2003; Cicek 2009; Gharbi et al. 2011). *Theileria parva* causing East Coast fever occurs in Sub-Saharan Africa and its main vector is *R. appendiculatus* (Dolan 1989). *Theileria annulata* causing tropical theileriosis occurs in northern Africa, southern Europe, the Middle, East, South and some parts of Asia and is transmitted mainly by *Hyalomma* species (George et al. 2015; Kumar et al. 2016; Dandasa et al. 2018).

*Theileria* go through three stages in their life cycle and each developmental stage elicits a different specific immune response (Pipano and Shkap, 2000). Sporozoites of *T. annulata* occur in salivary glands of ticks, schizonts are found in monocytes (macrophages) and lymphocytes (Sager et al. 1998), and merozoites in erythrocytes of the vertebrate hosts (Pipano and Shkap 2006). The most serious damage to cattle is caused by the schizont stage. The schizonts react with the microtubules of the host cells and divide with the host cell during mitosis, thus ensuring the persistence of the infection. In the case of long-term cultivation of lymphoid cells that are infected with schizonts, their virulence is lost, i.e.
schizonts from the blood elicit a stronger immune response than cultured ones (Pipano and Shkap 2000). It is against this stage that attenuated vaccines have been developed in many countries around the world (Gupta et al. 1998; Darghouth 2008). Theileria annulata affects livestock, mainly cattle and buffaloes (Darghouth et al. 2011). For example, in untreated calves, daily live weight gain was found to be reduced by 14.7% and milk yields of only 0.7 L per day were also registered (Gharbi et al. 2011).

Currently, acaricides as well as vaccination with attenuated T. annulata-infected cell lines are used to control the spread of infected ticks (Gharbi et al. 2011; Darghouth et al. 2011; Mhadhbi et al. 2010; Bilgic et al. 2019). For example, the attenuation of the Tunisian cell line infected with T. annulata schizonts protected almost 90% of Holstein cattle (Darghouth 2008). Although attenuated vaccines may even have an efficacy of about 100% in homologous parasites, their efficacy decreases in heterogeneous ones (Darghouth et al. 1996). Veal breeds in Sudan immunized with attenuated T. annulata only protected cattle from possible death (El Hag 2010). Importantly, not all live vaccines can eliminate the infection, they can only reduce the risk of tropical theileriosis (Gharbi et al. 2020).

Both Theileria species mentioned above are transmitted through tick saliva, infect lymphocytes and form macro-schizonts in the vertebrate host. This causes uncontrolled cell proliferation and immortalization (Spooner et al. 1989; Zweygarth et al. 2020). The disease they cause has the same diversity of the vaccine (Hemmink et al. 2016; Steinaa et al. 2016). However, the efficacy of this vaccine was not sufficient high (Sitt et al. 2015). This may be due to the limited diversity of the vaccine (Hemmink et al. 2016; Steinaa et al. 2018; Bilgic et al. 2019; Roy et al. 2019, 2021).

**Ticks and COVID-19 transmission**

In December 2019, a serious pneumonia disease, SARS-CoV-2, developed in Rhinolophus affinis (Horsfield, 1823) bats and spread around the world in a short time. Ticks have been suggested as one of the vectors of this disease (Aghajani et al. 2020; Villar et al. 2020). They could, for example, transmit the virus to pets, dogs, or cats. However, these pets are dead-end hosts and do not pose any risk of transmitting this pathogen to humans. Ectoparasitic cat fleas could be another SARS-CoV-2 rescuer (Villar et al. 2020).

Other results suggest that passive transmission of the virus is also possible by contact with surfaces that are contaminated with SARS-CoV-2 (Lam et al. 2021). This is also due to the stability of the virus (Goldman, 2020; Fernández-de-Mera et al. 2021).

To date, however, there is no reliable evidence that ticks or other ectoparasitic arthropods are involved in transmission of COVID-19 coronavirus infection (Wormser et al. 2021).

**Potential targets exprimed in tick digestive system for novel vaccine research**

Understanding the regulation of the tick's digestive mechanisms is currently the most powerful tool for combating the tick as a parasite. For example, the B. burgdorferi spirochaete is a highly motile pathogen that is able to move through host tissues and fluids (Nakamura 2020). If ticks are feeding on an infected host, the pathogens use chemotaxis and migrate toward or away from a chemical stimulus and as a result, transmission of the infection occurs (Murfin et al. 2019). It is the tick gut that is the primary site of pathogen colonization (Sharma et al. 2019) and can avoid the immune response of ticks and other hosts (Smith et al. 2016; Shaw et al. 2017). The spirochetes remain in the gut lumen during tick feeding and subsequently pass into the salivary glands (De Silva and Fikrig 1995).

Tick salivary gland secretions are essential for suppressing host immune responses (Bishop et al. 2002; Trimnell et al. 2005). The salivary glands form clusters of three types of acini: the agranular acini type I, which do not change in size during food intake and the granular acini type II and III, which increase in size with food intake. They are involved in the synthesis of bioactive components and the excretion of excess water and ions (Binnington 1978; Krolak et al. 1982). Saliva production is regulated by central neuropeptides present in innervation of the salivary glands from the synganglion (Ŝimo et al. 2013, 2014).

**Neuropeptides**

Neuropeptides and peptide hormones belong to the signalling molecules that control cellular communication in all multicellular organisms (Garczynski et al. 2019). Neuropeptide production begins with the synthesis of an inactive precursor molecule in the endoplasmic reticulum. Subsequently, this precursor is transported to the Golgi
apparatus and secretory granules are formed there. These secretory granules are transported from the Golgi apparatus by endocytosis. Secretory vesicles contain precursor proteins with convertases that cleave bioactive peptides from the precursor molecule (Hökfelt et al. 2000). The finished signalling molecules act on target cells through interaction with specific membrane receptors, for example G-protein coupled receptors (GPCRs) (Hewes and Taghert 2001). Tick neuropeptides are suitable target molecules for the design of vaccines or drugs that regulate the transmission of pathogens into the host's blood. However, because some arthropod neuropeptide sequences are similar to those in mammals, it is necessary to exclude the effects of antibodies to the homologous sequences of mammalian peptides when selecting a target antibody to a particular tick neuropeptide. In ticks, neuropeptides are produced by neurosecretory cells, interneurons of the central nervous system (CNS), as well as motor and sensory neurons, but also by endocrine cells of the intestine and cells of other peripheral organs (Schoofs et al. 2017). Insects temporarily store neuropeptides in their neurohormonal organs of the retrocerebral complex: corpora cardiaca (CC) and corpora allata (CA) and transverse nerves from where they are secreted into the haemolymph (Stay and Tobe 2007). They are produced in the form of prepropeptide precursors that are post-translationally cleaved and modified in the endoplasmic reticulum (Baggerman et al. 2005). The size of mature peptides in insects is 5 to 80 amino acids.

The regulation of cellular mechanisms by neuropeptides is complex. One neuropeptide can perform multiple functions. Conversely, multiple neuropeptides may be involved in the regulation of a single process (Schoofs et al. 2017). For a closer look, some immunoreactive neuropeptides found in various species of ticks are further described, which are also studied as potential "target" molecules for defence against the transmission of pathogens to the host. Initial application studies of neuropeptides in vaccine production were performed by Almazán et al. (2020). The effect of multiple antigen peptide (MAP) was tested based on the neuropeptides MIP and SIFamide (Hewes and Taghert 2001). Tick neuropeptides are suitable target molecules for the design of vaccines or drugs that regulate the transmission of pathogens into the host's blood. However, because some arthropod neuropeptide sequences are similar to those in mammals, it is necessary to exclude the effects of antibodies to the homologous sequences of mammalian peptides when selecting a target antibody to a particular tick neuropeptide. In ticks, neuropeptides are produced by neurosecretory cells, interneurons of the central nervous system (CNS), as well as motor and sensory neurons, but also by endocrine cells of the intestine and cells of other peripheral organs (Schoofs et al. 2017). Insects temporarily store neuropeptides in their neurohormonal organs of the retrocerebral complex: corpora cardiaca (CC) and corpora allata (CA) and transverse nerves from where they are secreted into the haemolymph (Stay and Tobe 2007). They are produced in the form of prepropeptide precursors that are post-translationally cleaved and modified in the endoplasmic reticulum (Baggerman et al. 2005). The size of mature peptides in insects is 5 to 80 amino acids.

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**Allatotropin**

Allatotropin (AT) has been identified in vitro as a stimulator of juvenile hormone (JH) biosynthesis in corpora allata (CA) in adult pharate females of *Manduca sexta* (Linnaeus, 1763) (Manse) (Kataoka et al. 1989). In addition to acting on CA, it accelerates the heart rate (cardioaccelerator function) (Koladich et al. 2002), stimulates the vibration of the ventral membrane, regulates movement, prevents ion transport through the middle intestinal epithelium and controls the release of digestive enzymes in the insect's midgut (Truesdell et al. 2000; Elekonich and Horodyski 2003; Weaver and Audsley 2007) and many others. AT and related neuropeptides regulate myoactive functions, development, food intake, muscle contractions, cardiovascular functions, circadian rhythms and inhibit ions (Bednár et al. 2017). AT has a myostimulatory effect at low concentrations and increase the amplitude of peristaltic movements (Duve et al. 1999). The pleiotropic function of AT peptides is indicated by the expression profile of the allatotropin receptor (ATR). ATR is highly expressed in the insect CA. The effect of AT on CA is mediated by Ca²⁺ phosphoinositide signalling (Rachinsky and Tobe 1996). To date, AT was found to elicit immune responses in two important vectors of pathogens, mosquitoes *Aedes aegypti* (Linnaeus, 1762) and *Anopheles albimanus* (Wiedemann, 1821) (Hernández-Martínez et al. 2017). The role of AT signalling may be expected in blood feeding ticks as well. AT immunoreactivity has been detected in the tick *R. appendiculatus* (Table 2, Fig. 1) in a number of synganglion neurons, as well as in peripheral nerves (Šimo et al. 2009a). A gene encoding ATs was identified in the genome of *I. scapularis* (Table 2, Fig. 1) (Šimo et al. 2014).

**Allatostatin A**

The main roles of insect allatostatins-A (AST-A) are myoinhibitory effects on internal organs, inhibition of endocrine functions and suppression of feeding (Duve et al. 1999; Bendena et al. 1999; Hergarden et al. 2012). Allatoregulatory peptides, including allatostatin A, B and C, are involved in the process of regulating tick feeding through the regulation of juvenile hormone in insects (Aguilar et al. 2003; Stay et al. 1992). AST-A in ticks was detected (Table 2) in peptidergic neurons in the synganglion and enteroendocrine cells in the midgut (Šimo et al. 2014). Some of the neurons (OsHG) innervate the hindgut of *R. appendiculatus* (Šimo and Park 2014). AST-A was also detected in *Dermacentor variabilis* (Say, 1821) (Zhu and Oliver 2001), *I. scapularis* (Fig. 1) and *R. microplus* (Donohue et al. 2010). In the genome of *I. scapularis*, four predicted and closely related AST-A receptor
| Neuropeptide       | Species of tick                                | Organ localization                                                                 | Function                                                                                                                                 |
|--------------------|-----------------------------------------------|------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| Allatostatin-A     | *D. variabilis* (Zhu and Oliver 2001)          | A large number of peptidergic neurons in the synganglion (Šimo et al. 2009a; Zhu and Oliver 2001), innervation of hindgut probably from the OsHG neuron (Šimo et al. 2014) | Expected function based on known properties in insects—myoinhibitory effect on movements of the gut or ovaries (Duve et al. 1999; Teal 2002) |
| (AST-A)            | *R. appendiculatus* (Šimo et al. 2009a)        |                                                                                    |                                                                                                                                          |
|                    | *R. microplus* (Donohue et al. 2010)          |                                                                                    |                                                                                                                                          |
|                    | *I. scapularis* (Šimo et al. 2014)            |                                                                                    |                                                                                                                                          |
| Allatotropin       | *R. appendiculatus* (Šimo et al. 2009a)        | A large number of peptidergic neurons in the synganglion and peripheral nerves (Šimo et al. 2009a), | Expected function based on known properties in insects—myostimulatory effect on peristaltic bowel movements or ovaries (Duve et al. 1999; Čižmár et al. 2019) |
| (AT)               |                                                                                      |                                                                                    |                                                                                                                                          |
| Pigment dispersing factor | *R. appendiculatus* (Šimo et al. 2009a) | Small neurons in the synganglion, innervation of SG type II acini from the neurons OsSG1,2 (Šimo et al. 2009a, b) | Currently unknown function                                                                                                               |
| (PDF)              |                                                                                      |                                                                                    |                                                                                                                                          |
| Myoinhibitory peptide | *R. appendiculatus* (Šimo et al. 2009a)    | Neurons in the synganglion, innervation of the SG acini type II, III by PcSG neuron (Šimo et al. 2009a, b), innervation of the rectal sac from PoHG1,2 neurons (Šimo et al. 2013, 2014) | SI Famide antagonist—reduces SI Fα-activated hindgut motility (Šimo and Park 2014)                                                     |
| (MIP)              | *I. scapularis* (Šimo et al. 2009b, 2014)    |                                                                                    |                                                                                                                                          |
| Tachykinin         | *R. appendiculatus* (Šimo et al. 2009a)       | Neurons in the synganglion which innervate its surface (Šimo et al. 2009a), and ducts in salivary glands (Mateos-Hernández, et al. 2021) | Currently unknown function                                                                                                               |
| (TK/TK-like)       | *I. scapularis* (Mateos-Hernández et al. 2021) |                                                                                    |                                                                                                                                          |
| SIF amide          | *R. appendiculatus* (Šimo et al. 2009a)       | Peptidergic neurons in the synganglion, innervation of the SG acini type II and III by PcSG neuron (Šimo et al. 2009a, b), innervation of the rectal sac from PoHG1,2 neurons (Šimo et al. 2013, 2014) | MIP antagonist, stimulates hindgut activity (Šimo and Park 2014)                                                                     |
| (SIFα)             | *I. scapularis* (Šimo et al. 2009b, 2014)    |                                                                                    |                                                                                                                                          |
| Insulin-like peptide | *O. parkeri* (Zhu and Oliver 1991)           | Neurosecretory cells and innervation in the synganglion (Zhu et Oliver 1991; Altschul et al. 1997; Egekwu et al. 2014) | The function of insulins is not yet known, the most detailed functions are described in insects, where they perform many physiological functions (Antonova et al. 2012; Strand et al. 2016; Nässel and Broeck 2016; Colombani, et al. 2012; Nuss and Brown 2018) |
| (ILP)              | *D. variabilis* (Altschul et al. 1997)        |                                                                                    |                                                                                                                                          |
|                    | *I. scapularis* (Egekwu et al. 2014)          |                                                                                    |                                                                                                                                          |
| Elevenin           | *I. scapularis* (Kim et al. 2018)             | PeSG neurons in the synganglion, innervation of SG acini type II, III (Kim et al. 2018) | May regulate salivary gland function associated with feeding (Kim et al. 2018)                                                             |
sequences (FGLa / ASTRs) were discovered (Šimo and Park 2014). Their sequences are similar as they contain a characteristic structure and seven transmembrane domains as well as all GPCR receptors (Šimo and Park 2014).

**SIFamide**

SIFamide (SIFa) immunoreactivity was observed (Table 2, Fig. 1) in several neurons in the synganglion of *I. scapularis* and *R. appendiculatus* (Šimo et al. 2009a; Šimo and Park 2014). The most prominent PcSG neurons innervate acini type II and III of the SGs. Other neurons producing SIFamide (PoHG1, 2) project axons through the opistosomal nerves (OsN) to the hindgut surface (Šimo and Park 2014). In vitro assay showed that application of SIFamide stimulated hindgut contractions. Increased gut motility may suggest the role of SIFa in ion and water transport (Šimo and Park 2014). The identified putative receptor for SIFamide is expressed in the synganglion, salivary glands and gut (Šimo et al. 2013).

### Table: Peptides, Sequences, and Sources

| Peptide          | Sequence                                                                 | Source                  |
|------------------|--------------------------------------------------------------------------|-------------------------|
| Allatotropin (AT)| MAALGRTSALVAAAALFLCLAAAGSETPEASDRGQIKGQXLRLSTARGKGLPPGALFLQRNQEPADPIPKGFKMKISTARGKGLKPEDDPLSLFELLENEIDDVPDLIKEEGIRRILSSTARGKGVSPGFSDQGQPSADQSGSGLWAEIAVWSDASSLGYSVGDSF | IscW_SIWCW017791        |
| Allatostatin A (AST-A)| MDMRPRCTVRSFRRMCPVCYTLLLFLMMAOYCAARADASPAQLEQENDKKYPAAMYGGFCAGAPFLFADDAAEQAERAESDEPDNLNDLPQGERPQHPRYFGFGLRRDQNYGPGSDHNRRRHRHHFGFGLGKGGKSEIE_DFMASHYNYGCQKASYGGDGERWKRSQSLSDH* | XP_029848989.1          |
| SIFamide (SIFa)  | MNSWKAFFMFGRVLVAMVMAWMNMACAYRRKPFNGSRKGLSRADLNADVYAMCENCNDCTQCFPQFQT_DGAQ* | Šimo et al. (2009)       |
| Myoinhibitory peptide (MIP) | MSPVSSRHRAGPRVPVATGESGRATTSASVVLRLVVLVLALLLCSSAEQPOCGKQDNWALSGMIWGGKASHWNRLSGMIWGGKAGAYGPQYALLAESDNGAGGHSARAAAPPGSRENHVNDLSGYWG* | Šimo et al. (2009)       |
| Elevenin (Elev)  | MKRTCIOALGVPFFAAALVHQHAEKCRKTFYFRCRGF-SARFPAITKMEAMSLKLYETDDKGNRRRPADA VLVWKNFYDIDFDPDEPLDATRSFKRELKY* | Kim et al. (2018)        |
| Insulin-like peptide 1 (ILP1)| MMAAVLVLCLCALTSSLHHRGASARSSVEKKNRYCGSNLNRVLDFEEYDPTKRGHTYRAPDLDPAWPVF PVLDANJDSKLGMFEAKAAQLQRLPSYVQCTTGRIVEECSHKSCSTLELLAYYEPRNNALQVLSQSDNTA | Sharma et al. (2019)     |
| Insulin-like peptide 3 (ILP3)| MALARHAADVVLVLAGATSVFVDLVEAPASSGAGTSSSSSSSTVRLGPVLWLVLMCDRGQGVNSHMID ALAGARPQPQVFRPSLPRDGEDQEDGSGSSRTTASIAANATHYLSHGSNGGIVGDECRRCKASCATSALAS CARPASSGASSLDFMNMLASPADEARSESAGMGDDAQAEFTPTEAPSWSVEVHQAEOVRNEHERGTPAERS RGDGSVGSTRVLPGANFRSLEDTRGPRIGRTSRHHRPPFFYVQAFAFFGIDTEALAA | Sharma et al. (2019)     |
| Insulin-like peptide 4 (ILP4)| MLRCAAVVLVLSVAVMDLASYTADTPWEEEFRRNNDDEWARVWHVRHRCYQHVLVSHMNVLCREdyKLRRDRDVAAKDPEMTDLFPLEAAGVLTGKLSQTVTRHNTRSTIDCEDCDevGSCWEEYAYCPRNAKRNRKR | Sharma et al. (2019)     |
| Insulin-like peptide 5 (ILP5)| MDHPEMKVVALSLVLLTLLTSLVTRSATQGSSVEVESNSRNEVEGVESEVLKELWGGDDLDGDDQDELEIMAADDFFAF AMRTCDPDSDLWDEDAFRAHFMGRGLAPAVSDFIAQLRRAVLOGRGGFTGMRGKRRKSTRTGKCHRAH ASTVARHGRSFLSEAESRPYY* | Šimo et al. (2019)       |
| Tachykinin (TK)   | MDMHVEMKVALSVALVSTTSSVTRGQGSSVGSVEVEPEPVEGTSEVSEVLKELWGGDDLDGDDQDELEIMAADDFFAF AMRTCDPDSDLWDEDAFRAHFMGRGLAPAVSDFIAQLRRAVLOGRGGFTGMRGKRRKSTRTGKCHRAH ASTVARHGRSFLSEAESRPYY* | Šimo et al. (2019)       |

**Fig. 1** An overview of selected *Ixodes scapularis* peptides. Accession numbers: AT (172aa), AST A (182 aa) XP_029848989.1; SIFa (74 aa) ADD92393.1;; MIP (131 aa); Elev (109 aa) AXL48134.1.; ILP1 (135 aa) QDB63964.1.; ILP3 (117 aa) QDB63965.1.; ILP4 (284 aa) QDB63966.1.; ILP5 (149 aa) QDB63967.1.; TK (169 aa) EL516783.1. The sequence of MIP1 N-terminus is unknown and the C-termini of the MIP3 is with missing amidation signal incomplete. Signal peptide is in the sequences underlined; yellow are A-chains of ILPs and turquoise are B-chains of ILPs; predicted dibasic cleavage sites are red; green are the sequences of mature neuropeptides and the asterisks stand for STOP codon.

**Myoinhibitory peptide**

Myoinhibitory peptide (MIP) is a neuropeptide that inhibits its visceral muscle contractions (Table 2) (Šimo and Park 2014). MIP was subsequently detected in *I. scapularis* (Fig. 1) and *R. appendiculatus* (Šimo et al. 2009a, b; Šimo and Park 2014). In all these ticks, MIP is expressed in PcGS synganglion neurons and passes into the SGs where the neurons innervate acini type II and III. It is acini type II and III that have specific cuticular valvae that open when ticks feed (Binnington 1978). Myoepithelial cells then push the ingested fluid into the salivary gland ducts (Coons et al. 1994; Lamoreaux et al. 2000). Available studies suggest that MIP could regulate salivary gland valve opening as well as myoepithelial cell function during tick feeding (Šimo et al. 2013).
et al. 2013). MIP-immunoreactivity was also detected in the hindgut innervation from PoHG_{1,2} neurons via opistosomal nerves, where it is colocalised with SIFamide (Šimó et al. 2014). Receptors specific for MIP were also identified (Šimó et al. 2013; Šimó and Park 2014). Because MIP and SIFamide are colocalized in terminal axons in acini type II and III in SG, we can assume that MIP will have an antagonistic effect on SIFamide-mediated increased motility. Following stimulation with SIFamide, the inhibitory effect of certain neuropeptides was tested. Only MIP was able to reduce intestinal motility by approximately 65% (Šimó and Park 2014). Based on described functions in insects and localization of MIP in ticks, we can assume that it has an inhibitory role in the tick salivary gland and gut (Šimó and Park 2014).

**Elevenin**

Elevenin (Elev) was first detected in the abdominal ganglia of *Aphysia californica* (Cooper, 1863) sea slugs, in the neuron L11 (Taussig et al. 1984). Its homologous peptides have also been identified in other molluscs and insects (Tanaka et al. 2014). In *Nilaparvata lugens* (Stal, 1854) (Insecta: Hemiptera) Elev was detected in the CNS and SGs (Uchiyama et al. 2018). It also plays an important role in cuticle melanisation and stimulates the production of intracellular cAMP (Uchiyama et al. 2018). Peptide elevenin (IsElev) and its two receptors, IsElevR1 and IsElevR2, were characterized in *I. scapularis* (Table 2, Fig. 1). The sensitivity of IsElevR1 to IsElev has been shown to be 560-fold higher than to IsElevR2 (Bauknecht and Jekely 2015; Kim et al. 2018). The gene encoding Elev peptide is expressed most abundantly in the synganglion and salivary glands of *I. scapularis*. In the synganglion of starving females, Elev was detected in PcSG neurons, which innervate the salivary gland acini type II and III (Kim et al. 2018). By qPCR IsElavR1 was detected in synganglion, salivary glands and ovary. The second receptor, IsElevR2 was detected in synganglion and ovary, but not in salivary glands (Kim et al. 2018). Based on immunoreactivity and expression levels in specific tissues, we can assume that Elev participates in processes in the tick salivary glands through its receptors and regulates the initial control of egg development processes.

**Insulin**

The insulin-like peptide (ILP) was detected by immunohistochemistry (Table 2) in the synganglion of the tick *Ornithodoros parkeri* (Cooley, 1936) (Zhu and Oliver 1991) and subsequently by BLAST search (Altschul et al. 1997) in several tick species (Šimó et al. 2014). Subsequently, a putative insulin transcript (IsILP1) was identified in *I. scapularis* (Egekwu et al. 2014) and similar is in *D. variabilis* (Donohue et al. 2010; Bissinger et al. 2011). Subsequently, other IsILP3, 4, and 5 were identified in *I. scapularis* (Fig. 1) (Sharma et al. 2019). IsILP5 and IsILP1 are expressed only in the synganglion and the highest level of IsILP3 and IsILP4 expression was detected in the salivary glands (Sharma et al. 2019). The insulin receptor IsInR in *I. ricinus* was detected especially in the ovaries, and knock-down of this receptor together with IrAKT and IrTOR reduced the amount of blood taken and reduced reproductive capacity (Kozelková et al. 2021). In nymphs, the expression of IsILP3 and IsILP4 increases upon detachment from the host. Increased expression after detachment suggests that it plays an important role during development of this stage to a higher instar (Sharma et al. 2019). Knockdown of the Insulin-like Growth Factor Binding Protein (IGFBP) prevented fully-engorgement of females *A. americanum* (Mulenga and Khumthong 2010). Interestingly, each insulin is characterized by a specific expression pattern (Sharma et al. 2019). The function of insulins in ticks is not yet known; however, the most detailed functions are described in insects, where they control many physiological functions (Antonova et al. 2012; Colombani et al. 2012; Strand et al. 2016; Nässel and Broeck 2016; Nuss and Brown 2018).

**Tachykinin**

Tachykinins (TKs) are widely distributed pleiotropic neuropeptides, which are present in vertebrates, and invertebrates (Nässel et al. 2019). Overexpression of human TK receptors (TKR) is connected to many diseases, such as depression, stress, cardiovascular diseases, Parkinson’s disease, and others (Feickert and Burckhardt 2019). TKs (Table 2, Fig. 1) are commonly expressed in the CNS and midgut enteroendocrine cells of insects and vertebrates (Nässel et al. 2019), but are also produced by the venom glands of wasps (Yoon et al. 2020; Arvidson et al. 2016). Interestingly, the salivary glands of the mosquito *A. aegypti* and octopus *Octopus vulgaris* (Cuvier, 1797) produce TK peptides with the FXGLMamide motif (Steinhoff et al. 2014; Nässel et al. 2019), related to vertebrate substance P derived from the preprotachykinin A precursor. It would be interesting to determine if ticks, as blood-feeding parasitic organisms, produce similar peptides in the salivary glands. TK immunoreactivity was observed in the synganglion and in the ducts of the salivary glands of *R. appendiculatus* (Šimó et al. 2009a) and a precursor encoding IscapTKs has been identified in the genome of *I. scapularis* (Šimó et al., 2014). Furthermore, infection of the ISE6 tick cell line with *A. phagocytophilum* led to strain-dependent changes in the TK expression (Mateos-Hernández et al. 2021). Possible involvement of TKs in the
tick-pathogen interactions make them even more interesting as neuropeptides for future studies.

**Conclusions**

The most appropriate strategy used to control the life cycle of ticks is to identify molecules that control feeding and digestion, as well as regulate complex interactions between ticks, pathogens and their hosts. This could provide efficient tools for interruption of pathogen transmission. Neuropeptides and their receptors participate in important signalling pathways which control crucial processes during feeding and associated transmission of pathogens. Recent data indicate that the feeding activity of the tick is under control of neuropeptide signalling that requires neuroendocrine interactions between the synganglion, salivary glands and gut. As the incidence of known tick-borne diseases is increasing from year to year, identification of bioactive molecules and their signalling pathways in ticks is essential for the targeted preparation of vaccines against ticks and tick-borne diseases.

**Acknowledgements** This study was supported by Slovak grant agencies, Agentúra na podporu výskumu a vývoja, (APVV-16-0395 and APVV-18-0201) and Vedecká grantová agentúra (VEGA 2/0080/18).

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