Features of Interaction Between Triatomines and Vertebrates Based on Bug Feeding Parameters

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Abstract Hematophagy is an unusual feeding strategy among arthropods occurring in <1.6% of phylum diversity. However, hematophagous arthropods possess great medical-veterinary relevance, as they act as vectors of important vertebrate diseases. Hematophagous species obtain blood from live vertebrates once they have successfully overcome key challenges, such as the penetration of mouthparts into the host epidermis, successful location of blood within the dermis, and pumping blood into the arthropod digestive tract. Despite a marked phylogenetic diversity among bloodsucking arthropods, adaptive convergences related to hematophagous behavior are observed, such as morphological adaptations of mouthparts, including structures selected for perforating, penetrating, and anchoring mouthparts in host skin, as well as the composition of saliva, which is rich in biomolecules capable of interfering with vertebrate physiology at the feeding site.

Triatomine bugs are temporary ectoparasites; their contact with their hosts is restricted to a blood meal. Blood is obtained directly from blood vessels in vertebrate skin. The following two physical sites of the triatomine–host interface are relevant during this process: a) the bug's "functional mouth" and host endothelium and b) the insect anterior midgut and host blood. In triatomines, most of the feeding time covers blood intake from host skin blood vessels to the insect anterior midgut. Blood intake is performed mainly through cibarial pump activity in these insects. The product of pumping frequency (F) and liquid volume ingested through each cibarial pump contraction (QLC) corresponds to the effective intake rate (EIR, mg/min) achieved by the insect during feeding. This is the triatomine feeding parameter that influences contact time with the host the most. The EIR of fifth instar nymphs fed on mouse abdominal skin can vary by more than 600% among triatomine spe-

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cies. The main parameter associated with differences in EIR between triatomine species, and among nymphal stages feeding in similar conditions, is QLC. Furthermore, cibarial pump frequency is the EIR component affected the most for a given species or development stage, by external factors such as host species (avian or mammal), feeding site characteristics (e.g., vessel diameter), and blood behavior within the midgut environment (e.g., coagulated/uncoagulated, aggregated/not aggregated). The likelihood that intestinal environmental factors affect feeding pump activity opens new perspectives for studying the impact of midgut colonization by different pathogens/microbiota on feeding performance, and/or the vectorial capacity of hematophagous arthropods.

Keywords Triatomines · Vertebrate host · Feeding parameters · Feeding site · Saliva · Insect anterior midgut

1 Initial Considerations

Hematophagy is an uncommon feeding behavior among arthropods, practiced by <1.6% of all ~1.21 million species described. It is believed that there are approximately only 14,500 hematophagous species distributed in the orders Diptera, Hemiptera, Siphonaptera, Lepidoptera, and Phthiraptera within the class Insecta. This strategy evolved solely in mites (~45,000 species) within the class Arachnida, where it is thought that less than 10% of species feed on blood (Ribeiro and Arca 2009; Mans 2011; Stork 2018). Therefore, the estimated number of blood-sucking arthropod[1] species is lower (<56%) than the ~33,000 species of major terrestrial vertebrate hosts. Since most hematophagous arthropods may be perceived, or even killed by the host during a blood meal, rapid feeding may be advantageous (Gillett 1969; Sant'Anna et al. 2001). Modifications of blood feeding performance in the vector appear to be common strategy found in many parasite–vector associations to enhance parasite circulation (Hurd 2003). In general, it is during hematophagy that pathogens circulate between the vertebrate host and their vectors. In addition, ingested blood may represent the only nutrient source for some arthropods (e.g., triatomines, ticks, lice, bed bugs) or as a nutritional stimulus for oocyte production (e.g., sandflies and mosquitoes). Therefore, differences in feeding performance directly affect vector competence and/or insect vector population dynamics.

In Hemiptera with sucking mouthparts, the presence of piercing elements and feeding pumps facilitated the emergence of different liquid diets. Although most hemipteran species (within the suborder Heteroptera) are phytophagous or predatory, obligatory hematophagy appears in three families (Cimicidae, Polyctenidae and Reduviidae). The largest number of hematophagous species (150) are within

[1] Hematophagous species of the class Crustacea, such as parasitic isopods of the family Gnathiidae, are not included.

the Reduviidae family, all of which were grouped into the subfamily Triatominae (Cobben 1978; Monteiro et al. 2018).

The topics covered in this chapter have been organized into two interrelated sections: the first one considers general aspects of hematophagy with an emphasis on triatomines, while the second, a compilation of published data on triatomine feeding behavior, aims to provide an understanding of how vectors and hosts interact at the feeding site, and how the midgut environment affects blood ingestion in hematophagous arthropods.

2 General View of Hematophagy

Hematophagy has arisen on at least 20 distinct occasions in the course of arthropod evolution. This promoted a series of independent, though functionally convergent physiological adaptations to solve common challenges relevant to hematophagous behavior, including a morphologically specialized feeding apparatus (Black and Kondratieff 2005) and various salivary biomolecules (Ribeiro and Francischetti 2003; Valenzuela 2004; Andrade et al. 2005; Ribeiro and Arca 2009; Arca and Ribeiro 2018) to enable feeding on blood from vertebrate host skin.

The skin of vertebrates is divided into an outermost layer called epidermis and two inner layers called dermis and hypodermis. The dermis is the thickest layer of the skin where blood vessels are located. However, only a fraction of the total skin area (<5%) is normally irrigated by blood vessels (Ribeiro, 1987). Blood is a tissue containing different types of cells, for example, red blood cells (RBCs), white blood cells (WBCs), and platelets or thrombocytes, and intercellular liquid (i.e., plasma) containing proteins, ions, and other molecules (Lewis 1996; Baskurt and Meiselman 2003).

Blood composition is relatively uniform among vertebrates with the exception of groups with nucleated red blood cells containing high levels of nucleic acids, such as birds, reptiles, and mammals of the family Camelidae. Whole human blood contains 80 g of water, 0.6 g of lipids, 0.08 g of carbohydrates, and 20.5 g of proteins per 100 mL (Lehane 2005). Thus, blood is composed of ~80% water and has a dry weight of ~97% protein.

Hematophagous species obtain blood from live vertebrates upon successfully overcoming key challenges, such as penetration of mouthparts into the host epidermis, location of blood in the host dermis, and pumping host blood into the arthropod digestive tract.

Despite morphological variations observed in mouthparts of hematophagous arthropod groups, it is possible to observe functional similarities, including structures selected for puncturing, penetrating, and anchoring, as well as sheath-like covering, a food canal, and a separated canal for saliva ejection owing to adaptive convergence to enable blood sucking (Krenn and Aspöck 2012). Insects possess a salivary canal that is independent of the food canal allowing them to simultaneously ingest blood and release saliva into host skin. In ticks, blood intake and saliva ejec-

tion occur alternately, as these arthropods use the food canal for both purposes (Lavoipierre and Riek 1955; Costa et al. 2016). Saliva is channeled through maxillary stylets only in fleas (Siphonaptera) and the order Hemiptera (Wenk 1953).

In triatomines, the proboscis is composed of a three-segment labium that encompasses a pair of mandibles and maxillae, both of which are long and needle-like. Mandible tips feature a row of teeth on their edges, whereas maxillae are thinner and have smoother structures (without teeth and bristles). Maxilla stylets articulate with each other to form two channels: a central food canal and a narrow alimentary canal (Lavoipierre et al. 1959). Triatomine mouthparts have unique features among Reduviidae bugs; their mandibles only have one row of strong teeth aligned along the midline, while their maxillary stylets lack bristles (Weirauch 2008). Such peculiar characteristics in relation to predatory members of Reduviidae can be morphological adaptations to hematophagous behavior in triatomines, as these allow a clean cut in the epidermis by the mandibles and less cellular destruction through smooth maxillae movements within the vertebrate host dermis.

After overcoming the epidermis barrier, hematophagous arthropods need to bring the alimentary canal opening (also called "functional mouth") into contact with blood. They use two basic mechanisms to achieve this, namely solenophagy ("vessel-feeding") and telmophagy ("pool feeding"), and these terms were proposed by Lavoipierre in 1965. In solenophagy, the alimentary canal opening is introduced directly into the blood vessel. Depending on the size of the blood vessel, it is possible to observe vibrations in its wall during blood ingestion by arthropods (Lavoipierre et al. 1959; Soares et al. 2014) (Fig. 1A). This feeding strategy is found in triatomines, mosquitoes, and lice (Lehane 2005).

Telmophagy consists of positioning the functional mouth in the skin region that contains blood from a vessel lacerated by the mechanical action of the arthropod's mouthparts. The formation of a feeding pool on mouse skin takes ~5% of total contact time (~ 40 min) in the soft tick *Ornithodoros rostratus*, with intense activity of the chelicerae and abundant salivation observed at this stage. Blood ingestion is characterized by a rhythmic variation in pool volume surrounding the tick's mouthparts (Costa et al. 2016) (Fig. 1A–C). Among all arthropods, the pool feeding strategy is present in sandflies, ticks, tabanids, ceratopogonids, tsetse flies, and blackflies (Lavoipierre and Riek 1955; Lehane 2005). Depending on feeding site conditions, certain vessel feeders may feed on blood extravasated from vessels, as reported in mosquitoes (Lavoipierre 1965).

It is the activity of the arthropod's mouthparts in search of blood that damages skin structures (cells and vessels) resulting in a release of or exposure to various host molecules in the invertebrate feeding site regardless of the feeding mechanism (solenophagy or telmophagy). Some of these molecules are mediators of host physiological responses (e.g., ADP, ATP, and collagen) that prevent blood loss (hemostasis) and promote tissue repair (inflammation).

Mammals have developed sophisticated mechanisms to limit blood loss after a vascular injury, a phenomenon known as hemostasis. This usually begins with a transient vasoconstriction episode followed by platelet adhesion/aggregation and blood coagulation (Ratnoff 1987). The injury of tissue (not only vascular), platelet

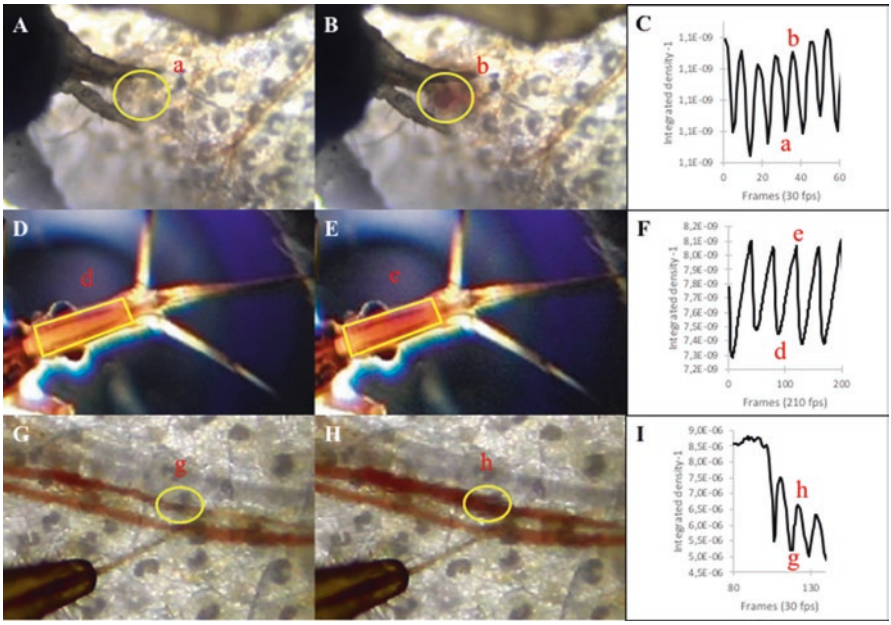

Fig. 1 Analysis of intravital images recorded during arthropod blood feeding on mouse skin A–B: Images of a 4th instar *Ornithodoros rostratus* feeding site, with the area around the feeding pool selected (marked by the yellow line) as minimal (a) or covered with blood (b). D–E: Images of a 5th instar *Rhodnius prolixus* head with the area around empty (d) or blood-filled (e) with the cibarial pump selected. G–H: Images of the feeding site of a 5th instar *Triatoma infestans*, with the area around the cannulated venule selected (marked by the yellow line) with minimal blood (g) or its lumen full of blood (h). C, F and I: The graphical representation of an estimate of the area occupied by blood within the selected sites in each frame over time. This calculation was performed using the Integrated Density function of ImageJ program (https://imagej.nih.gov/), according to Paim et al. (2017)

activation/aggregation, together with the activation of blood coagulation triggers the induction of inflammation, which is characterized by cardinal features such as rubor (redness) and tumor (swelling), as well as calor (heat) and dolor (pain). All of these involve changes in microcirculation: redness and heat reflect vasodilation, while pain is produced by the stimulation of nociceptors located in the inflamed tissue, which can be intensified by the swelling caused by increased vascular permeability (Ley 2008; Ribeiro and Francischetti 2003; Ribeiro and Arca 2009).

In addition, hemostasis and inflammatory reactions may be exacerbated by the development of immune responses (both innate and adaptive) against the arthropod's salivary antigens released during a blood meal (Ribeiro 1987, 1995). Therefore, during hematophagy, arthropods encounter some or all of these reactions, which hamper blood acquisition, such as by decreasing blood availability at the arthropod's feeding site (e.g., hemostasis reactions), or trigger defensive host behavior (e.g., reactions to itch and pain).

In contrast, insect saliva is rich in bioactive molecules capable of interfering with hemostasis, inflammation, and vertebrate host immunity, aiding the acquisition of a blood meal (Ribeiro and Francischetti 2003). Fleas and most blood feeding nematocerans (mosquitoes, sandflies, and blackflies) are estimated to have between 100 and 200 types of protein in their saliva; insects from the suborder Brachycera (tsetse flies and tabanids) have about 250–300, triatomines have >300, and ticks have more than 500 bioactive proteins to aid hematophagy (Francischetti et al. 2009; Arca and Ribeiro 2018). Such differences in salivary composition could be related to feeding type (vessel feeding or pool feeding) and blood meal duration (Arca and Ribeiro 2018).

Lipocalins are the most abundant and diverse family of proteins present in the saliva of triatomines and ticks. They have been shown to bind small molecules such as biogenic amines, nucleotides, and eicosanoids, which are effectors of the host's hemostasis and inflammation (Andersen and Ribeiro 2017).

In most hematophagous species, the pattern of salivation during different phases of feeding, as well as information on the saliva deposition site (whether it is inside or outside the vessels) within the host skin, is poorly known. In a study that described the bed bug (*Cimex lectularius*) feeding mechanism visualizing the insect's blood meal through the skin of a mouse ear, Dickerson and Lavoipierre (1959) commented: *"We looked particularly for the discharge of saliva, but we never observed the ejection of any fluid from the mouthparts, either during probing or whilst the insect was feeding from a blood-vessel."*

Salivation in *Rhodinus prolixus* begins at the moment of the bite, continues until the end of the blood meal (Friend and Smith 1971), and shows two distinct patterns. During the probing phase, salivation is abundant and constant, whereas during the engorgement phase, saliva is slowly released into the blood vessels in low frequency pulses (Soares et al. 2006; Sant'Anna et al. 2017). Part of the saliva released by the triatomine is ingested along with the diet, enabling the salivary biomolecules to act on blood stored in the insect midgut (Ribeiro and Garcia 1980; Soares et al. 2006).

After locating the blood, the insect needs to pump it from host blood vessels to the insect midgut, where the meal is initially stored. In liquid feeding insects, the cibarial and/or pharyngeal regions are modified to form a feeding pump. The walls of these regions are reinforced in these cases and normally have resilin (a rubber-like protein) deposits (Edwards 1983) linked to cibarial dilator muscles (extrinsic visceral muscles). Dilator muscles are antagonized by circular intrinsic muscles in pharyngeal pumps lacking intrinsic muscles (retractors), whereas muscle activity results from the elasticity of the muscle wall in cibarial pumps (Smith 1985; Chapman et al. 2013). The cibarial pump is well developed, whereas the pharyngeal pump is poorly developed in hematophagous Hemiptera. Both are well developed in female mosquitoes (Chapman et al. 2013).

Over the past decade, the integrated use of different 3D imaging and analysis techniques (particle image velocimetry [PIV] and synchrotron X-ray microscopic computed tomography) has yielded a detailed description of the coordinated operation of both the suction pumps (cibarial and pharyngeal) of the arthropod. Mosquitoes have two pumping patterns, which can produce a continuous ingestion flow through

multiple small suction motions, and a burst mode involving a single high-volume stroke (Kim et al. 2011; Lee et al. 2009; Kikuchi et al. 2018). However, the context in which mosquitoes shift their pumping pattern (of small or high-volume strokes) is unclear.

A recent study of *R. prolixus* synchrotron X-ray images showed that the frequency of the reduced pharyngeal pump matches that of the cibarial pump, although it makes inverted contraction-relaxation movements during fluid intake by the insect (Lahondère et al. 2017).

In contrast to bloodsucking insects and argasid ticks that normally spend a few minutes to <2 h on feeding, it takes several days for ixodid ticks to complete their blood meal on the host. The size of blood meal varies greatly between different groups of hematophagous arthropods. The gain in adult female body weight after a blood feed can reach ~110–140% in culicids, ~130% in cimicids, ~200–300% in triatomines and over 1000% in ixodids (Lehane 2005; Flynn and Kaufman 2015; Sant'Anna et al. 2017). The volume of blood ingested can be influenced by several factors such as the age, body size, nutritional status, and reproductive state of the arthropod. Under conditions where no physical restrictions to maintain blood pumping exist, triatomine and culicid blood meal sizes are regulated by the activation of abdominal stretch receptors, which signal the optimal blood meal volume to be ingested into the insect's midgut to the brain (Maddrell 1964; Gwadz 1969).

For temporary ectoparasites, visiting the host for a blood meal is very dangerous. Any appropriate strategy must include the maximization of nutrient intake rates, and the minimization of the host's contact time to overcome risks (Lehane 2005), while the role of feeding mechanics in determining constraints of such optimal strategies is often neglected (Daniel and Kingsolver 1983).

From a mechanical point of view, blood can be considered a solid–liquid suspension with cellular elements (primarily red blood cells) being the solid phase. Apparent blood viscosity depends on the shear forces present (since blood behaves like a non-Newtonian fluid), and it is determined by factors such as blood hematocrit (the volume percentage of RBC in blood), plasma viscosity, RBC aggregation, and RBC deformability. The viscosity of vertebrate blood decreases with hematocrit reduction and with an increase in temperature and shear rates, and the latter results from the movement of blood cells within vessels. Red blood cells tend to aggregate and adhere to each other (erythrocyte aggregation) with an intensity varying between species. The less aggregated the red blood cells, the more easily the particle suspension can move along a blood vessel (Kingsolver and Daniel 1995; Baskurt and Meiselman 2003).

Few models have been proposed to describe blood feeding mechanics to date, with those put forward mainly considering physical characteristics of the proboscis (mainly the diameter/length of the initial section of the alimentary canal), and the difference in pressure generated by the insect's feeding pumps (Kornev et al. 2017). An analysis of these models based on the description of blood flow through narrow tubes suggested that the biomechanics of blood feeding can be explained by the Hagen–Poiseuille equation for Newtonian fluids (Bennet-Clark 1963a, b; Daniel and Kingsolver 1983; Lehane 2005). According to Kingsolver and Daniel (1995),

parameters such as negative pressures produced by the cibarial pump, insect food canal dimensions, blood viscosity, the host's red blood cell size, and deformation capacity can all influence blood ingestion rate.

Although there are important differences in blood meal parameters (size, duration, and periodicity) between blood feeding arthropod groups, a similar sequential pattern of events is observed during feeding by most bloodsucking insects and argasid ticks. Thus, based on the movement of mouthparts and/or feeding pump activity, the arthropod's blood feeding process can be divided into at least four basic steps: bite, probing, engorgement, and interruption. Bite corresponds to the moment when mouthparts penetrate the outer part of the skin. Probing occurs when the arthropod actively moves its mouthparts searching for blood in the host dermis. Probing is preceded by the bite, but it can also occur at any time during the feeding process without the need for the arthropod to remove mouthparts from the host skin. Engorgement is the period in which the arthropod effectively ingests blood with feeding pumps in operation. Interruption is the moment when blood intake is stopped without the withdrawal of mouthparts from the host skin, but with feeding pumps inactive (in the case of argasid ticks, apparently there is no saliva ejection). With the exception of interruption, all events appear at least once during blood feeding, although they can happen several times during the blood meal.

3 Triatomine Blood Feeding Characteristics

Triatomine bugs are temporary ectoparasites, and their contact with their hosts, both for immature instars and adults, is restricted to a blood meal. Feeding motivation is rhythmically modulated, with individuals more likely to feed during the first hours of the night (Lazzari et al. 2013). These insects are vessel feeders obtaining their blood meals directly from blood vessels (venules or arterioles) of their vertebrate hosts. After the bug pierces host skin, a probing period, characterized by rapid whip-like intradermal movements of the maxillae, can be observed (Lavoipierre et al. 1959). During this initial phase, *R. prolixus* samples the liquid around its maxillae periodically, analyzing its composition to detect the presence of phagostimulants (cues of blood presence—ATP and ADP nucleotides). Eight gustatory sensillae located in the cibarium are used for this purpose (Friend and Smith 1971; Pontes et al. 2016). Once a suitable vessel is found, probing ceases and the bug engorges with blood (Lavoipierre et al. 1959). The ingestion of blood through the food canal is aided by the cibarial pump, the filling of which is accomplished by extending muscles from the dorsal wall of the head, while emptying is achieved by returning the piston under the force of the elastic ligament (Bennet-Clark 1963a, b).

Fifth instar nymphs of *R. prolixus* can achieve high ingestion rates (~20 mg/min at a flow rate of ~0.33 μL s^{-1}) despite a narrow food canal at the apex (8–10 μm in diameter) (Bennet-Clark 1963a, b). Conservative calculations suggest that the cibarial pump of *R. prolixus* is able to generate pressure differences of 1–2 atm during feeding (Kingsolver and Daniel 1995).

At the beginning of the blood meal, the abdominal cuticle of *R. prolixus* becomes less rigid (plasticization) allowing the abdomen to dilate, thereby accommodating a proportionately large amount of food in the anterior midgut (Bennet-Clark 1962; Maddrell 1966). Cuticle plasticization, as several other feeding-related physiological responses, are under neural control mediated by hormones secreted into the hemolymph, occurring as soon as the insect touches/bites host skin (Maddrell 1966; Reynolds 1974; Ianowski et al. 1998).

Orchard (2006) argued that the neurohormone serotonin (5-HT) released by nerve terminations coordinates the steps of a blood meal in *R. prolixus*, acting as an effector, additive or synergic agent in several feeding-related events such as: a) salivation; b) water and ion transport from the anterior midgut to the hemolymph, and the concentration of ingested blood cells and plasma proteins; c) cuticle plasticization; d) the elevation of dorsal vessel frequency, which is important for increased hemolymph circulation to hormone-target tissues (potential energy supply to the cibarial pump), and for insect thermoregulation (see Lahondère et al. 2017); e) urine formation and post-prandial diuresis (Orchard 2006).

The technique (electrical penetration graph [EPG]) to study feeding parameters in triatomine bugs was standardized by Smith and Friend (1970), registering changes in electrical resistance between bugs and their feeding source. This methodology was modified by Smith (1979) and Guarneri et al. (2000) for recording signals generated by the cibarial pump's musculature, similar to an electromyogram (Araujo et al. 2011).

Combined analysis using electrical signal profiles and intravital microscopy (targeting cibarial pump activity and vessel wall movements, see Fig. 1) obtained during the insect's blood meal combined the information from both methodologies to describe different phases of triatomine blood feeding in detail (Soares et al. 2014) (see details in Table 1).

Total contact time (TCT) of bugs with their feeding source (corresponding to "feeding time") is briefly divided into two parts: the ingestive (IP) and non-ingestive periods (NIP). The former comprises the sum of all engorgement periods (with the cibarial pump in activity), while the latter is the sum of probing and interruption events that occur during blood feeding (cibarial pump downtime). Using the bug's weight gain (WG) upon feeding (corresponding to "blood meal size"), two distinct ingestion rates can be calculated: the total ingestion rate (TIR), related to TCT (TIR = WG/TCT), and the effective ingestion rate (EIR), related to IP (EIR = WG/IP). The effective ingestion rate can also be obtained by multiplying the frequency of pumping (F) by the quantity of liquid ingested by each cibarial pump contraction (corresponding to "stroke volume"). Thus, TCT can be calculated from EIR ($TCT = [WG \times (F \times QLC)^{-1}] + NIP$). This equation allows for a good understanding of the feeding process through relationships among different feeding parameters.

To evaluate host influence (host species or feeding site) or the impact of insect characteristics (feeding apparatus, developmental stage, and salivary gene) on feeding parameters, we compiled electromyogram feeding behavioral data for seven triatomine species (three *Triatoma* and four *Rhodnius*), four species of host (three mammals and one avian) with different feeding site characteristics, with hosts treated with anti-hemostatic drugs (heparin) or insects injected with dsRNA (RNAi) for salivary genes (Table 1).

Table 1 Blood-feeding parameters (see the definitions below) of different triatomines (species, stages, treatments) fed on live hosts

Species[a]	Stage	TCT (min)	IP %	NIP %	WG (mg)	EIR (mg/min)	F (Hz)	QLC (ηL)	Host[b]	Site[c]	Refs[d]	Line
T. brasiliensis	1st	18.3	89.0	11.0	6.5	0.4	2.8	2.7	H	FS	3	1
T. brasiliensis	2nd	18.7	91.4	8.6	24.0	1.4	3.3	7.1	H	FS	3	2
T. brasiliensis	3rd	25.5	91.0	9.0	46.3	2.0	2.6	12.6	H	FS	3	3
T. brasiliensis	4th	23.5	96.2	3.8	85.9	3.8	2.4	26.9	H	FS	3	4
T. brasiliensis	5th	33.9	93.8	6.2	359.3	11.3	3.0	62.8	H	FS	3	5
T. brasiliensis	5th	20.2	76.6	23.4	264.7	17.1	3.9	71.4	P	BS	2	6
T. brasiliensis	5th	29.3	70.8	29.2	271.8	13.1	3.0	70.5	M	AS	2	7
*T. brasiliensis**	5th	34.0	100.0	0.0	384.2	11.3	3.0	62.8	H	FS	4	8
*T. brasiliensis**	5th	15.7	72.6	27.4	70.7	6.2	2.1	49.2	Tl	AS	4	9
*T. brasiliensis***	5th	37.6	100.0	0.0	413.6	11.0	2.7	66.3	H	FS	4	10
*T. brasiliensis***	5th	23.2	77.6	22.4	127.6	7.1	2.3	51.4	Tl	AS	4	11
T. infestans	5th	9.9	90.2	9.8	250.0	28.0	4.3	109.0	P	BS	2	12
T. infestans	5th	14.2	64.8	35.2	195.9	21.3	3.4	102.1	M	AS	2	13
T. pseudomaculata	5th	34.5	88.0	12.0	157.9	5.2	3.5	26.0	P	BS	2	14
T. pseudomaculata	5th	36.2	77.5	22.5	115.0	4.1	3.0	23.6	M	AS	2	15
R. nasutus	5th	17.8	63.4	36.6	123.0	10.9	4.3	42.4	P	BS	5	16
R. nasutus	5th	17.6	73.7	26.3	129.0	9.9	4.0	41.3	M	AS	5	17
R. neglectus	5th	25.2	84.3	15.7	140.0	6.6	3.6	30.4	P	BS	5	18
R. neglectus	5th	46.5	73.5	26.5	120.0	3.5	1.9	30.5	M	AS	5	19
R. prolixus	5th	14.6	84.3	15.7	262.0	21.3	6.0	59.1	P	BS	5	20
R. prolixus	5th	24.6	69.8	30.2	260.0	15.1	4.8	52.5	M	AS	5	21
*R. prolixus****	5th	20.7	93.2	6.8	237.0	12.8	3.7	57.7	M	DS	1	22
*R. prolixus****	5th	17.4	100.0	0.0	270.0	16.0	4.7	56.7	M	TBV	1	23
R. prolixus	5th	43.9	84.3	15.7	229.0	8.6	2.7	53.1	M	DS	1	24
R. prolixus	5th	18.1	100.0	0.0	242.0	14.8	4.5	54.8	M	TBV	1	25

Species[a]	Stage	TCT (min)	IP %	NIP %	WG (mg)	EIR (mg/min)	F (Hz)	QLC (ηL)	Host[b]	Site[c]	Refs[d]	Line
R. robustus	5th	23.7	68.7	31.3	258.0	15.9	4.4	60.0	P	BS	5	26
R. robustus	5th	37.9	59.7	40.3	232.0	10.2	2.7	62.9	M	AS	5	27
T. brasiliensis	Male	17.2	81.4	18.6	145.3	10.4	2.5	67.9	H	FS	3	28
T. brasiliensis	Female	21.1	79.6	20.4	228.1	13.6	2.5	88.9	H	FS	3	29

Feeding parameters definition:
Total contact time [TCT (min)] is defined as the total time which the mouthparts of the bugs remain into the host skin.
Ingestive period [IP (min)] comprises the sum of all engorgement periods with the cibarial pump in activity
Non-ingestive period [NIP (min)] is defined as the period when insects are not pumping, thus comprising probing plus any interruption events
Weight gain [WG (mg)], is calculated by the weight of the insect after meal minus its initial weight (before feeding)
Total intake rate [TIR (mg/min)] is calculated by the weight gain divided by total contact time (TIR = WG/TCT)
Effective intake rate [EIR (mg/min)] is calculated by the weight gain divided by the ingestive period (EIR = WG/IP)
Frequency [F (Hz)] is the total number of cibarial pump contractions divided by the ingestive period (IP)
Quantity of liquid ingested by cibarial pump contraction [QLC (ηl)] is obtained by dividing the weight gain by the total number of cibarial pump contractions during the feeding process, considering blood densities as 1 mg/ml
[a]*domestic population; **sylvatic population; ***Nitrophorins$_{(1-4)}$ knockdown
[b]H: human; M: mouse; P: Pigeon; Ti: *Thrichomys laurentius*
[c]AS: Abdominal skin; BS: Breast skin; DS: Dorsal skin; FS = Forearm skin; TBV: Tail base vein
[d]1: Araujo et al. (2009a, b, c); 2: Guarneri et al. (2000); 3: Guarneri et al. (2003); 4: Guarneri et al. (2011); 5: Sant'Anna et al. (2001)

Table 1 provides an overview of feeding parameters presented by triatomines fed on live hosts, where a large variation between species is observed.

4 Total Contact Time

An important factor influencing the interaction between hematophagous arthropods and their hosts is TCT, as defensive host behavior may reduce blood feeding success or even kill the insect. When triatomines feed on unanesthetized hosts, the reduction in blood meal size due to an increase in insect density is modulated by the host's perception of the bugs resulting in shorter feeding times (Schofield 1994; Pereira et al. 1995, 1998). Therefore, species with higher blood ingestion rates possess a better capacity to exploit blood resources from available hosts inside human dwellings, thereby reaching higher population densities. A high feeding performance observed for both *T. infestans* and *R. prolixus* allowed these species to spread and become the most important vectors of *Trypanosoma cruzi* in South and Central America (Sant'Anna et al. 2001; Pereira et al. 2006).

Smith (1979) showed the effect of diet viscosity on *R. prolixus* feeding parameters when bugs were fed on an artificial feeder. He observed that an elicited increase in diet viscosity (0.8 to 6.5 cP) increased TCT by reducing TIR (~68%) and WG (~33%) in fifth instar nymphs. This TIR decline was due to a decrease in both F (~48%) and QLC (~38%).

Notably, TIR and EIR are similar when NIP (i.e., probing and interruption events during engorgement periods) is short, as observed with *Rhodnius* species when fed on an artificial feeder (Friend and Smith 1971; Smith 1979; Sant'Anna et al. 2001). However, this is not the case with the *Triatoma* species studied. They present frequent interruptions and partial engorgement, suggesting that they may need other stimuli to keep the cibarial pump functioning when feeding outside living hosts (Lazzari and Nuñez 1989; Guarneri et al. 2000). Therefore, it is desirable, when possible, to use EIR, as this parameter reflects only IP.

Firstly, data from seven triatomine species (four *Rhodnius* and three *Triatoma*) from the same developmental stage (fifth instar nymphs) were analyzed under similar experimental conditions using two hosts (pigeon and mouse) to evaluate how TCT behaves when bugs feed on live hosts, where all species were able to obtain similar WG values (Table 1. Lines: 6-7, 12-21 and 26-27). A comparison of these species showed a large variation in TCT (9.9–46.5 min) regardless of host species. Pearson's correlation coefficients of the feeding parameters of these species obtained indicated, that TCT mainly correlated with EIR (-0.81; $p < 0.05$), and that no significant correlation was found between TCT and WG (-0.35; n.s.) (Table 2). In turn, the difference in EIR (F × QLC) between species was more influenced by QLC (0.90; $p < 0.05$) than the frequency of the cibarial pump (0.60; $p < 0.05$) (Table 2).

Contrastingly, TCT for different nymphal stages of *T. brasiliensis* fed on human hosts ranged from 18 to 34 min (Table 1. Lines: 1–5). In contrast to that observed in fifth instar nymphs of different species, variation of EIR values among *T. brasiliensis*

Table 2 Pearson correlation coefficients of feeding parameters of seven triatomine species fed on pigeon and mouse[1]

	TCT (min)	WG (mg)	EIR (mg/min)	F (Hz)	QLC (nL)
TCT (min)	1				
WG (mg)	−0.346[b]	1			
EIR (mg/min)	−0.807[a]	0.712[a]	1		
F (Hz)	−0.747[a]	0.433[b]	0.598[a]	1	
QLC (nL)	−0.619[a]	0.660[a]	0.902[a]	0.214[b]	1

For variable definitions, see Table 1
[1]Data analyzed are shown in lines 6-7, 12-21 and 26-27 of Table 1
[a]$p < 0.05$ data
[b]not significant ($p > 0.05$)

Table 3 Pearson correlation coefficients of feeding parameters of *T. brasiliensis* nymphal stages fed on human hosts[1]

	TCT (min)	WG (mg)	EIR (mg/min)	F (Hz)	QLC (nL)
TCT (min)	1.00				
WG (mg)	0.93[a]	1.00			
EIR (mg/min)	0.93[a]	1.00[a]	1.00		
F (Hz)	−0.09[b]	0.15[b]	0.11[b]	1.00	
QLC (nL)	0.93[a]	0.98[a]	0.99[a]	0.02[b]	1.00

Variable definitions are used. See Table 1
[1]Data analyzed are in lines 1–5 of Table 1
[a]$p < 0.05$
[b]not significant ($p > 0.05$)

nymphal stages was similar to that between EIR (0.93; $p < 0.05$) and WG (0.93; $p < 0.05$) (Table 3). This difference can be explained by the fact that the anterior intestine and the cibarial pump, even though not in equal proportion, follow body growth during insect development. Values of WG for nymphal stages in *Triatoma* species was approximately 5 × of pre-feeding weight (Sant'Anna et al. 2017). Cibarial pump contraction is among the main factors responsible for EIR variation observed during the post-embryonic development of triatomines (0.99; $p < 0.05$). However, no significant correlation was found between EIR and F (0.11; n.s.) (Table 3). In *T. brasiliensis* adults, males and females presented marked sexual dimorphism regarding QLC. Females show better feeding performance compared to males, because they have higher QLC (Guarneri et al. 2000) (Table 1. Lines: 28-29).

In all of the triatomine-host associations analyzed above, most of the contact time was spent on the process of blood ingestion by cibarial pump action (ingestive period), corresponding to an average ~ 80% of TCT. Although the non-ingestive period (NIP) generally represents less than 20% of TCT, it is a critical phase, as it includes the initial probing step following the bite, corresponding to the host's most perceived feeding period (Schofield et al. 1986). Interruptions during IP are usually followed by a "secondary probing" in triatomines, even when the insect does not remove mouthparts from host skin.

Table 4 Pearson's correlation coefficients of the feeding parameter ratio (pigeon/mouse) obtained by feeding each triatomine species on both hosts[1]

	TCT (min)	WG (mg)	EIR (mg/min)	F (Hz)	QLC (nL)
TCT (min)	1.00				
WG (mg)	0.11[b]	1.00			
EIR (mg/min)	−0.79[a]	0.16[b]	1.00		
F (Hz)	−0.74[b]	0.10[b]	0.97[a]	1.00	
QLC (nL)	0.24[b]	0.26[b]	−0.41[b]	−0.62[b]	1.00

For variable definitions, see Table 1

[1]The data analyzed were obtained by dividing respective feeding parameters of each line, as follows: 6/7; 12/13; 14/15; 16/17; 18/19; 20/21 and 26/27 of Table 1

[a]$p < 0.05$

[b]not significant ($p > 0.05$)

5 Birds Versus Mammal Hosts

A comparison of feeding parameters obtained in the two hosts (pigeon and mouse) showed profound differences among triatomine species (Table 1). For example, TCT spent by *R. nasutus* on obtaining similar WG on mouse was almost equal to that spent on pigeon, while it was ~85% higher in *R. neglectus* (Table 1. Lines: 16-17 and 18-19). Interestingly, these two species belong to the same species complex (*R. prolixus* complex) and are phylogenetically very close.

Despite the differences found, all species presented better feeding performance in pigeons except for *R. nasutus*, evidenced by an average TCT decrease of ~30% and by an increase of 27% to 87% in EIR. The analysis of feeding parameter ratio (pigeon/mouse) obtained by feeding triatomine species on both hosts showed that the difference in TCT between the two hosts was more correlated with EIR (− 0.8; $p < 0.05$) and F (−0.7; $p = 0{,}056$) than the other parameters (Table 4). Although QLC is the main parameter associated with the differences in EIR found between species, and among nymphal stages (see above), the impact of host change (pigeon × mouse) in EIR in the same species is more related to F variation (0.97; $p < 0.05$), suggesting that cibarial pump frequency is the main indicator of impacts of external factors, such as different host species on insect feeding performance (Table 4).

These results demonstrate that most triatomine species show better feeding performance (higher EIR/F) when they take a blood meal from pigeons than from mice.

6 Saliva and Salivation During Blood Feeding

Ribeiro and Garcia (1981) were the first to show that triatomine saliva reduces probing and feeding times. They showed that salivectomized *R. prolixus* specimens fed equally well on blood presented in an artificial feeder (without host hemostasis/inflammation responses), whereas they fed slower in rabbits in comparison to sham-operated controls (Ribeiro and Garcia 1981).

However, marked differences in expressed molecules and salivary gland morphology exist within the subfamily Triatominae, observed particularly between the Rhodniini and Triatomini tribes (Ribeiro et al. 1998; Lacombe 1999).

Although nitrophorins (nitric oxide [NO] carrier hemeproteins) are the most abundant salivary lipocalins in the genus *Rhodnius*, this family of lipocalins does not occur in species of the Triatomini tribe (Ribeiro et al. 2012). In contrast, only the sialotranscriptomes of Triatomini species identified secreted protease sequences (Ribeiro et al. 2012).

Nitrophorins are responsible for the characteristic red coloration of Rhodniini salivary glands, in contrast to the colorless glands of Triatomini. Besides presenting anti-coagulant and anti-histaminic activities, *Rhodnius* salivary nitrophorins release NO into host skin, a potent vasodilator and anti-platelet aggregation agent (Champagne et al. 1995). Interestingly, only *Cimex* species (Cimicide) have NO as a vasodilator molecule among hematophagous Hemiptera apart from Rhodniini species, using completely different nitrophorins to carry this unstable gas (Ribeiro et al. 2012). Although NO elicits a potent and transient relaxation of vascular smooth muscles, long-term vasodilation is observed at an *R. prolixus* feeding site even after the end of the insect's feeding. One possible explanation for this long-lasting effect of saliva is that part of the NO released by nitrophorins binds to host proteins such as albumin, forming S-nitroso-proteins (S-nitroso-albumin). These in turn slowly release NO ensuring long-term vasodilation in host skin, ultimately enabling efficient hematophagy for longer periods of time (Paim et al. 2017).

Differences found in the composition of bioactive salivary molecules (such as NO, nitrophorins, and proteases) between the Rhodniini and Triatomini tribes will reflect the biological activity of their saliva. Comparative studies have shown quantitative and/or qualitative differences in triatomine saliva in properties such as anticoagulant, antiplatelet aggregation, vasodilatory, proteolytic, and inhibitory of the classical complement pathway (Pereira et al. 1996; Ribeiro et al. 1998; Amino et al. 2001; Cavalcante et al. 2003; Barros et al. 2009).

Comparisons of salivary gland morphology also show differences between species of the two tribes, in which salivary glands of Triatomini species consist of three lobes (D1, D2, and D3), whereas the D3 lobe is absent in Rhodniini species (Lacombe 1999).

The impact of salivary molecules on triatomine feeding parameters has been demonstrated in recent studies using knockdown insects (RNAi). While most knockdown *R. prolixus* specimens for salivary nitrophorins$_{(1\text{-}4)}$ probed 2–20 times before starting the engorgement phase in the dorsal surface of mouse skin, all insects from the control group presented only a single probing activity. The rate of effective ingestion was also affected by reduced nitrophorin expression by lowering pumping frequency in bugs feeding on dorsal mouse skin (Araujo et al. 2009a). Interestingly, the negative impact of silencing salivary nitrophorins on feeding performance was not observed, when the insects probed into a large mouse tail vessel (Table 1, Lines: 22-23), reiterating the importance of endothelial stress at the insect feeding site in triggering host hemostasis and inflammatory responses, as discussed here.

Triatomines feature an interesting mechanism regulating the release of saliva at their feeding site. The salivary pump of *R. prolixus* works intermittently during IP and has a low frequency (~0.5 Hz) compared with the cibarial pump (~ 4.5 Hz) when feeding on mouse skin. This large difference in frequencies implies that during periods when the insect is effectively ingesting blood, most of the egested saliva is ingested. However, at times when F is too low or blood pumping is interrupted, the quantity of released saliva increases within the canulated vessel. This mechanism modulates the quantity of saliva deposited into microcirculation, minimizing host immune response against salivary antigens (Soares et al. 2006).

Several substances aiding hematophagy have been described from triatomine saliva, including anticoagulants, vasodilators, antihistamines, sodium channel blockers, as well as of platelet aggregation inhibitors induced by ADP, thrombin, serotonin, arachidonic acid, platelet-activating factor (PAF), epinephrine, and norepinephrine. In addition to these substances, others such as compounds with immunosuppressive activity, a sialidase enzyme, and a pore-forming protein have also been described, as well as anti-complement activity aiding the feeding process, all reducing immune and inflammatory responses of the vertebrate host (Ribeiro 1995; Andrade et al. 2005; Ribeiro and Arca 2009; Ribeiro et al. 2012).

7 Triatomine–Host Interface

The host–parasite interface is the site of molecular exchange between the two organisms, operating in a two-way path where nutrients from the host enter the parasite and molecules from the parasite (e.g., metabolic wastes) enter the host. A detailed representation of this interface was described using a few models, mainly intracellular parasites, which have revealed complex interactions taking place (Trager 1986).

As shown here, triatomine bugs are temporary ectoparasites with most of their feeding time (TCT) used for pumping blood (IP) from host skin blood vessels to the insect anterior midgut. Therefore, the functional interface with the host involves both the blood feeding site and the insect midgut in this group.

7.1 Triatomine–Host Endothelium

As triatomines are "vessel feeders," the most enduring connection within the triatomine–host interface at the feeding site is established between the insect maxillae tips ("functional mouth"), with the release of saliva on one side and the vertebrate's vascular endothelium at the other side.

Vessel diameter decreases immediately upon the introduction of triatomine mouthparts, and visible pulsation promptly begins evidencing the onset of IP. The intensity of vessel wall movements varies considerably. Sometimes they are imperceptible, while other sometimes so accentuated that they can be easily observed

(Lavoipierre et al. 1959). Therefore, IP is preceded by vessel wall injury allowing the subendothelial matrix to be exposed to blood components, whereas cibarial pump operation in the insect causes large local oscillations in blood flow (wall shear stress) in the cannulated vessels.

The endothelium is a single layer of cells lining the lumen of vertebrate blood vessels. Mammalian endothelial cells play a crucial role in maintaining a balance between the activation and inhibition of the hemostatic system and inflammation. Under normal conditions, mammalian endothelial cells have a non-thrombogenic surface that does not support platelet adhesion or fibrin generation. They produce and release powerful soluble platelet activation inhibitors, nitric oxide, and prostacyclin. They also express ADPases (CD39) and thrombin inhibitors (i.e., thrombomodulin), which in turn rapidly metabolize two major platelet agonists, ADP and thrombin, respectively. This antithrombotic environment, however, may change rapidly after endothelium activation by vascular injury, or even in response to changes in fluid shear stress. The activated endothelium (i.e., a procoagulant environment) can actively facilitate fibrin generation and the recruitment of platelets and leukocytes through tissue factor expression, exposing ligands such as P-selectin and vWf through the synthesis of pro-inflammatory mediators (Nesbitt et al. 2006; Watson 2009).

Therefore, endothelial cells trigger hemostatic and inflammatory reactions once activated. These host responses may explain the increase in vascular permeability, platelet aggregation, and rolling/adherent leukocytes in the venular endothelium adjacent to the *R. prolixus* feeding site, as observed by an intravital microscopy study (Soares et al. 2014). It can also explain the accentuated accumulation of leukocytes around the region where *R. prolixus* maxilla are inserted into the vessel, as observed by histological techniques (Lavoipierre et al. 1959).

When triatomines take blood from host skin, mainly of mammals (mice), cibarial pump frequency does not remain constant throughout the feeding, but shows a tendency to decrease in its final period (Sant'Anna et al. 2001). The analysis of a collation of images of the cibarial pump in the head of *R. prolixus* (Fig. 1D–F), or of vessel wall movements (Fig. 1G–I), together with electromyographic records, allowed us to infer that changes in F observed during insect feeding are mainly due to variations in pump filling time (Soares et al. 2014).

Endothelial activation at the bug feeding site can generate a procoagulant environment suitable for platelet aggregation and thrombin generation, thereby changing rheological characteristics of blood. The blood clotting process basically consists of a transition from fluidic blood to a solid clot. Once the blood coagulation cascade has been initiated, a rapid increase in blood viscosity occurs (Puckett et al. 2005; Ranucci et al. 2014). It is known that an increase in diet viscosity leads to a decline in cibarial pump frequency (Smith 1979). Thus, it is plausible that an increase in blood viscosity at the feeding site could explain the increase in pump filling time as observed during *R. prolixus* blood meal on mouse skin (Soares et al. 2014).

Furthermore, given that the opening of the feeding channel is narrow (8–10 μm), the formation of microaggregates of platelets at the feeding site could increase the friction of food along the channel wall, which could also prolong the time of pump filling.

Within this context, it is expected that the smaller the vessel diameter of the vertebrate host, the greater the mechanical stress caused by insect cibarial pump activity (i.e., high amplitude of vessel wall movements and strong oscillations of flow). This could explain the fact that triatomines had greater difficulty in feeding from small vessels in mouse dorsal skin (Ø ~ 20–40 μm) compared with a large lateral vein (Ø > 500 μm) of the tail base (Table 1. Lines: 24-25) (Araujo et al. 2009a).

Following this logic, the best feeding performance was achieved in triatomines that fed on pigeon blood (>EIR/F) in comparison to mice, which might be related to differences between bird and mammal hemostasis. Although basic hemostatic mechanisms are conserved among vertebrates, marked differences among different vertebrate hosts exist. For example, the intrinsic coagulation pathway is a highly essential hemostatic mechanism in mammals, but less important in birds (Lewis 1996).

However, this greater difficulty for triatomines, especially in maintaining F during blood feeding when changing pigeons to mice, must be more related to differences found between platelets and thrombocytes, considering the relevant role of platelets in the coordination of hemostatic and inflammatory responses in mammals.

Thrombocytes in birds perform a similar function to platelets, but spread and/or aggregate less efficiently upon ADP, collagen, ristocetin, arachidonic acid addition, or thrombin exposure than those of mammals (Lewis 1996; Schmaier et al. 2011). Avian thrombocytes express lower levels of integrin α2bβ3 that plays an important role in aggregation (Schmaier et al. 2011), and do not have the cytoplasmic GP1b-alpha domain that is important for cytoskeletal rearrangement required for changing platelet shape (Ribeiro et al. 2015). These features make thrombocytes slower to induce blood clots to control bleeding, and their aggregates are less resistant to high shear forces in fluid than platelets (Schmaier et al. 2011; Ribeiro et al. 2015).

7.2 Triatomine–Host Blood

The second site of the vector–host interface relevant during blood feeding is formed between insect anterior midgut contents (intestinal and salivary molecules) and vertebrate blood components. Midgut microvilli are covered with an unusual structure called perimicrovillar membrane in hemipterans, extending toward the luminal compartment with a dead end. The perimicrovillar membrane is well developed in blood-fed, but is poorly developed in unfed insects (Terra et al. 1996).

The blood ingested (plus saliva) during triatomine feeding is stored in the wide anterior part of the midgut, from which water and ions are transported to the hemolymph and Malpighian tubules before excretion via the rectum. Concentrated blood passes slowly into the digestive and absorptive part of the posterior midgut in small amounts (Kollien and Schaub 2000). The blood ingested remains in the anterior midgut until the end of digestion, which may take several days. For example, *T. infestans* adult females can ingest a blood meal ~2.1 times their own weight, the digestion of which takes an average of 14 days (Lehane 2005). It is intestinal

phenomena occurring during the blood meal, that may affect blood pumping by the insect cibarial pump, which will be emphasized here.

Blood clotting was triggered experimentally in the anterior midgut by the administration of exogenous thrombin or knockdown of a specific intestinal anticoagulant named brasiliensin (a thrombin inhibitor) in *T. brasiliensis* fourth instar nymphs (Araujo et al. 2007). Upon gene knockdown by RNAi, the bug's blood meal size (WG) on hamsters was considerably reduced (39–55%). After a dissection of the insects, clots were observed in their anterior midgut, while blood was only compacted in control bugs, as 'clots' were easily disrupted by shaking. The presence of fibrinolytic activity in the triatomine midgut was demonstrated by Hellmann and Hawkins (1964), and this activity could explain why the presence of blood clots in the anterior midgut of blood fed *T. brasiliensis*, which had brasiliensin knocked down by dsRNA injections did not prevent blood transference and digestion in the posterior midgut.

Electromyogram analysis revealed that *T. infestans* and *R. prolixus* had a higher EIR/F in comparison to *T. brasiliensis*. The reduced TIR for *T. brasiliensis* was associated with the poor ability of the species to maintain F during blood ingestion, even when the feeding site is favorable (such as mouse lateral tail vein) (Paim et al. 2011). This higher TIR/F value observed for *T. brasiliensis* when fed on mice previously treated with heparin reinforces the importance of maintaining fluid blood in the anterior midgut for successful blood feeding. When *T. brasiliensis* nymphs were knocked down for brasiliensin and fed from lateral tail vein, they showed difficulty during feeding presenting lower TIR, longer NIP and a blood meal size 2x lower than control nymphs. This difficulty observed for silenced insects could be reverted by treating mice with heparin (Paim et al. 2011).

Besides coagulation, other mechanisms such as platelet aggregation can influence blood viscosity in the insect anterior midgut. As thrombin is also a potent activator of platelet aggregation (Ribeiro and Francischetti 2003), it is possible that the reduction in brasiliensin levels in silenced insects elicited platelet aggregation that could have helped in clot formation in the intestine.

Similar results were obtained for the bedbug *Cimex hemipterus*, which showed improved feeding parameters when fed on pigeons previously treated with a systemic anticoagulant (warfarin). *C. hemipterus* presented a blood meal approximately twice as large in size when fed on treated birds (WG = 3.7 mg) as opposed to untreated pigeons (WG = 1.7 mg) (Araujo et al. 2009b).

On the whole, results obtained for *T. brasiliensis* knocked down for brasiliensin support the idea that blood must remain fluid enough during the ingestion period, as backpressure induced by increased viscosity caused by blood clots prevent the successful pumping of blood into the midgut. Once the blood coagulation cascade has been initiated, viscosity rises rapidly during the clotting process (Puckett et al. 2005). Molecules present in the anterior midgut can also exert a hemagglutination effect on erythrocytes (RBC) of certain vertebrate hosts (Pereira et al. 1981; Gregorio and Ratcliffe 1991; Araujo et al. 2009c; Moreira et al. 2018).

When comparing feeding performance of fifth instar *T. brasiliensis* nymphs fed either on humans or *Thrichomys apereoides* (= *Thrichomys laurentius*, an abundant

species of wild rodent that inhabits the same type of rock burrows as *T. brasiliensis* in Northeast Brazil, see Roque et al. 2005), the insects had more difficulty (<EIR and WG) obtaining a blood meal from wild rodents (Guarneri et al. 2011) (Table 1. Lines: 8-9 and 10-11). Similar results were obtained for third instar nymphs fed on rats or *T. laurentius*, where insects fed on wild rodents showed lower TIR than those fed on murine hosts (Araujo et al. 2009b). The first evidence that hemagglutination can interfere with insect feeding came from the observation that *T. brasiliensis* anterior midgut contents are unable to agglutinate RBC from *T. laurentius* blood compared with rat blood.

Thus, experiments were carried out where *T. brasiliensis* specimens were fed on artificial diets prepared with cattle RBC (such as *T. Laurentius*, RBC are not agglutinated) or rat RBC (which were agglutinated) resuspended in plasma from rat blood, to maintain similar characteristics (e.g., hematocrit, RBC size, plasma viscosity). Higher TIR values showed by insects fed on rat RBC suspension demonstrated that the occurrence of intestinal hemagglutination can positively impact bug feeding performance. The inability of *T. brasiliensis* to agglutinate *T. laurentius* RBC may partly explain this great difficulty (low TIR/EIR and a reduced blood meal) to obtain a blood meal from this wild rodent (Araujo et al. 2009c; Guarneri et al. 2011).

The impact of intestinal hemagglutination on obtaining a successful blood meal, demonstrated for triatomines, is also present in other hematophagous species. Feeding performance in three *Anopheles* species was higher in those with intestinal hemagglutination activity (Chege and Beier 1998). For example, *A. albimanus* and *A. freeborni* agglutinate human RBC feed more rapidly than *A. gambiae,* which is lacking the ability to agglutinate human blood.

The effect of hemagglutination on blood feeding performance may be related to the alteration of blood viscosity. Blood flow decreases dramatically after passing through the narrow anterior region of the food canal and reaching the broad anterior midgut; it even stops in some regions. Under these conditions, the rapid agglutination of RBC together with the deposition of agglutinates formed in the intestinal wall reduce the number of suspended red blood cells ("hematocrit") within the insect anterior midgut. Due to a decreased viscosity of the posterior part of the liquid feed column, these regions with reduced hematocrit create lower resistance for the cibarial pump to propel blood through the food canal.

The natural capacity of erythrocytes of certain mammalian species to form rolls, also known as erythrocyte aggregation (not hemagglutination), can also change blood viscosity at low shear conditions. Erythrocyte aggregation varies greatly among vertebrate species, with the highest level observed in horse blood followed by cat, human, dog, and pig blood. Low levels of erythrocyte aggregation were observed of RBC from rabbit, mouse, rat, cattle, and sheep (Weng et al. 1996; Windberger et al. 2003).

As soon as blood is exposed to the anterior midgut wall, the host complement system is activated. The alternative pathway is triggered by carbohydrates from the glycocalyx of intestinal cells, whereas the classical pathway is triggered by the nonspecific binding of natural antibodies to these carbohydrates or other intestinal mol-

ecules. The presence of carbohydrates covering intestinal membranes can also trigger the lectin pathway by binding to the mannose-binding protein. As insect enterocytes forming the intestinal epithelium are in a single layer, preventing complement activation is essential for the insect to survive, as it could lead to the rupture of the digestive tract and even to insect death. Both saliva and intestinal contents from all triatomines studied to date (*T. brasiliensis*, *T. infestans*, and *R. prolixus*) are capable of inhibiting C3b deposition by classical and alternative pathways of the human complement system (Barros et al. 2009).

8 Final Comments

A major proportion of triatomine total contact time (TCT) is used for pumping blood (IP) from the host skin blood vessel to the insect anterior midgut. Two physical sites of the triatomine–host interface are relevant during this process: a) the triatomine "functional mouth" and host endothelium and b) the insect anterior midgut and host blood. Considering these sites, it is possible to identify features, important in the triatomine–host interaction, that modulate insect feeding performance.

Effective intake rate (EIR) is the feeding parameter that affects the time of contact with the host the most in triatomines. Differences in EIR (F × QLC) between triatomine species can vary greatly if any of the factors such as host species, evolutionary stage, and feeding site are considered in these comparisons. For example, the EIR of fifth instar nymphs fed on mouse abdominal skin varies from 3.5 mg/min for *R. neglectus* to 21.3 mg/min for *T. infestans*, representing a 6.1-fold difference in EIR.

Although QLC (i.e., the bug's intrinsic characteristics) is the main parameter associated with differences in EIR found between triatomine species or among nymphal stages, the impact of host change (pigeon × mouse) on EIR within the same species/stage is associated more with F variation.

These findings show that the main indicator of the impact of external factors, such as host species (avian or mammals), feeding site characteristics (e.g., vessel diameters), and/or blood characteristics in the midgut environment (e.g., coagulated/uncoagulated, aggregated/unaggregated) on triatomine feeding performance is cibarial pump frequency.

Since the food channel is continuous and blood is incompressible any obstacle to blood flow between the triatomine functional mouth and the anterior midgut can interfere with the blood intake rate. Therefore, to produce a true model capable of describing triatomine blood feeding biomechanics, it is necessary to consider the entire insect gut, since current models include the initial parts of the alimentary canal only.

The modification of vector blood feeding performance by parasites to enhance parasitic circulation appears to be a common strategy found in many parasite–vector associations; thus, an additional aspect worth investigating is whether intestinal colonization by pathogens or microbiota affects the ability of the arthropod to feed.

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