Evolution of salivary secretions in haematophagous animals

Francesca L. Ware* and Martin R. Luck

School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicester LE12 5RD, UK

*Corresponding author: 27 Braishfield Gardens, Bournemouth, Dorset BH8 0QA, UK. Email: francesca.ware@yahoo.co.uk

Supervisor: Prof. Martin Luck, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicester LE12 5RD, UK.

Haemostasis is the prevention of blood fluidity in vertebrates and is the first stage of wound healing. Haematophagous animals use the blood of vertebrates as their sole source of nutrition and have evolved many salivary constituents to counteract the haemostatic response of their prey. These animals and their saliva have been studied for many years, with some applications in medicine. The purpose of this study is to compare the salivary constituents of leeches (Hirudinae), ticks (Argasidae and Ixodidae) and vampire bats (Desmodontinae) and to consider their evolutionary origin. Salivary constituents include plasminogen activators (PAs), anticoagulants (activated factor X, FXa; inhibitors), vasodilators, platelet aggregation inhibitors (PAgI) and thrombin inhibitors. The animals studied all tend to possess an anticoagulant and a form of apyrase (PAgI) to assist with blood feeding. Ticks and vampire bats have a form of PA but the leech does not. The vampire bat has a PAgI but no vasodilator. The animals studied are from taxonomically unrelated groups but exploit similar mechanisms of action to facilitate their haematophagy.

Given that the haematophagous lifestyle of these animals developed much later than their common ancestors, we conclude that their mechanisms for haematophagy have arisen by convergent evolution. Some molecules, e.g. serine proteases found in invertebrate saliva, are probably derived from a common ancestral gene. The possible paths that have led to evolution of vampire bat salivary components are considered. Further research into the homology of these salivary constituents is required to give insight into how these animals adapted to haematophagy and their further therapeutic potential.

Key words: haematophagous, saliva, evolution, leech, tick, vampire bat

Submitted on 21 September 2015; editorial decision on 28 November 2016

Introduction

Haematophagous animals are those which rely on blood from other animals as their only source of nutrition. These parasitic creatures have evolved highly specific salivary molecules that counteract the haemostatic response of the host and also exert limited behavioural control. Whilst considerable research has been carried out on the salivary components and their potential for the treatment of human disease, few papers have explored their evolution. Because this unusual feeding method is displayed across the animal kingdom but in a limited number of species, we have chosen to examine examples representing distinctly different phyla. We describe and compare the range of active components in the saliva of haematophagous animals in the following three groups: leeches (Hirudinae), ticks (Ixodidae and Argasidae) and vampire bats (Desmodontinae). We then consider whether these components may have arisen through convergent or divergent evolution.

Haemostasis (Fig. 1) is the process by which blood flow stops at the site of an injury. It is one of the first responses to vascular damage and initiates further processes including wound repair. Briefly, coagulation (reviewed by Palta, Saroa and Palta, 2014)
Leeches are invertebrate, segmented worms from the phylum Annelida, class Clitellata, subclass Hirudinae (reviewed by Abdulkader et al., 2013). Leeches live in slow flowing streams and fresh water ponds (reviewed by Hildebrandt and Lemke, 2011). Young leeches, having left the safety of the cocoon, feed on amphibians. When their mouth parts have matured, they move on to feed on animals with thicker skin and more nutritious blood, including birds and mammals (Hildebrandt and Lemke, 2011).

Leeches, specifically the medicinal leech Hirudo medicinalis, have been used by physicians as a medicinal therapy for various diseases since early civilization (reviewed by Munshi et al., 2008). Haycraft (1884) reported that the H. medicinalis produced a substance with anticoagulant properties. In fact, the leech produces several anticoagulants and thrombolytics, stored in the salivary glands (Chopin et al., 2000).

The leech attaches to its prey using the anterior portion of its sucker. It begins periodic tilting movements of its three jaws, in order to slice open the skin. The pumping action of the pharyngeal muscles sucks the blood from the destroyed blood vessels and lymph of the host into the leech’s crop (Lent et al., 1988). These jaw movements also initiate the secretion of saliva from its unicellular salivary gland cells, located anteriorly in segments three and nine (Hildebrandt and Lemke, 2011). The mechanism of protein release from gland cells and the biochemical events needed to synthesize salivary proteins are unknown (Hildebrandt and Lemke, 2011). Serotonin may stimulate saliva excretion (Marshall and Lent, 1988; Hildebrandt and Lemke, 2011) and pharyngeal peristalsis (Lent et al., 1988).

The ingestion of blood lasts roughly 25 min (Lent et al., 1988). Only the red blood cells and plasma proteins are of nutritious value to the leech. Plasma and haem derivatives are excreted over the following 4–6 days, ensuring efficient digestion. Table 1 shows the haematophagy-relevant components of saliva in various leech species and their function in the host.

Anticoagulants

The majority of the anticoagulants found in leeches are inhibitors of FXa preventing the conversion of prothrombin to thrombin of the vertebrate coagulation cascade (common pathway). Examples are Therostatin (isolated from Theromyzon tessulatum; Chopin et al., 2000) and Antistatin. Antistatin has also been proposed to have anti-metastatic properties (Tuszynski, Gasic and Gasic, 1987). Ghilanten is an anticoagulant (FXa inhibitor) obtained from the leech Haementeria globulosa. A cuglobulin called plasminogen (profibrinolysin) is found within the plasma proteins trapped in the clot. Plasminogen is converted into plasmin (fibrinolysin) by tissue-type (t-PA) or urokinase-type (u-PA) plasminogen activators (PAs; both are serine proteases). Plasmin is the active component of the plasminogen (fibrinolytic) system (reviewed by Chapin and Hajjar, 2015), responsible for the eventual breakdown of fibrin fibres, fibrinogen, FV, FVIII, FXII and prothrombin at the start of the wound repair process (Tellgren-Roth et al., 2009; Hall, 2011; Barrett et al., 2012).

**Figure 1.** Haemostasis in vertebrates. A simplified account of haemostasis in vertebrates, including mammals, identifying possible targets for the salivary molecules of haematophagous animals.

Adapted from Law, Ribeiro and Wells (1992, review article) and Barrett et al. (2012). Dashed arrows indicate inhibition. 5-HT, serotonin; TXA2, thromboxane A2.
| Leech species                      | Molecule          | Function in host                                | Reference                                                                 |
|-----------------------------------|-------------------|------------------------------------------------|---------------------------------------------------------------------------|
| *Hirudo medicinalis* (European medicinal leech) | Hirudin           | Thrombin inhibitor                              | Jacobi (1904), Markwardt (1957), Nawarskas and Anderson (2001), Coppens et al. (2012), Jiang et al. (2013) |
|                                   | Bufrudin          | Thrombin inhibitor                              | Electricwala et al. (1991), Abdualkader et al. (2013)                     |
|                                   | Apyrase           | Agonist of platelets                            | Rigbi, Orevi and Eldor (1996), Hildebrandt and Lemke (2011)               |
|                                   | Theromin          | Thrombin inhibitor                              | Salzet et al. (2000)                                                     |
|                                   | Mammalian-type collagenase | Reduce platelet adherence                  | Rigbi et al. (1987)                                                      |
|                                   | Calin             | Platelet adhesion and activation inhibition     | Munro, Jones and Sawyer (1991), Abdualkader et al. (2013)                |
| *Haementeria vizottoi*            | Vizottin          | Anticoagulant (FXa inhibitor)                   | Oliveira et al. (2012)                                                   |
|                                   | Hyaluronidase     | Digests hyaluronic acid present in the ECM      | Linker, Hoffman and Meyer (1957), Hovingh and Linker (1999), Hildebrandt and Lemke (2011) |
|                                   | Inhibitor of C1 complement system component | Anti-inflammatory                          | Baskova et al. (1988), Boskova and Zavalova (2001)                      |
|                                   | LCI               | Regulator of fibrinolysis rate or inhibits carboxypeptidase | Reverter et al. (1998), Hildebrandt and Lemke (2011)                     |
|                                   | Eglin-c           | Impairment of neutrophils (alpha-chymotrypsin, subtilisin, chymosin, granulocyte proteinases, elastase and cathepsin G inhibitor) | Seemüller et al. (1977), Snider et al. (1985), Braun et al. (1987), Zaidi et al. (2011) |
|                                   | LDTI              | Possible role in supressing cell-mediated inflammatory reactions | Mühlhahn et al. (1994), Sommerhoff et al. (1994), Stubbs et al. (1997), Hildebrandt and Lemke (2011) |
|                                   | Hirustasin        | Antistatin-type serine protease inhibitor. Also trypsin, alpha-chymotrypsin and neutrophil cathepsin G inhibitor | Söllner et al. (1994), Boskova and Zavalova (2001)                       |
| *Haementeria oficinaalis* (Mexican Leech) | Antistatin        | Anticoagulant (FXa inhibitor) and anti-metastasis | Tuszynski, Gasic and Gasic (1987)                                        |
| *Haementeria ghilianii* (Great/Giant Amazon Leech) | Ghilanten         | Anticoagulant (FXa inhibitor) and anti-metastasis | Brankamp et al. (1990), Blankenship et al. (1990)                        |
|                                   | Saratin           | PAgI                                           | Barnes et al. (2001), Abdualkader et al. (2013)                          |
|                                   | Hementin          | Fibrinogenolytic metalloproteinase              | Swadesh, Huang and Budzynski (1990)                                     |

Continued
ghilianii (Blankenship et al., 1990; Brankamp et al., 1990), and is also believed to have anti-metastasic properties. Lefaxin is another anticoagulant (FXa inhibitor) from Haementeria depressa (Faria et al., 1999). The N-terminal chain of lefaxin shows no homology to antistatin or ghishtanen.

Another FXa inhibitor isolated from Haementeria vizottoi, called Vizottin, displays a different effect compared with other leech antistatin-like inhibitors (Oliveira et al., 2012). Vizottin prevents the formation of FXa by the extrinsic tenase complex (FVIIa: tissue factor) and also inhibits free and bound FXa, probably by interacting with the active site of FXa (Oliveira et al., 2012).

**Thrombin inhibitors**

A direct thrombin inhibitor is an anticoagulant, which binds to thrombin directly and blocks its activity (reviewed by Coppens et al., 2012). Hirudin (Jacobi, 1904) was isolated from H. medicinalis by Markwardt (1957; reviewed by Zaidi et al., 2011). Hirudin is the most potent naturally occurring thrombin inhibitor (Zaidi et al., 2011; Jiang et al., 2013) and many of its synthetic derivatives are used in clinics on a daily basis (reviewed by Nawarskas and Anderson, 2001; Zaidi et al., 2011). Subsequently, a similar anti-thrombin called Bufrudin has also been isolated (Electricwala et al., 1991; Abdulkadher et al., 2013). Hirudin and bufrudin have slightly different structural (N-terminal amino acid sequence) and immunological properties (Electricwala et al., 1991; Zaidi et al., 2011).

Hirustasin is an antistatin-type serine protease inhibitor and the first tissue kallikrein inhibitor identified in the leech (Söllner et al., 1994; Kallikrein-kinin system discovered by Abelous and Bardier, 1909, as cited by review Su, 2014 and Kraut, Frey and Werle, 1930). Despite similarities to antistatin (FXa inhibitor; differing reactive site sequence and proteinase activity), hirustasin does not inhibit blood coagulation in *vitro*, nor is it amido-lytic of isolated FXa. This suggests that the specificity of antistatin-type proteinases influences coagulation (reviewed by Söllner et al., 1994; Boskova and Zavalova, 2001).

A further thrombin inhibitor, Theromin, has been isolated from the Duck Leech Theromyzon tessulatum. It has no sequence homology with any other thrombin inhibitor (Salzet et al., 2000).

**Analgesia**

Some kinins are potent activators of nociceptive nerve cells, which induce or enhance pain sensations (Steranka et al., 1988; Hildebrandt and Lemke, 2011). Tissue kallikreins are proteases that cleave inactive kininogens to become active kinins (Hildebrandt and Lemke, 2011). The presence of antistatin-type substances (e.g. Hirustasin) in leech saliva may be an indication that it could reduce local tissue kallikrein activity in host tissue around the feeding site, therefore preventing the production of pain-inducing kinins. It may have an analgesic effect on the host (Hildebrandt and Lemke, 2011). An alternative explanation for kinin suppression is the
release of kininases into the wound to inactivate kinins by proteolytic cleavage. This removal of pain-inducing or sensitizing agents from the feeding site would explain the analgesic effect of leeches.

**Fibrinolysis/increasing blood flow**

Leech carboxypeptidase inhibitor (LCI; Reverter et al., 1998), inhibits a metalloproteinase (Carboxypeptidase B) responsible for the cleavage of kinin and therefore its inactivation in blood plasma. By inhibiting carboxypeptidase B, this increases kinin presence, which may result in increased pain and blood flow to the feeding site. Whilst the increased blood flow would be beneficial to the leech, the increase in pain (kinin) would not. Therefore, the LCI effect may be more relevant to inhibiting carboxypeptidase B effect on fibrinolysis (Hildebrandt and Lemke, 2011).

**Inhibitors of host immune system**

The complement system is part of the innate immune system of vertebrates and is the first defence against invaders (e.g. bacteria; Buchner, 1891). An inhibitor of complement component C1 (60–70 kDa), from leech saliva, blocks both the classic and alternative pathways of the complement system (Baskova et al., 1988; as cited and by Boskova and Zavalova, 2001) and is anti-inflammatory (Hildebrandt and Lemke, 2011).

Leech saliva contains a specific inhibitor of mast cells, leech-derived trypsin inhibitor (LDTI; Mühlhahn et al., 1994; Sommerhoff et al., 1994; Stubbs et al., 1997; Hildebrandt and Lemke, 2011). It exists in three isoforms with differing C-termini. Its biological function is not yet characterized, but it may suppress cell-mediated inflammation in host tissues at the feeding site (Hildebrandt and Lemke, 2011).

A secretory protein found in leeches named Eglin-c (Seemüller et al., 1977) binds to human neutrophils (Snider et al., 1985; Braun et al., 1987). Neutrophils are the most abundant white blood cell in mammals and part of the innate immune system (Murphy, 2012). Eglin-c stops neutrophils at the feeding site from entering the surrounding tissue, preventing inflammation. It is therefore proposed as an anti-inflammatory agent, which protects host tissues from destruction by endogenous neutrophils (Hildebrandt and Lemke, 2011).

Hyaluronidase (Linker, Hoffman and Meyer, 1957; Hovingh and Linker, 1999) digests hyaluronic acid present in the extracellular matrix of host tissues. Its secretion in leech saliva facilitates the distribution of other salivary molecules. These interfere with immune cell function in the host by mobilizing water molecules from proteoglycans and destabilizing the matrix (Hildebrandt and Lemke, 2011).

**Inhibitors of platelet activation and adherence**

Leech saliva contains two substances, which reduce host platelet adherence to vessel walls at the feeding site: a mammalian-type collagenase (Righi et al., 1987) and apyrase, an adenosine 5′-diphosphate (ADP) diphosphohydrolase. The latter removes the terminal phosphate group from ADP, generating AMP that does not bind to purinergic receptors, thereby suppressing platelet adhesion (Righi, Orevi and Eldor, 1996; Hildebrandt and Lemke, 2011).

**Ticks**

Ticks are ectoparasites of the order Parasitiformes, sub-order Ixodida (Black and Piesman, 1994), which have an obligate haematophagous lifestyle (reviewed by Kazimirová and Štíbraniová, 2013). There are two major families, the Ixodidae (hard; Black and Piesman, 1994; reviewed by Francischetti et al., 2009) and Argasidae (soft). Ixodidae ticks are further partitioned into the metastricate (Dermacentor or Rhipicephalusgenera spp.), which have short mouth parts and secrete a cement for attachment to the host (Francischetti et al., 2009; Kazimirová and Štíbraniová, 2013; reviewed by Sauer, 1977), and prostricate ticks, which use long, barbed mouth parts to stay attached to their host (Francischetti et al., 2009; Kazimirová and Štíbraniová, 2013).

Adult Ixodidae ticks prefer large mammals like humans and especially, ruminant livestock (Maina et al., 2014). Immature Ixodidae ticks also feed on smaller mammals, birds and reptiles (Maina et al., 2014). Soft ticks (Argasidae) feed on a wide range of vertebrates including amphibians, reptiles and birds (Kazimirová and Štíbraniová, 2013).

The constituents of tick saliva diversify as they mature, and quantities of various substances vary between each moult (instar) until sexual maturity is reached (Lloyd and Walker, 1995; Due et al., 2013; Juckett, 2013).

The active constituents of tick saliva are shown in Table 2. Ticks form a haemorrhagic pool within the tissues of the host from which they feed (reviewed by Ribeiro, 1995). When a tick is not attached to a host, salivary gland lobes produce hygroscopic saliva. This helps the tick remain hydrated whilst it waits for a host, sometimes for years (reviewed by Bowman and Sauer, 2004; Francischetti et al., 2009). Salivation is believed to be under nervous control, involving cAMP and calcium (Sauer, 1977). Ticks alternate in cycles of feeding and salivation, each lasting 5–20 min (Gregson, 1967). After feeding, they fall off their hosts and become inactive and unwilling to reattach (Bowman and Sauer, 2004). It was long believed, as early as 27–79 AD, that ticks would die after gorging on blood due to lack of an anus. However, ticks do possess an anus and excrete mainly guanine and other nitrogenous waste in small amounts (Bowman and Sauer, 2004).

**Vasodilators and inhibitors of immune system function**

A vasodilator assists the tick in its blood feeding by increasing blood flow to feeding site (Ribeiro, Makoul and Robinson, 1988). The salivary vasodilators from Ixodes scapularis/dammini are the arachidonic acid derivatives prostacyclin (PGI2;
Table 2. Haematophagy-related substances found in saliva of hard (*Ixodidae*) and soft (*Argasidae*) ticks and their functions in the host

| Tick species | Molecule | Function in host | Reference |
|--------------|----------|------------------|-----------|
| **Hard ticks (*Ixodidae*)** | | | |
| *Ixodes scapularis* or *Ixodes dammini* (Deer/Blacklegged tick) | PGI$_2$ | Platelet inhibitor | Ribeiro, Makoul and Robinson (1988), Kazimirová and Štíbrániová (2013) |
| | PGE$_2$ | Vasodilator | Ribeiro et al. (1985), Law, Ribeiro and Wells (1992) |
| | Metalloprotease | Inhibits angiogenesis | Valenzuela et al. (2002), Kazimirová and Štíbrániová (2013) |
| |Ixolaris TFPI | | Lai et al. (2004), Francischetti et al. (2002b) |
| |- | Kininase enzyme (analgesic effect) | Ribeiro et al. (1985), Ribeiro and Mather (1998), Francischetti et al. (2009) |
| **Amblyomma americanum** (Lone Star tick) | PGE$_2$ | Vasodilator | Ribeiro et al. (1992), Law, Ribeiro and Wells (1992), Ribeiro (1995) |
| | PGF$_{2\alpha}$ | Vasodilator | Ribeiro et al. (1992) |
| **Amblyomma hebraeum** (South African Bont Tick) | Amblin | Thrombin inhibitor | Lai et al. (2004) |
| **Amblyomma variegatum** (Tropical Bont tick) | Variegin | Thrombin inhibitor | Koh et al. (2007), Koh and Kini (2008) |
| | Peptide AP18 | Enhances thrombin amidolytic activity | Koh et al. (2007) |
| |- | Anti-IL-8 | Hajnická et al. (2001) |
| **Rhipicephalus/Boophilus microplus** (Cattle/Southern cattle tick) | PGE$_2$ | Vasodilator | Dickinson et al. (1976), Higgs et al. (1976), Law, Ribeiro and Wells (1992), Tatchell and Binnington (1973) |
| | Boophilin | Thrombin inhibitor; serine protease inhibitor; potential anticoagulant | Macedo-Ribeiro et al. (2008); Liao et al. (2009) |
| |Ixodidin | Antimicrobial single-domain inhibitor/protease inhibitor | Fogaça et al. (2006) |
| **Rhipicephalus evertsi evertsi** (Red-legged tick) | Neurotoxin | Paralysis of host | Viljoen et al. (1986), Lloyd and Walker (1995) |
| **Hyalomma dromedarii** (Camel tick) | NTI-1 | Thrombin inhibitor | Ibrahim et al. (2001) |
| | NTI-2 | | |
| **Haemaphysalis Longicornis** (Cattle/Bush/Shrub tick) | Longistatin | PA and anticoagulant properties. | Anisuzzaman et al. (2010, 2011) |
| | (isolated from midgut) | Hemalin | Thrombin inhibitor | Liao et al. (2009) |
| **Dermacentor variabilis** (American dog/Wood tick) | Variabilin | Platelet inhibitor (Disintegrin) | Wang et al. (1996), Francischetti et al. (2009) |
| **Dermacentor andersoni** (Rocky Mountain wood tick) |- | Anti-TNFα | Ramachandra and Wikel (1995) |

Continued
Ribeiro, Makoul and Robinson, 1988) and PGE₂ (Ribeiro et al., 1985; reviewed by Valenzuela, 2004). PGE₂ is also found in the saliva of *Amblyomma americanum* (Ribeiro et al., 1992) and *Boophilus microplus* (Dickinson et al., 1976; Higgs et al., 1976; reviewed by Tatchell and Binnington, 1973; Law, Ribeiro and Wells, 1992). These may be involved in the generalized lymphocyte suppression of tick-infested species (Ribeiro et al., 1985; Law, Ribeiro and Wells, 1992).

Tick histamine-binding proteins (HBPs) are lipocalins, which trap cationic, hydrophilic molecules. This is in contrast to most lipocalins that bind lipophilic compounds. This is presumably an adaptation to haematophagy; the salivary glands of most if not all species of tick contain highly histamine-specific binding proteins (Paesen et al., 1999, 2000). Evidence suggests that expression of histamine is linked to an acquired tick resistance (D. andersoni; reviewed by Wikel, 1982; Brossard and Wikel, 2004).

The metalloprotease found in *I. scapularis* inhibits angiogenesis, the process of forming new blood capillaries (Valenzuela et al., 2002; Kazimírová and Štíbrániová, 2013). Cytokines, including chemokines, interferons and tumour necrosis factor (TNF), are important in vertebrate host immune responses, its development and expression. Tick saliva also contains anti-TNF (Ramachandra and Wikel, 1995), anti-IL-8 (interleukin 8; Hajímká et al., 2001) and blocks other cytokine binding activities (Brossard and Wikel, 2004).

### Anticoagulant and PA

*Haemaphysalis longicornis* secretes a PA, characterized and named Longistatin (Anisuzzaman et al., 2010, 2011). This has high specificity to fibrin clot-bound plasminogen and has been proposed to have anticoagulant function also (Anisuzzaman et al., 2011). This combination of functions suggests that it is a critical component in the maintenance of blood pools in the feeding process of *Ixodid* ticks (Anisuzzaman et al., 2011).

### Platelet aggregation inhibitors

Tick saliva contains apyrase, a platelet agonist (Kazimírová and Štíbrániová, 2013) and platelet inhibitors such as disintegrins and prostacyclin. These inhibitors, for example Variabilin, contain the arginine-glycine-aspartic acid (RGD) motif, which prevents the binding of fibrinogen to platelets (Wang et al., 1996; Francischetti et al., 2009). A further platelet inhibitor called Moubatin is capable of weakly inhibiting collagen-induced platelet aggregation (reviewed by Waxman and Connolly, 1993; Basanova, Baskova and Zavalova, 2002).

### Thrombin inhibitors

Two non-competitive thrombin inhibitors, NTI-1 (3.2 kDa) and NTI-2 (14.9 kDa), have been isolated from *Hyalomma dromedarii* (Ibrahim et al., 2001). A potent thrombin inhibitor found in the tropical bont tick (*Amblyomma variegatum*), called Variegin is a 32 residue polypeptide and one of the smallest thrombin inhibitors discovered in nature (Koh et al., 2007, 2013).
reviewed by Koh and Kini, 2008). It has no structural similarities with thrombin inhibitors found in other haematophagous animals, including hirudin, Rhodnin and Theromin, even though they have the same binding site on thrombin (Koh et al., 2007). Peptide API18 (a synthetic peptide of variegin) has been reported to bind to exosite-I, slightly enhancing thrombin amidolytic activity (Koh et al., 2007).

Boophilus are one-host ticks, which feed on cattle (Jongejan and Uilenberg, 2004). Boophilin, a proteinase inhibitor, inhibits thrombin and interferes with serine proteases: trypsin and plasmin (Macedo-Ribeiro et al., 2008). Hemalin (Liao et al., 2009), a thrombin inhibitor of the Kunitz-type family, has high homology with boophilin (Macedo-Ribeiro et al., 2008). The thrombin inhibitor Amblin (Lai et al., 2004) has been isolated from the haemolymph of ixodid tick Amblyomma hebraeum and displays sequence similarities to boophilin (Macedo-Ribeiro et al., 2008) and Ixolaris (a tissue factor pathway inhibitor, TFPI; Francischetti et al., 2002b). Inhibitors of the Kunitz family with bovine-pancreatic-trypsin inhibitor domains are also common in ticks (reviewed by Corral-Rodriguez et al., 2009). Savignin is a thrombin inhibitor found in Ornithodoros savignyi, which inhibits thrombin-induced platelet aggregation (Nienaber, Gaspar and Neitz, 1999). It is a competitive, slow, tight-binding inhibitor that interacts with thrombin exosite-I and blocks its catalytic site (as does leech hirudin; Nienaber, Gaspar and Neitz, 1999).

Other

Neurotoxins found in the saliva of Rhipicephalus evertsi evertsi cause host paralysis (Viljoen et al., 1986; Lloyd and Walker, 1995) and have presumably evolved to prevent the host interrupting feeding sessions. Tick anticoagulant peptide (TAP), isolated from Ornithodoros moubata, functions as an inhibitor of FXa (Waxman et al., 1990). Ixodes scapularis contains a kininase enzyme, which may contribute to the analgesic effect of bites (Ribeiro et al., 1985; Ribeiro and Mather, 1998; Francischetti et al., 2009).

Vampire bats

Three species of bat have evolved a haematophagous lifestyle: Diphylia ecaudata, Desmodus rotundus and Diaemus youngi. These ‘vampire’ bats are mammals of the order Chiroptera, sub-order Microchiroptera, sub-family Desmodontinae (other members of the sub-order are insectivores). Avian hosts are the only prey of the hairy-legged vampire bat (D. ecaudata; Tellgren-Roth et al., 2009; Low et al., 2013). The common vampire bat (D. rotundus) feeds substantially only on mammalian hosts, including livestock and humans (Low et al., 2013). The white-winged vampire bat (D. youngi) feeds on both mammalian and avian blood (Low et al., 2013). They are distributed widely over central and South America, typically living in caves, tree hollows and abandoned mines (Low et al., 2013) in colonies of 30–100 individuals (Greenhall and Schutt, 1996; Altringham, 2011; reviewed by Wimsatt, 1959).

Vampire bats often roost in a group of 8–12 females; these are related and unrelated individuals with whom they perform reciprocal altruism, the reduction of one animal’s fitness to increase that of another with the expectation that this behaviour will be returned (Altringham, 2011). The fact that vampire bats perform this with those to whom they are not closely related suggests a highly stable, social composition within the colony (Altringham, 2011).

Once the bat has located its prey (using long-range vision, olfaction, acute hearing and echolocation), it uses close-range thermal and mechanical sensitivity strategies to locate blood capillaries below the skin surface (Jones, Teeling and Rossiter, 2013; Low et al., 2013). They attack only resting or sleeping prey and generally feed without disturbance (Wimsatt, 1959). The process must therefore be painless (Wimsatt, 1959), suggesting an analgesic in the saliva. Each species possesses a characteristic lower lip median groove, which facilitates feeding (Wimsatt, 1959) but with small differences in anatomy (Greenhall and Schutt, 1996).

Desmodus rotundus possess upper and lower incisors. The upper incisors diverge to create an upside-down ‘V’ shape. This creates a crater-like ‘divot’ 1–2 mm deep in the host’s tissue. The bat laps up the blood via two straw-like ducts on the ventral side of the tongue (Wimsatt, 1959), whilst saliva is released from the dorsal side of the tongue from the sub-maxillary gland (Low et al., 2013). It takes 20–30 min for a bat to take in ~25 ml, which is approximately 60% of their bodyweight (Altringham, 2011). To prevent themselves from being grounded by the weight and open to potential predators, their kidneys excrete excess plasma from the already ingested blood before they finish a feed (Altringham, 2011).

The concoction of anticoagulants and other biochemicals in the bat’s saliva causes the normal response to this type of injury in the host to be delayed from minutes to hours (Low et al., 2013). The overall anticoagulant activity of the saliva decreases progressively after daily salivation and is restored after 4 days (Fernandez et al., 1998). Multiple feedings from an individual prey animal may increase its resistance to the anticoagulants, indicating that an immune response can be acquired (Delpietro and Russo, 2009).

Anticoagulants

Draculin is an anticoagulant found in vampire bat saliva (Apitz-Castro et al., 1995; Fernandez et al., 1998, 1999). It requires glycosylation of the native molecule to become a biologically active, non-competitive, tight-binding inhibitor of FXa (Fernandez et al., 1998, 1999). It is also an inhibitor of FXa of the intrinsic pathway of blood coagulation (Fernandez et al., 1998; Basanova, Baskova and Zavalova, 2002; Oliveira et al., 2012).

Recent transcriptome and proteome studies of D. rotundus glands (Ma et al., 2013) revealed a 2-Kunitz-domain type
inhibitor, named Desmolaris (Ma et al., 2013). Desmolaris is a naturally deleted form of Kunitz-I-domainless TFPI and has similar actions: it tightly binds FXIa of the extrinsic pathway of the coagulation cascade (Ma et al., 2013).

Platelet aggregation inhibition and fibrinolytic activity

A platelet aggregation inhibitor (PAgl) (ADP-induced and thrombin-induced) in vampire bat saliva exerts its effects by blocking ADP-binding sites or by specifically acting on the plasma cofactor involved in the platelet aggregation reaction (Hawkey, 1967). This molecule could be the apyrase or phosphatase recently found in the saliva of D. rotundus (Francischetti et al., 2013).

PA/fibrinolytic activity

The fibrinolytic activity of vampire bat saliva has been known since 1932 (Bier, 1932). A PA (a serine protease) has been found and named D. rotundus salivary plasminogen activator (DSPA), Desmokinase (Cartwright, 1974; Low et al., 2013) or Desmoteplase (reviewed by Paciaroni, Medeiros and Bogousslavsky, 2009). It has been characterized (Hawkey, 1966; Gardell et al., 1989) and cloned (Krätzschmar et al., 1991; Gulba, Prus and Witt, 1995; Francischetti et al., 2013). Four DSPAs (α1, α2, β and γ) have been isolated and characterized (reviewed by Piechowski-Jozwiak and Bogousslavsky, 2013); they are encoded by four different highly conserved genes, each with distinct structure and properties (Krätzschmar et al., 1991). All are homologous to human t-PA, except that the DSPAs are single-chain molecules and are dependent on fibrin as a cofactor (Liberatore et al., 2003).

DSPA-α1 has been investigated for its pharmaceutical potential and was in phase III clinical trials (Piechowski-Jozwiak and Bogousslavsky, 2013). Several substances found in D. rotundus saliva (Table 3) could be of potential use in thrombolytic therapy.

Discussion

Haematophagous animals have evolved mechanisms, which counteract the haemostatic response of their hosts. The Triatominae sub-family of haematophagous bugs possesses many beneficial compounds in their saliva to counteract haemostasis. Lipocalin, for example, also found in many other species of blood-feeding arthropods (Santos et al., 2007). The variety of mechanisms is extensive and several have been exploited in medical applications (Abdulkader et al., 2013; reviewed by Cherniack, 2011; Piechowski-Jozwiak and Bogousslavsky, 2013). The three types of animal discussed in this review represent taxonomically distinct groups and it is clear that they exhibit similarities and differences in the mechanisms they employ. Other haematophagous parasites not discussed (Table 4) such as sandflies (Lutzomyia longipalpis) also possess anti-coagulation mechanisms (Collin et al., 2012). This raises the question of whether divergent or convergent evolutionary mechanisms may have been in play.

We take divergent evolution to be the emergence in a species of an apomorphy not present in an ancestor (or the retention of a characteristic in one descendant and its loss in another descendant). This mechanism can be reliably invoked when unique characteristics and mechanisms occur in a species or higher taxonomic group. We take convergent evolution to be evidenced by the presence of an apomorphy in more than one species when it is unlikely to have been present in their closest common ancestor.

The three animal groups represented here (mammals, arthropods and annelids) apparently diverged early in animal evolution (reviewed by Sanetra et al., 2005). Mammals are deuterostomes while arthropods and annelids are protostomes (diverged from bilateria); arthropods are ecdysozoa and annelids are lophotrochozoa (diverged from protostomia). These major divergences probably occurred during the pre-Cambrian, Ediacaran era around 600 million years ago (m.y.a.; Erwin and Davidson 2002; Peterson et al., 2008). In addition, all three animals prey on amniote homeotherms—mammals and birds—which emerged considerably later than the parasite clades: 300–250 m.y.a. (Hellenius and Ruben 2004; Laurin and Reisz, 2011).

Given that haematophagy is a rare phenomenon in each of the three groups, the likelihood that it is a characteristic which has been retained from nearest common ancestors is extremely small. Furthermore, assuming that the vertebrate hosts for the haematophagous leeches and ticks emerged much later than the common ancestor of these parasites and that the mammalian parasite-host relationships of the vampire bats must also have emerged later, we take the haematophagy of our three animals groups to be a convergently evolved lifestyle.

Given this conclusion, it would appear that any similarities between the species in the mechanisms they employ to achieve haematophagy (preventing blood coagulation, nociception, would healing, etc.) must also have arisen convergently. Note, however, that the fundamental processes of coagulation, nociception and wound healing, against which haematophagous animals work, are largely common to mammals and birds. They therefore define and limit the biochemical mechanisms, which must have evolved for the haematophagous lifestyle to be successful. A further possibility is that the mechanisms haematophagous animals use are co-opted from, and adaptations of, commonly existing biochemical processes. To this extent, they represent divergent evolution from their non-haematophagous cousins as well as convergent evolution towards unrelated similarity.

Most haematophagous species possesses at least one anticoagulant, which inhibits one or multiple factors involved in the mammalian coagulation cascade (Tables 1–4). Anticoagulants, most of which are FXa inhibitors, are the dominant constituents of leech saliva. Although the N-terminal sequence of lefaxin showed no homology with other
leech anticoagulants it does show homology with Prolixin S derived from *Rhodnius prolixus* (Kissing bug; Hellmann and Hawkins, 1965). The anticoagulant and anti-metastatic agents antistatin and ghilanten are found in different species of leech so it is most likely that these properties have developed separately to facilitate blood feeding in these species. Whilst evidence of analgesia is currently not available for leeches, it is for *Ixodes* ticks (Ribeiro et al., 1985; Hildebrandt and Lemke, 2011). Similar feeding physiology of leeches has been observed in *R. prolixus*, suggesting that similar processes may be required for efficient blood feeding, also acquired convergently (Lent et al., 1988).

Table 3. Haematophagy-related molecules found in the sub-maxillary and accessory glands of the vampire bat (*D. rotundus*)

| Molecule                        | Function in host                                      | Reference                                                                 |
|--------------------------------|-------------------------------------------------------|---------------------------------------------------------------------------|
| Draculin                       | Anticoagulant (FXa and FIXa inhibitor)                 | Apitz-Castro et al. (1995), Fernandez et al. (1998, 1999), Basanova, Baskova and Zavalova (2002) |
| Desmolaris                     | Anticoagulant (FXIa inhibitor)                        | Ma et al. (2013)                                                          |
| Desmoteplase (DSPA-alpha-1)    | PA (fibrinolytic/thrombolytic)                        | Bier (1932), Gardell et al. (1989), Krätzschmar et al. (1991), Schleuning et al. (1992), Gulba, Praus and Witt (1995), Paciaroni, Merdeiros and Bogoussalvsky (2009); Tellgren-Roth et al. (2009); Francischetti et al. (2013), Piechowski-Jozwiak and Bogoussalvsky (2013), reviewed by Patel, Ipsiglou and Apostolakis (2014) |
| Phosphatase                    | Anti-platelet                                         | Hawkey (1967)                                                             |
| Apyrase                         | Anti-platelet                                         | Hawkey (1967)                                                             |
| Pituitary adenylate cyclase activating peptide (PACAP) | Vasodilator                                           |                                                                           |
| C-type natriuretic peptide (CNP) | Vasodilator                                           |                                                                           |
| TNFα-stimulated gene 6 (TSG-6) | Anti-inflammatory                                     |                                                                           |
| Lipophilin/secretoglobin precursors (several) | Anti-inflammatory                                     |                                                                           |
| Lipocalin and other lipid carriers | Anti-inflammatory                                     |                                                                           |
| Cystatin                       | Anti-inflammatory (cysteine-type inhibitor)            |                                                                           |
| Chemokine CCL28                | Antimicrobial (broad spectrum)                        |                                                                           |
| β-Defensin, BPI/LBP/CETP family lymphotoxin | Antimicrobial                                         |                                                                           |
| Kunitz domain (protease inhibitor domain) | Kunitz inhibitor                                      |                                                                           |
| Neuroserpins                   | Modulates activation of fibrinolysis triggered by DSPA |                                                                           |
| Chymase                        | Serine protease                                       |                                                                           |
| Clotting pathways serine proteases | Protease                                              |                                                                           |
| DNAse                          | Effects neutrophil function                           |                                                                           |

Source: Francischetti et al. (2013) unless otherwise stated specified. Further research is needed to confirm the function of some of these molecules in the host.

aSerine proteases are included because PAs belong to this family of enzymes.

FIXa, activated coagulation factor IX; FXIa, activated coagulation factor XI; CCL28, (C-C motif) ligand 28; BPI/LBP/CETP family, bactericidal permeability-increasing protein/lipopolysaccharide-binding protein/cholesteryl ester transfer protein family; DNAse, deoxyribonuclease; PA, plasminogen activator; DSPA, D rotundus salivary plasminogen activator.
Table 4. Molecules in the saliva of haematophagous and non-haematophagous animals other than the three groups considered in detail in this review

| Species                          | Molecule | Function in host | Reference                                                                 |
|----------------------------------|----------|-----------------|---------------------------------------------------------------------------|
| **Haematophagous animals**       |          |                 |                                                                           |
| Lutzomyia longipalpis (Sand fly) | Maxadilan| Vasodilator<sup>3</sup> | Ribeiro et al. (1989b), Law, Ribeiro and Wells (1992), Lerner et al. (1991). |
|                                  | Lufaxin  | Anticoagulant (FXa inhibitor) | Collin et al. (2012).                                                    |
|                                  | -        | Anti-complement | Cavalcante, Pereira and Gontijo (2003).                                  |
|                                  | Apyrase   | PAgI            | Ribeiro, Rossignol and Spielman (1986), Ribeiro (1995).                  |
| Phlebotomus papatasi (Sand fly)  | Apyrase   | PAgI            | Ribeiro et al. (1989a), Ribeiro (1995).                                  |
| Phlebotomus argenteipes          | Apyrase   | PAgI            | Ribeiro et al. (1989a), Ribeiro (1995).                                  |
| Phlebotomus perniciosus          | Apyrase   | PAgI            | Ribeiro et al. (1989a), Ribeiro (1995).                                  |
| Glossina morsitans (Savannah Tsetse fly) | S′nucleotidase-related Apyrase | PAgI | Mant and Parker (1981), Caljon et al. (2010).                           |
|                                  | -        | Thrombin inhibitor | Parker and Mant (1979), Ribeiro (1995).                                 |
| Glossina austeni (Savannah Tsetse fly) | - | PA             | Hawkins (1966), Hawkey (1967).                                           |
| Simulium vittatum (Black fly/Buffalo gnat) | - | Thrombin inhibitor | Jacobs et al. (1990), Ribeiro (1995).                                   |
|                                  | -        | Anticoagulant (FVII inhibitor) | Makonnen et al., unpublished observations as cited by Ribeiro (1995) |
|                                  | -        | Anticoagulant (FXa inhibitor) | Jacobs et al. (1990).                                                   |
|                                  | -        | Anticoagulant (FVa inhibitor) | Abebe et al. (1996), Basanova, Baskova and Zavalova (2002)              |
| Aedes aegypti (Yellow-fever mosquito) | - | Anticoagulant (serine protease inhibitor of FXa) | Stark and James (1995).                                                |
|                                  | Apyrase   | PAgI            | Ribeiro et al. (1984), Vachereau and Ribeiro (1989).                    |
| Oropsylla bacchii (Flea)         | Apyrase   | PAgI            | Ribeiro, Vaughan and Azad (1990b), Ribeiro (1995).                      |
| Orchopea howardii (Squirrel Flea)| Apyrase   | PAgI            | Ribeiro, Vaughan and Azad (1990b), Ribeiro (1995).                      |
| Xenopsylla cheopsis (Oriental Rat Flea) | Apyrase | PAgI | Ribeiro, Vaughan and Azad (1990b), Ribeiro (1995).                      |
| Rhodniini/Triatomini (Kissing/Traitomines/Assassin/Conenose bug) | Apyrase | PAgI | Ribeiro, Marinotti and Gonzales (1990a), Law, Ribeiro and Wells (1992), Santos et al. (2007) |
|                                  | Dimiconin| Anticoagulant (FXII inhibitor) | Ishimaru et al. (2012).                                                |

Continued
Table 4. Continued

| Species                                      | Molecule                        | Function in host                                      | Reference                                                        |
|----------------------------------------------|---------------------------------|------------------------------------------------------|-----------------------------------------------------------------|
| **Prolixin S (NP2)**                         | Anticoagulant                   | Hellmann and Hawkins (1965), Ribeiro, Schneider and Guimarães (1995) |
| **Triabin**                                  | Thrombin inhibitor              | Noeske-Jungblut et al. (1995), Ishimaru et al. (2012) |
| **Rhodniin**                                 | Thrombin Inhibitor              | Friedrich et al. (1993)                               |
| **Pallidipin 1 and 2**                       | Collagen-induced PAgI           | Noeske-Jungblut et al. (1994), Basanova, Baskova and Zavalova (2002), Ishimaru et al. (2012) |
| **R. prolixus aggregation inhibitor-1 (RPAl-1)** | PAgI                            | Francischetti et al. (2000), Francischetti, Andersen and Ribeiro (2002a), Francischetti et al. (2009), Ishimaru et al. (2012) |
| **Triafestin-1 and -2**                      | Plasma kallikrein-kinin system inhibitors (FXII and kininogen inhibitor) | Isawa et al. (2007), Ishimaru et al. (2012). |
| **NP1-4**                                    | Vasodilator                     | Ribeiro, Marinotti and Gonzales (1990a), Champagne, Nussenzeig and Ribeiro (1995), Basanova, Baskova and Zavalova (2002), reviewed by Champagne (2005) |
| **Lipocalins**                               | Transports small hydrophobic molecules | Santos et al. (2007) |
| **Serine protease**                          | Protease                        | Santos et al. (2007)                                  |
| **Eutriatoma maculatus** (Assassin bug)      | Maculatin                       | Hellmann and Hawkins (1966)                           |
| **Culicoides sonorensis** (Biting midge)     | TFPI1 and TFPI2                | Protease inhibitors                                   | Campbell et al. (2005)                                           |
| **Non-haematophagous animals**               |                                 |                                                      |                                                                  |
| **Bombus terrestris** (Buff-tailed/Large earth Bumblebee) | **Br-KTI**                     | Serine protease inhibitor (acts as a plasmin inhibitor) | Qiu et al. (2013)                                                 |
| **Scolopendra subspinipes mutilans** (Chinese red-head/Chinese red-headed centipede) | **-**                           | Anticoagulant (FXa inhibitor)                         | Kong et al. (2013)                                               |
|                                              | **Scolonase**                   | Serine protease                                      | You et al. (2004)                                                |

Continued
The PAs in the vampire bats are highly conserved, especially between the two species which feed upon mammals (D. rotundus and D. youngi; Tellgren-Roth et al., 2009). Evolution of the plasminogen gene may have led to the wide utilization of mammalian livestock by these two species and consequently led to the rise of D. rotundus as the common vampire bat (Tellgren-Roth et al., 2009). Absence of a PA in leeches suggests that the PA activity in the saliva of H. longicornis, Eutriatoma maculatus, R. proluxus (Hellmann and Hawkins, 1964; Hawkins and Hellman, 1966) and D. rotundus is the result of convergent evolution. If it originated from a common ancestral gene, it is unclear why the leech would lose a potentially beneficial feature during its evolution.

The dual function of tick Longistatin, as a PA and anticoagulant, indicates the importance of these components to species survival. It also suggests that other PAs are the result of replication and division over the course of evolution. The evolutionary split of the PAs and anticoagulants might have led to the now separate entities which can be observed in other invertebrates. Further investigation into the homology between Longistatin and other PAs and anticoagulants may provide further insight (Bowman and Sauer 2004; Anisuzzaman et al., 2011). The thrombin inhibitor variegin, from the tick A. variegatum, has no homology with other haematophagous animal thrombin inhibitors and presumably evolved independently. Peptide AP18 binding to exosite-I also in A. variegatum ticks is comparible to hirudin C terminus behaviour of leeches (Maragano et al., 1989; Naski et al., 1990), suggesting that these two sequences have a similar mechanism of action even though the two species are phylogenetically distant (Koh et al., 2007).

The vampire bat uses a distinct PAgI to block ADP-induced platelet aggregation. The PAgI of other haematophagous animals uses different biochemical mechanisms. Apyrase, which hydrolyses ATP and ADP, is commonly found in haematophagous insects including the tsetse fly Glossina morsitans (Mant and Parker, 1981; Caljon et al., 2010). Amounts of apyrase activity are elevated in blood-feeding animals compared with non-blood-feeding insects, suggesting exploitation of a pre-existing mechanism (Francischetti et al., 2009).

Both haematophagous animals and non-haematophagous animals possess some sort of serine protease and serine protease inhibitor (Qiu et al., 2013). The presence of serine protease inhibitors in diverse groups of insects (honey bee, mosquito; Table 4; Qiu et al., 2013) suggests that endogenous suppressors of serine proteases, of which PA is a family member, are required at some stage of insect life and have been conserved for reasons unconnected with blood feeding. Insects heal wounds using cross-linking enzymes including transglutaminase, phenoloxidase (reviewed by Theopold et al., 2004) and lipophorin (Li et al., 2002) to form haemolymph clots. No true orthologues of vertebrate blood clotting factors have been found in insects but several proteins with similar functional domains have been detected (Theopold et al., 2004).

In line with the similarities between serine proteases and domains of coagulation factors, some serine proteases in the protochordate Botryllus schlosseri (star ascidian/golden star tunicate) are homologous to vertebrate blood-coagulation proteases (Ponczek, Bijak and Nowak, 2012). They participate in reactions involving the provoked aggregation of different cell-type colonies leading to cell clumping at the site of contact. This suggests that a cascade of activated serine proteases was initially a defence mechanism (not associated with vascular injury; Wan et al., 2013), which evolved into the vertebrate complement system and haemostatic response (Oren et al., 2008; Ponczek, Bijak and Nowak, 2012).

Although the evidence suggests that PAs in invertebrates were derived from a common ancestor, this does not seem likely for the vampire bats. One hypothesis for the transition of vampire bats to haematophagy is arboreal feeding (Schutt, 2008): they may have evolved from carnivorous bats whose prey had grown too large for them to hunt by their usual methods. A behavioural shift allowed the proto-vampires to utilize these larger animals as a food resource.

We hypothesize two alternative mechanisms for the development of the necessary salivary biochemicaly in vampire bats to support this transition:
(1) Adaptation of blood haemostatic molecules into anti-haemostatic salivary molecules. Because PAs have been found in all vertebrates investigated so far (reviewed by Schleuning, 2001), it is possible that molecules present in a common ancestor could have evolved separate but parallel functions. The mechanism for this would be gene duplication followed by mutation and selection. Since other haematophagous animals possess different salivary components, the convergence to haematophagy, facilitated by duplication, must have resulted from selection according to different mammalian lifestyles or habitat. Whether this would have been possible in the relatively short evolutionary time available (between 6000 and 2 000 000 years, with the Phyllostomidae family diverging at 35 m.y.a.; Schutt, 2008; Jones, Teeling and Rossiter, 2013) is not clear.

(2) Exploitation of host blood proteins. Anti-haemostatic proteins could be obtained directly from the blood of the hosts and exploited adaptively. For this to occur, bats would need to absorb the materials intact and without destruction by their immune system, and be able to exploit them in subsequent feeding. The blood coagulation factors FVII, FX and FIX have similar sequence and domain arrangements in all vertebrates (Ponczek, Bijak and Nowak, 2012) so it is possible that those derived from the host were well tolerated.

Conclusion

Haematophagous animals possess many salivary constituents, which are capable of preventing haemostasis in their host, exploiting a range of different mechanisms. Given that haematophagy is considered to have evolved independently several times, the active salivary components presumably emerged by convergent evolution. The derivation of these molecules has yet to be defined, but the salivary components of leeches and ticks at least could be adaptations of common ancestral genes.

Recent advances in large-scale transcriptome analysis and structural proteomic analysis have made the identification of such molecules easier. Identification of new molecules and comparative homology of current proteins, especially those with anti-metastasis and analgesic properties, may help explain their origin as well as the evolution to haematophagy.

Author biography

Francesca graduated with a 2:1 BSc (Hons) Animal Science degree from the University of Nottingham in 2014. It was during this time that she developed a passionate interest in immunology, infection and wound healing. Francesca was awarded one of fifteen School of Life Sciences Taught MSc Scholarship and has now completed her MSc in Immunology and Allergy at the University of Nottingham. She hopes to continue in the field of immunology research, by one day completing a PhD, to ultimately become a lecturer in immunology. F.L.W. designed the study, carried out the research and wrote up the paper. M.R.L. assisted in writing up the paper.

References

Abdualkader, A. M., Ghawi, A. M., Alaama, M. et al. (2013) Leech therapeutice applications. Indian Journal of Pharmaceutical Sciences, 75 (2), 127–137.
Abebe, M., Ribeiro, J. M., Cupp, M. S. et al. (1996) Novel anticoagulant from salivary glands of Simulium vittatum (Diptera: Simuliidae) inhibits activity of coagulation factor V. Journal of Medical Entomology, 33 (1), 173–176.
Abelous, J. E. and Bardier, E. (1909) ‘Les substances hypotensives de l’urine humaine normale’. CR Société de Biologie (in French), 66, 511–520.
Altringham, J. D. (2011) Bats: From Evolution to Conservation, 2nd edn, Oxford University Press Inc, New York, USA, pp. 29–30, 160–161.
Anisuzzaman, Islam, M. K., Alim, M. A. et al. (2011) Longistatin, a plasminogen activator, is key to the availability of blood-meals for ixodid ticks. PLoS Pathogens, 7 (3), e1001312.
Anisuzzaman, Islam, M. K., Miyoshi, T. et al. (2010) Longistatin, a novel EF-hand protein from the ixodid tick Haemaphysalis longicornis, is required for acquisition of host blood-meals. International Journal for Parasitology, 40 (6), 721–729.
Aptiz-Castro, R., Béguin, S., Tablante, A. et al. (1995) Purification and partial characterization of draculin, the anticoagulant factor present in the saliva of vampire bats (Desmodus rotundus). Thrombosis and Haemostasis, 73 (1), 94–100.
Barnes, C. S., Krafft, B., Frech, M. et al. (2001) Production and characterization of saratin, an inhibitor of von Willebrand factor-dependent platelet adhesion to collagen. Seminars in Thrombosis and Haemostasis, 27 (4), 337–348.
Barrett, K. E., Barman, S. M., Boitano, S. et al. (2012) Ganong’s Review of Medical Physiology, 24th edn, McGraw-Hill Companies Inc, China, pp. 564–569.
Basanova, A. V., Baskova, I. P. and Zavalova, L. L. (2002) Vascular-platelet and plasma hemostasis regulators from bloodsucking animals. Biochemistry (Moscow), 67 (1), 143–150.
Baskova, I. P., Nikonov, G. I., Mirkamali, E. G. et al. (1988) Influence of a preparation from the medicinal leech on phagocytosis and complement system. Kazansky Medicinsky Zhurnal, 5, 334–336.
Bier, O. G. (1932) Action anticoagulante et fibrinolytique de l’extrait des glandes salivaires d’une chauve-souris hémophage (Desmodus rufus). Comptes Rendus Hebdomadaires des Séances – Société de Biologie, 110, 129–131.
Black, W. C. 4th and Piesman, J. (1994) Phylogeny of hard- and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences.
Bowman, A. S. and Sauer, J. R. (2004) Tick salivary glands: function, Bioscience Horizons
Brossard, M. and Wikel, S. K. (2004) Tick immunobiology.
Boskova, I. P. and Zavalova, L. L. (2001) Proteinase inhibitors from the medi-
Chapin, J. C. and Hajjar, K. A. (2015) Fibrinolysis and the control of
Blankenship, D. T., Brankamp, R. G., Manley, G. D. et al. (1987) Kinetic studies on the
Buchner, H. (1891) Zur Nomenklatur der schützenden Eiweisskörper.
Campbell, C. L., Vandyke, K. A., Letchworth, G. J. et al. (2005) Midgut and sal-
Cartwright, T. (1974) The plasminogen activator of vampire bat saliva. Blood, 43 (3), 317–326.
Cavalcanте, R. R., Pereira, M. H. and Gontijo, N. F. (2003) Anti-
Champagne, D. E. (2005) Anhemostatic molecules from saliva of blood-feeding arthropods. Pathophysiology of Haemostasis and Thrombosis, 34 (4–5), 221–227.
Champagne, D. E., Nussenzeig, R. H. and Ribeiro, J. M. C. (1995) Purification, partial characterization, and cloning of nitric oxide-carrying heme proteins (nitrophorins) from salivary glands of the blood-sucking insect Rhodnius prolixus. The Journal of Biological Chemistry, 270 (15), 8691–8695.
Chapin, J. C. and Hajjar, K. A. (2015) Fibrinolysis and the control of blood coagulation. Blood Reviews, 29 (1), 17–24.
Cherniack, E. P. (2011) Bugs as drugs, part two: worms, leeches, scorpions, snails, ticks, centipedes, and spiders. Alternative Medicine Review: A Journal of Clinical Therapeutic, 16 (1), 50–58.
Chopin, V., Salzet, M., Baert, JI et al. (2000) Therostasin, a novel clotting factor Xa inhibitor from the rhynchobdellid leech, Theromyzon tessulatum. Journal of Biological Chemistry, 275 (42), 32701–32707.
Chudzinski-Tavassi, A. M., Kelen, E. M., de Paula Rosa, A. P. et al. (1998) Fibrin(o)genolytic properties of purified hemeretin, a metallopro-
teinase from the leech Haementeria depressa. Thrombosis and Hemostasis, 80 (1), 155–160.
Collin, N., Assumpção, T. C. F., Mizurini, D. M. et al. (2012) Lufaxin, a novel factor Xa inhibitor from the salivary gland of the sand fly Lutzomyia longipalpis blocks protease-activated receptor 2 activation and inhibits inflammation and thrombosis in vivo. Arteriosclerosis, Thrombosis and Vascular Biology, 32 (9), 2185–2198.
Coppens, M., Eikelboom, J. W., Gustafsson, D. et al. (2012) Translational success stories: development of direct thrombin inhibitors. Circulation Research, 111, 920–929.
Corral-Rodríguez, M. A., Macedo-Ribeiro, S., Barbosa Pereira, P. J. B. et al. (2009) Tick-derived Kunitz-type inhibitors as antithrombotic factors. Insect Biochemistry and Molecular Biology, 39 (9), 579–595.
Delpiejo, H. A. and Russo, R. G. (2009) Acquired resistance to saliva anticoagulants by prey previously fed upon by vampire bats (Desmodus rotundus): evidence for immune response. Journal of Mammalogy, 90 (5), 1132–1138.
Dickinson, R. G., O’Hagan, J. E., Schotz, M. et al. (1976) Prostaglandin in the saliva of the cattle tick Boophilus microplus. The Australian Journal of Experimental Biology and Medical Science, 54 (5), 475–486.
Due, C., Fox, W., Medlock, J. M. et al. (2013) Tick bite prevention and tick removal. British Medical Journal (Clinical Research ed.), 347, f7123.
Erwin, D. H. E. and Davidson, E. H. (2002) The last common bilaterian ancestor. Development, 129, 3021–3032.
Electricwala, A., Sawyer, R. T., Jones, C. P. et al. (1991) Isolation of thrombin inhibitor from the leech Hirudinaria manillensis. Blood Coagulation and Fibrinolysis, 2 (1), 83–89.
Faria, F., Kelen, E. M., Sampaio, C. A. et al. (1999) A new factor Xa inhibi-
tor (ilefaxin) from the Haementeria depressa leech. Thrombosis and Haemostasis, 82 (5), 1469–1473.
Fernandez, A. Z., Tablante, A., Bartoli, F. et al. (1998) Expression of bio-
ticantin, an anticoagulant factor from vampire bat saliva, is strictly dependent on the appropriate glycosylation of the native molecule. Biochimica et Biophysica acta, 1425 (2), 291–299.
Fernandez, A. Z., Tablante, A., Beguin, S. et al. (1999) Draculin, the anti-
coagulant factor in vampire bat saliva, is a tight-binding, non-
competitive inhibitor of activated factor X. Biochimica et Biophysica acta, 1434 (1), 135–142.
Fogaça, A. C., Almeida, I. C., Eberlin, M. N. et al. (2006) Ixodidin, a novel antimicrobial peptide from the hemocytes of the cattle tick Boophilus microplus with inhibitory activity against serine protei-
nases. Peptides, 27 (4), 667–674.
Francischetti, I. M., Andersen, J. F. and Ribeiro, J. M. (2002a) Biochemical and functional characterization of recombinant Rhodnius prolixus platelet aggregation inhibitor 1 as a novel lipocalin with high
affinity for adenosine diphosphate and other adenine nucleotides. Biochemistry, 41, 3810–3818.

Francischetti, I. M. B., Assumpção, T. C. F., Ma, D. et al. (2013) The ‘Vampirome’: Transcriptome and proteome analysis of the principal and accessory submaxillary glands of the vampire bat Desmodus rotundus, a vector of human rabies. Journal of Proteomics, 82, 288–319.

Francischetti, I. M. B., Ribeiro, J. M., Champagne, D. et al. (2000) Purification, cloning, expression, and mechanism of action of a novel platelet aggregation inhibitor from the salivary gland of the bloodsucking bug, Rhodinus prolixus. Journal of Biological Chemistry, 275, 12639–12650.

Francischetti, I. M. B., Sa-Nunes, A., Mans, B. J. et al. (2009) The role of saliva in tick feeding. Frontiers in Bioscience (Landmark Edition), 14, 2051–2088.

Francischetti, I. M. B., Valenzuela, J. G., Andersen, J. F. et al. (2002b) Isolariis, a novel recombinant tissue factor pathway inhibitor (TFPI) from the salivary gland of the tick, ixodes scapularis: identification of factor X and factor Xa as scaffolds for the inhibition of factor VIIa/tissue factor complex. Blood, 99 (10), 3602–3612.

Kraut, H., Frey, F. K. and Werle, E. (1930) Der Nachweis eines Kreislaufhormons in der Pankreasdrüse. (IV. Mitteilung über dieses Kreislaufhormon.). Hoppe-Seylers Zeitschrift für Physiologische Chemie, 189 (3–4), 97–106.

Friedrich, T., Kroger, B., Bialojan, S. et al. (1993) A Kazal-type inhibitor with thrombin specificity from Rhodinus prolixus. Journal of Biological Chemistry, 268 (1993), 16216–16222.

Fu, Z., Zhang, L., Liu, X. et al. (2013) Comparative proteomic analysis of the sun- and freeze-dried earthworm Eisenia fetida with differentially thrombolytic activities. Journal of Proteomics, 83, 1–14.

Gardell, S. J., Duong, L. T., Diehl, R. E. et al. (1989) Isolation, characterization, and cDNA cloning of a vampire bat salivary plasminogen activator. The Journal of Biological Chemistry, 264 (30), 17947–17952.

Geng, P., Lin, L., Li, Y. et al. (2014) A novel fibrinogenolytic trypsin-like protease from Chinese oak silkworm (Antheraea pernyi): purification and characterization. Biochemical and Biophysical Research Communications, 445 (1), 64–70.

Greenhall, A. M. and Schutt, W. A. (1996) Dixenium youngi. Mammalian Species, 533, 1–7.

Gregson, J. D. (1967) Observations on the movement of fluids in the vicinity of the mouthparts of naturally feeding Dermacentor andersoni Stiles. Parasitology, 57, 1–8.

Gulba, D. C., Praus, M. and Witt, W. (1995) DSPA alpha—Properties of the plasminogen activators of the vampire bat Desmodus rotundus. Fibrinolysis, 9 (Suppl 1), 91–96.

Hajnichká, V., Kocáková, P., Sláviková, M. et al. (2001) Anti-interleukin-8 activity of tick salivary gland extracts. Parasite Immunology, 23 (9), 483–489.

Hall, J. E. (2011) Guyton and Hall’s Textbook of Medical Physiology, 12th edn, Saunders Elsevier, Philadelphia, PA, USA, pp. 451–460.

Hawkey, C. (1966) Plasminogen activator in saliva of the vampire bat Desmodus rotundus. Nature, 211 (5047), 434–435.

Hawkey, C. (1967) Inhibitor of platelet aggregation present in saliva of the vampire bat Desmodus rotundus. British Journal of Haematology, 13 (6), 1014–1020.

Hawkins, R. I. (1966) Factors affecting blood clotting from salivary glands and crop of Glossina austeni. Nature (London), 212, 738.

Hawkins, R. I. and Hellman, K. (1966) Investigations on a plasminogen activator in Two-blood-Suckers, Rhodinus prolixus Stål and Hirudo medicinalis,. British Journal of Haematology, 12, 86.

Haycraft, J. B. (1884) On the action of a secretion obtained from the medicinal leech on the coagulation of the blood. Proceedings of the Royal Society of London, 36, 478–487.

Hellenius, W. J. and Ruben, J. A. (2004) The Evolution of endothermy in terrestrial vertebrates: Who? When? Why? in Physiological and Biochemical Zoology, 77, pp. 1019–1042.

Hellmann, K. and Hawkins, R. I. (1964) Anticoagulant and fibrinolytic activities from Rhodinus Prolixus Stål. Nature, 201, 1008–1009.

Hellmann, K. and Hawkins, R. I. (1965) Prolixins-S and prolixin-G; two anticoagulants from Rhodinus prolixus Stål. Nature, 207 (994), 265–267.

Hellmann, K. and Hawkins, R. I. (1966) An antithrombin (maculatin) and a plasminogen activator extractable from the blood-sucking hemipteran, Eletriatoma maculatus. British Journal of Haematology, 12 (4), 376–384.

Higgs, G. A., Vane, J. R., Hart, R. J. et al. (1976) Prostaglandins in the saliva of cattle tick, Boophilus microplus (Canestrini) (Acarina, Ixodidae). Bulletin of Entomological Research, 66, 665–670.

Hildebrandt, J. P. and Lemke, S. (2011) Small bite; large impact–saliva and salivary molecules in the medicinal leech, Hirudo medicinalis. Naturwissenschaften, 98, 995–1008.

Hovingh, P. and Linker, A. (1999) Hyaluronidase activity in leeches (Hirudinea). Comparative Biochemistry and Physiology, Part B, Biochemistry and Molecular Biology, 124 (3), 319–326.

Ibrahim, M. A., Ghazy, A. H., Maharem, T. et al. (2001) Isolation and properties of two forms of thrombin inhibitor from the nymphs of the camel tick Hyalomma dromedarii (Acari: Ixodidae). Experimental and Applied Acarology, 25 (8), 675–698.

Isawa, H., Orito, Y., Jingushi, N. et al. (2007) Identification and characterization of plasma kallikrein-kinin system inhibitors from salivary glands of the blood-sucking insect Triatoma infestans. The FEBS Journal, 274 (16), 4271–4286.

Ishimaru, Y., Gomez, E. A., Zhang, F. et al. (2012) Dimicon, a novel coagulation inhibitor from the kissing bug, Triatoma dimidiata, a vector of Chagas disease. Journal of Experimental Biology, 215 (Pt 20), 3597–3602.

Jacobi, Y. (1904) Über Hirudin. Deutsche Medizinische Wochenschrift, 30, 1786–1787.
Jacobs, J. W., Cupp, E. W., Sardana, M. et al. (1990) Isolation and characterization of a coagulation factor Xa inhibitor from black fly salivary glands. *Thrombosis and Haemostasis*, 64 (2), 235–238.

Jiang, S., Jiao, J., Zhang, T. et al. (2013) Pharmacokinetics study of recombinant hirudin in the plasma of rats using chromogenic substrate, ELISA, and radioisotope assays. *PLoS One*, 8 (6), e64336.

Jones, G., Teeling, E. C. and Rossiter, S. J. (2013) From the ultrasonic to the infrared: molecular evolution and the sensory biology of ticks. *Frontiers in Physiology*, 4, 117.

Jongejan, F. and Uilenberg, G. (2004) The global importance of ticks. *Parasitology*, 129, S3–14.

Juckett, G. (2013) Arthropod bites. *American Family Physician*, 88 (12), 841–847.

Kazimirová, M. and Štibrániová, I. (2013) Tick salivary compounds: their role in modulation of host defences and pathogen transmission. *Frontiers in Cellular and Infection Microbiology*, 3, 43.

Koh, C. Y., Kazimirova, M., Trimnell, A. et al. (2007) Variegin, a novel fast-acting anticoagulant from the salivary gland of the cattle tick *Aedes albopictus*. *Expert Review of Hematology*, 1 (2), 135–139.

Koh, C. Y. and Kini, R. M. (2008) Anticoagulants from hematophagous animals. *Expert Review of Hematology*, 1 (2), 135–139.

Kong, Y., Shao, Y., Chen, H. et al. (2013) A novel factor Xa-inhibiting peptide from Centipedes Venom. *International Journal of Peptides Research and Therapeutics*, 19, 303–311.

Krätzschmar, J., Haenderl, B., Langer, G. et al. (1991) The plasminogen activator family from the salivary gland of the vampire bat *Desmodus rotundus*: cloning and expression. *Gene*, 105 (2), 229–237.

Lai, R., Takeuchi, H., Jonczy, J. et al. (2004) A thrombin inhibitor from the ixodid tick, *Amblyomma hebraeum*. *Gene*, 342 (2), 243–249.

Laurin M. and Reisz R. R. (2011) Synapsida. Mammals and their extinct relatives, accessed at: http://tolweb.org/Synapsida/14845.[Last updated 14/8/11]. (9 March 2015).

Law, J. H., Ribeiro, J. M. C. and Wells, M. A. (1992) Biochemical insights derived from diversity in insects. *Annual Review of Biochemistry*, 61, 87–111.

Lent, C. M., Fliegner, K. H., Freedman, E. et al. (1988) Ingestive behaviour and physiology of the medicinal leech. *Journal of Experimental Biology*, 137, 513–527.

Lerner, E. A., Ribeiro, J. M., Nelson, R. J. et al. (1991) Isolation of a potent vasodilatory peptide from the salivary glands of the sand fly *Luotzymya longipalpis*. *Journal of Biological Chemistry*, 266 (17), 11234–11236.

Li, D., Scherfer, C., Korayem, A. M. et al. (2002) Insect hemolymph clotting: evidence for interaction between the coagulation system and the prophenoloxidase activating cascade. *Insect Biochemistry and Molecular Biology*, 32 (8), 919–928.

Li, G., Wang, K. Y., Li, D. et al. (2012) Cloning, expression and characterization of a gene from earthworm *Eisenia fetida* encoding a blood-clot dissolving protein. *PLoS One*, 7 (12), e53110.

Liao, M., Zhou, J., Gong, H. et al. (2009) Hemalin, a thrombin inhibitor isolated from a midgut cDNA library from the hard tick *Haemaphysalis longicornis*. *Journal of Insect Physiology*, 55 (2), 164–173.

Liberatore, G. T., Samson, A., Bladin, C. et al. (2003) Vampire bat salivary plasminogen activator (desmoteplase): a unique fibrinolytic enzyme that does not promote neurodegeneration. *Stroke*, 34 (2), 537–543.

Linker, A., Hoffman, P. and Meyer, K. (1957) The hyaluronidase of the leech: an endoglucuronidase. *Nature*, 180 (4590), 810–811.

Lloyd, C. M. and Walker, A. R. (1995) Salivary glands and saliva of *Amblyomma variegatum* ticks: comparison of immatures and adults in relation to the pathogenesis of dermatophilosis. *Veterinary Parasitology*, 59, 59–67.

Low, D. H. W., Sunagar, K., Undheim, E. A. B. et al. (2013) Dracula’s children: Molecular evolution of vampire bat venom. *Journal of Proteomics*, 89, 95–111.

Ma, D., Mizununi, D. M., Assumpção, T. C. F. et al. (2013) Desmolaris, a novel factor Xαa anticoagulant from the salivary gland of the vampire bat (*Desmodus rotundus*) inhibits inflammation and thrombosis *in vivo*. *Blood*, 122 (25), 4094–4106.

Macedo-Ribeiro, S., Almeida, C., Calisto, B. M. et al. (2008) Isolation, cloning and structural characterisation of boophilin, a multifunctional Kunitz-type proteinase inhibitor from the cattle tick. *PLoS One*, 3 (2), e1624.

Maina, A. N., Jiang, J., Omulo, S. A. et al. (2014) High prevalence of *Rickettsia Africae* variants in *Amblyomma variegatum*. Ticks from domestic mammals in rural western Kenya: implications for human health. *Vector-Borne and Zoonotic Diseases* (Larchmont, N.Y.), 14 (10), 693–702.

Mans, B. J., Gasper, A. R. M. D., Louw, A. I. et al. (1998a) Apyrase activity and platelet aggregation inhibitors in the tick *Ornithodoros savignyi* (Acarina: Argasidae). *Experimental and Applied Acarology*, 22, 353–366.

Mans, B. J., Gasper, A. R. M. D., Louw, A. I. et al. (1998b) Purification and characterisation of apyrase from the tick, *Ornithodoros savignyi*. *Comparative Biochemistry and Physiology. Part B: Biochemistry & Molecular Biology*, 120, 617–624.

Mant, M. J. and Parker, K. R. (1981) Two platelet aggregation inhibitors in tsetse (Glossina) saliva with studies of roles of thrombin and citrate in *in vitro* platelet aggregation. *British Journal of Haematology*, 48 (4), 601–608.

Maragane, J. M., Chao, B., Joseph, M. L. et al. (1989) Anticoagulant activity of synthetic hirudin peptides. *Journal of Biological Chemistry*, 264, 8692–8698.

Markwardt, F. (1957) Die Isolierung und chemische Charakterisierung des Hirudins. *Hoppe-Seyler’s Zeitschrift für Physiologische Chemie*, 308, 147–156.
Marshall, C. G. and Lent, C. M. (1988) Excitability and secretory activity in the salivary gland cells of jawed leeches (Hirudinea: Gnathobdellida). Journal of Experimental Biology, 137, 89–105.

Mihara, H., Sumi, H. and Yoneta, T. et al. (1991) A novel fibrinolytic enzyme extracted from the earthworm, Lumbricus rubellus. The Japanese Journal of Physiology, 41 (3), 461–472.

Mühlhahn, P., Czisch, M., Morenweiser, R. et al. (1994) Structure of leech derived trypsinase inhibitor (LDTI-C) in solution. FEBS Letters, 355 (3), 290–296.

Munro, R., Jones, C. P. and Sawyer, R. T. (1991) Calin–a platelet adhesion inhibitor from the salivary glands of the medicinal leech. Blood Coagulation and Fibrinolysis: An International Journal in Haemostasis and Thrombosis, 2 (1), 179–184.

Munshi, Y., Ara, I., Rafique, H. et al. (2008) Leeching in the history—a review. Pakistan Journal of Biological Sciences, 11 (13), 1650–1653.

Murphy, K. (2012) Janeway's Immunobiology, 8th edn, Garland Science, New York, USA, p.7.

Naski, M. C., Fenton, J. W., Maraganore, J. M. et al. (1990) The COOH-terminal domain of hirudin. An exosite-directed competitive inhibitor of the action of alpha-thrombin on fibrinogen. Journal of Biological Chemistry, 265, 13484–13489.

Nawarskas, J. J. and Anderson, J. R. (2001) Bivalirudin: a new approach to anticoagulation. Heart Disease (Hagerstown, Md), 3 (2), 131–137.

Nienaber, J., Gaspar, A. R. and Neitz, A. W. H. (1999) Savignin, a potent thrombin inhibitor isolated from the salivary glands of the tick Ornithodoros savignyi (Acari: Argasidae). Experimental Parasitology, 93 (2), 82–91.

Noeske-Jungblut, C., Haendler, B., Donner, P. et al. (1995) Triabin, a highly potent exosite inhibitor of thrombin. Journal of Biochemical Chemistry, 270 (48), 28629–28634.

Noeske-Jungblut, C., Krätzschmar, J., Haendler, B. et al. (1994) An inhibitor of collagen-induced platelet aggregation from the saliva of Triatoma pallidipennis. The Journal of Biological Chemistry, 269 (7), 5050–5053.

Oliveira, D. G., Alvarez-Flores, M. P., Lopes, A. R. et al. (2012) Functional characterisation of vizzontin, the first factor Xa inhibitor purified from the leech Haementeria vizottai. Thrombosis and Haemostasis, 108 (3), 570–578.

Oren, M., Escande, Ml., Paz, G. et al. (2008) Urochordate histoincompatible interactions activate vertebrate-like coagulation system components. PLoS One, 3 (9), e3123.

Paciaroni, M., Medeiros, E. and Bogousslavsky, J. (2009) Desmoteplase. Expert Opinion on Biological Therapy, 9 (6), 773–778.

Paeesen, G. C., Adams, P. L., Harlos, K. et al. (1999) Tick histamine-binding proteins: isolation cloning and three-dimensional structure. Molecular Cell, 3 (5), 661–671.

Paeesen, G. C., Adams, P. L., Nuttall, P. A. et al. (2000) Tick histamine-binding proteins: lipocalins with a second binding cavity. Biochimica et Biophysica Acta (BBA) – Protein Structure and Molecular Enzymology, 1482 (1–2), 92–101.

Palta, S., Saroa, R. and Palta, A. (2014) Overview of the coagulation system. Indian Journal of anaesthesia, 58 (5), 515–523.

Park, S. Y., Kye, K. C., Lee, M. H. et al. (1989) Fibrinolytic activity of the earthworm extract. Thrombosis and Haemostasis, 62, 545–550.

Parker, K. R. and Mant, M. J. (1979) Effects of tsetse (Glossina morsitans morsitans Westw.) (Diptera: Glossinidae) salivary gland homogenate on coagulation and fibrinolysis. Thrombosis and Haemostasis, 42 (2), 743–751.

Patel, R., Ispongou, S. and Apostolakis, S. (2014) Desmoteplase as a potential treatment for cerebral ischaemia. Expert Opinion on Investigational Drugs, 23 (6), 865–873.

Peterson, K. J., Cotton, J. A., Gehling, J. G. et al. (2008) The Ediacaran emergence of bilaterians: congruence between the genetic and the geological fossil records. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 363 (No. 1496), 1435–1443.

Piechowski-Jozwiak, B. and Bogousslavsky, J. (2013) The use of desmoteplase (bat saliva) in the treatment of ischaemia. Expert Opinion on Biological Therapy, 13 (3), 447–453.

Ponczek, M. B., Bijak, M. Z. and Nowak, P. Z. (2012) Evolution of thrombin and other hemostatic proteases by survey of protochordate, hemichordate, and echinoderm genomes. Journal of Molecular Evolution, 74 (5–6), 319–331.

Qiu, Y., Lee, K. S., Choo, Y. M. et al. (2013) Molecular cloning and anti-fibrinolytic activity of a serine protease inhibitor from bumblebee (Bombus terrestris) venom. Toxicon, 63, 1–6.

Ramachandra, R. N. and Wikel, S. K. (1995) Effects of Dermacentor andersoni (Acari: Ixodidae) salivary gland extracts on Bos indicus and B. taurus lymphocytes and macrophages: in vitro cytokine elaboration and lymphocyte blastogenesis. Journal of Medical Entomology, 32, 338–345.

Reverter, D., Vendrell, J., Canals, F. et al. (1998) A carboxypeptidase inhibitor from the medical leech Hirudo medicinalis. Isolation, sequence analysis, cDNA cloning, recombinant expression, and characterization. The Journal of Biological Chemistry, 273 (49), 32927–32933.

Ribeiro, J. M. C. (1995) Blood-feeding arthropods: live syringes or invertebrate pharmacologists? Infectious Agents and Disease, 4, 143–152.

Ribeiro, J. M., Evans, P. M., MacSwain, J. L. et al. (1992) Amblyomma americanum: characterization of salivary proaglandins E2 and F2 alpha by RP-HPLC/bioassay and gas chromatography-mass spectrometry. Experimental Parasitology, 74 (1), 112–116.

Ribeiro, J. M. C., Modi, G. B., Tesh, R. B. et al. (1989a) Salivary apyrase activity of some old world phlebotomine sand flies. Insect Biochemistry, 19 (4), 409–412.

Ribeiro, J. M., Makoul, G. T., Levine, J. et al. (1985) Antihemostatic, anti-inflammatory, and immunosuppressive properties of the saliva of a tick, Ixodes dammini. Journal of Experimental Medicine, 161, 332–344.
Ribeiro, J. M., Makoul, G. T. and Robinson, D. R. (1988) *Ixodes dammini*: evidence for salivary prostacyclin secretion. *The Journal of Parasitology*, 74 (6), 1068–1069.

Ribeiro, J. M., Marinotti, O. and Gonzales, R. (1990a) A salivary vasodilator in the blood-sucking bug, *Rhodnius prolixus*. *British Journal of Pharmacology*, 101 (4), 932–936.

Ribeiro, J. M., Rossignol, P. A. and Spielman, A. (1986) Blood-finding strategy of a capillary-feeding sandfly, *Lutzomyia longipalpis*. *Comparative Biochemistry and Physiology. A, Comparative Physiology*, 83 (4), 683–686.

Ribeiro, J. M., Sarkis, J. J., Rossignol, P. A. et al. (1984) Salivary apyrase of *Aedes aegypti*: characterization and secretory fate. *Comparative Biochemistry and Physiology. B, Biochemistry and Molecular Biology*, 79 (1), 81–86.

Ribeiro, J. M., Schneider, M. and Guimarães, J. A. (1995) Purification and characterization of prolixin S (nitrophenin 2), the salivary anticoagulant of the blood-sucking bug *Rhodnius prolixus*. *The Biochemical Journal*, 308 (Pt 1), 243–249.

Ribeiro, J. M., Vachereau, A., Modi, G. B. et al. (1989b) A novel vasodilatory peptide from the salivary glands of the sand fly *Lutzomyia longipalpis*. *Science (New York, N.Y)*, 243 (4888), 212–214.

Ribeiro, J. M., Vaughan, J. A. and Azad, A. F. (1990b) Characterization of the salivary apyrase activity of three rodent flea species. *Comparative Biochemistry and Physiology. B, Comparative Biochemistry*, 95 (2), 215–219.

Rigbi, M., Levy, H., Iraqi, F. et al. (1987) The saliva of the medicinal leech Hirudo medicinalis–I. Biochemical characterization of the high molecular weight fraction. *Comparative Biochemistry and Physiology. B, Comparative Biochemistry*, 87 (3), 567–573.

Rigbi, M., Orevi, M. and Eldor, A. (1996) Platelet aggregation and coagulation inhibitors in leech saliva and their roles in leech therapy. *Seminars in Thrombosis and Hemostasis*, 22, 273–278.

Salzet, M., Chopin, V., Baert, J. et al. (2000) Theromin, a novel leech thrombin inhibitor. *Journal of Biological Chemistry*, 275 (40), 30774–30780.

Sanetra, M., Begemann, G., Becker, Mb. et al. (2005) Conservation and co-option in developmental programmes: the importance of homology relationships. *Frontiers in Zoology*, 2, 15.

Santos, A., Ribeiro, J. M., Lehane, M. J. et al. (2007) The sialotranscriptome of the blood-sucking bug *Triatoma brasiliensis* (*Hemiptera, Triatominae*). *Insect Biochemistry and Molecular Biology*, 37 (7), 702–712.

Sauer, J. R. (1977) Acarine salivary glands—Physiological relationships. *Journal of Medical Entomology*, 14, 1–9.

Schleuning, W. D. (2001) Vampire bat plasminogen activator DSPA-alpha-1 (desmeteoptase): a thrombolytic drug optimized by natural selection. *Haemostasis*, 31 (3–6), 118–122.

Schleuning, W. D., Alagon, A., Biodol, W. et al. (1992) Plasminogen activators from the saliva of *Desmodus rotundus* (common vampire bat): unique fibrin specificity. *Annals of the New York Academy of Sciences*, 667, 395–403.

Schutt, B. (2008) *Dark Banquet; Blood and the Curious Lives of Blood-Feeding Creatures*, Three Rivers Press, New York, USA, pp. 49–58.

Seemüller, U., Meier, M., Ohlsson, K. et al. (1977) Isolation and characterization of a low molecular weight inhibitor (of chymotrypsin and human granulocytic elastase and cathepsin G) from leeches. *Hoppe-Seyler’s Zeitschrift für Physiologische Chemie*, 358 (9), 1105–1107.

Sharma, A., Sonah, H., Deshmukh, R. K. et al. (2011) Cloning of fibronectin-like protease-0 (Efp-0) gene from diverse earthworm individuals. *Indian Journal of Biotechnology*, 10 (3), 270–273.

Sniider, G. L., Stone, P. I., Lucey, E. C. et al. (1985) Eglin-c, a polypeptide derived from the medicinal leech, prevents human neutrophil elastase-induced emphysema and bronchial secretory cell metaplasia in the hamster. *The American Review of Respiratory Disease*, 132 (6), 1155–1161.

Söllner, C., Mentele, R., Eckerskorn, C. et al. (1994) Isolation and characterization of hirustasin, an antistasin-type serine-protease inhibitor from the medical leech *Hirudo medicinalis*. *European Journal of Biochemistry*, 219 (3), 937–943.

Sommerhoff, C. P., Söllner, C., Mentele, R. et al. (1994) A Kazal-type inhibitor of human mast cell tryptase: isolation from the medical leech *Hirudo medicinalis*, characterization, and sequence analysis. *Biological Chemistry Hoppe Seyler*, 375, 685–694.

Stark, K. R. and James, A. A. (1995) A factor Xa-directed anticoagulant from the salivary glands of the yellow fever mosquito *Aedes aegypti*. *Experimental Parasitology*, 81 (3), 321–331.

Steranka, L. R., Manning, D. C., DeHaas, C. J. et al. (1988) Bradykinin as a pain mediator: receptors are localized to sensory neurons, and antagonists have analgesic actions. *Proceedings of the National Academy of Sciences of USA*, 85, 3245–3249.

Stubbs, M. T., Morenweiser, R., Stürzebecher, J. et al. (1997) The three-dimensional structure of recombinant leech-derived tryptase inhibitor in complex with trypsin. Implications for the structure of human mast cell tryptase and its inhibition. *Journal of Biological Chemistry*, 272, 19931–19937.

Su, J. B. (2014) Different cross-talk sites between the renin–angiotensin and the kallikrein–kinin systems. *Journal of the Renin-Angiotensin-Aldosterone System*, 15 (4), 319–328.

Swadesh, J. K., Huang, I. Y. and Budzynski, A. Z. (1990) Purification and characterization of hementin, a fibrinogenolytic protease from the leech *Haementeria ghilianii*. *Journal of Chromatography*, 502 (2), 359–369.

Tasiemski, A., Vandenbulcke, F., Mitta, G. et al. (2004) Molecular characterization of two novel antibacterial peptides inducible upon bacterial challenge in an annelid, the leech *Theromyzon tessulatum*. *The Journal of Biological Chemistry*, 279 (30), 30973–30982.
Tatchell R. J. and Binnington K. C. (1973) An active constituent of the saliva of the cattle tick, *Boophilus microplus*; *Proceedings of the 3rd International Conference of Acarology*, 1971, 745.

Tellgren-Roth, A., Dittmar, K., Massey, S. E. et al. (2009) Keeping the blood flowing-plasminogen activator genes and feeding behavior in vampire bats. *Naturwissenschaften*, 96 (1), 39–47.

Theopold, U., Schmidt, O., Söderhall, K. et al. (2004) Coagulation in arthropods: defence, wound closure and healing. *Trends in Immunology*, 25 (6), 289–294.

Tuszynski, G. P., Gasic, T. B. and Gasic, G. J. (1987) Isolation and characterization of antistasin. An inhibitor of metastasis and coagulation. *Journal of Biological Chemistry*, 262 (20), 9718–9723.

Vachereau, A. and Ribeiro, J. M. C. (1989) Immunoreactivity of salivary gland apyrase of *Aedes aegypti* with antibodies against a similar hydrolase present in the pancreas of mammals. *Insect Biochemistry*, 19 (6), 527–534.

Valenzuela, J. G. (2004) Exploring tick saliva: from biochemistry to ‘sialomes’ and functional genomics. *Parasitology*, 129 (Suppl), S83–S94.

Valenzuela, J. G., Francischetti, I. M. B., Pham, V. M. et al. (2002) Exploring the sialome of the tick *Ixodes scapularis*. *Journal of Experimental Biology*, 205, 2843–2864.

van de Locht, A., Stubbs, M. T. and Bode, W. (1996) The ornithodorin-thrombin crystal structure, a key to the TAP enigma? *The EMBO Journal*, 15 (22), 6011–6017.

Viljoen, G. J., Bezuidenhout, J. D., Oberem, P. T. et al. (1986) Isolation of a neurotoxin from the salivary glands of female *Rhipicephalus evertsi evertsi*. *Journal of Parasitology*, 72, 865–874.

Wan, H., Lee, K. S., Kim, B. Y. et al. (2013) A spider-derived Kunitz-type serine protease inhibitor that acts as a plasmin inhibitor and an elastase inhibitor. *PLoS One*, 8 (1), e53343.

Wang, X., Coons, L. B., Taylor, D. B et al. (1996) Variabilin, a novel RGD-containing antagonist of glycoprotein IIb-IIIa and platelet aggregation inhibitor from the hard tick *Dermacentor variabilis*. *The Journal of Biological Chemistry*, 271 (30), 17785–17790.

Waxman, L. and Connolly, T. M. (1993) Isolation of an inhibitor selective for collagen-stimulated platelet aggregation from the soft tick *Ornithodoros moubata*. *The Journal of Biological Chemistry*, 268 (8), 5445–5449.

Waxman, L., Smith, D. E., Arcuri, K. E. et al. (1990) Tick anticoagulant peptide (TAP) is a novel inhibitor of blood coagulation factor Xa. *Science*, 248 (4955), 593–596.

Wikel, S. K. (1982) Histamine content of tick attachment sites and the effect of H1 and H2 histamine antagonists on the expression of resistance. *Annals of Tropical Medicine and Parasitology*, 76, 179–185.

Wimsatt, W. A. (1959) Portrait of a vampire. *Ward’s Natural Science Bulletin*, 32, 35.

You, W. K., Sohn, Y. D., Kim, K. Y. et al. (2004) Purification and molecular cloning of a novel serine protease from the centipede, *Scolopendra subspinipes mutilans*. *Insect Biochemistry and Molecular Biology*, 34 (3), 239–250.

Zaidi, S. M., Jameel, S. S., Zaman, F. et al. (2011) A systematic overview of the medicinal importance of Sanguivorous leeches. *Alternative Medicine Review*, 16 (1), 59–65.