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Short Communication

Effect of Pilocarpine on Tick Saliva Extraction

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ABSTRACT

Tick fauna of Pakistan are rich in a number of genera and species. Despite this richness and suitability of the climatic conditions, little systematic work has been performed to investigate taxonomy, bionomics, seasonal and regional occurrence of ticks infestation in the Pakistani livestock. In this study, investigations were made to determine the effect of pilocarpine on tick saliva extraction, owing to the fact that saliva of ticks plays important role in research on tick-transmitted diseases. The ticks were collected from the surrounded area of Tandoallayar and Hyderabad, Pakistan. The saliva production through ordinary method (without pilocarpine) was 0.54 µl per tick and the saliva production using pilocarpine was 0.81 µl per tick. Results further showed that there is 0.27 µl difference present between the saliva extracted by applying pilocarpine as compared to saliva extracted without the use of pilocarpine (ordinary method). These results indicated that pilocarpine could be a choice for the researchers to obtain higher amounts of tick saliva for their works, however, chemical quality of tick saliva obtained through pilocarpine should be investigated through advanced qualitative techniques.

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Livestock is an important and sometimes overlooked element of the livelihood strategies of poor people in the world. As much as 70 percent of the rural population depends on livestock in the world.(Delgado et al., 1999). Livestock is diverse and includes cattle, goats, sheep, pigs, poultry, horses, camels, yaks, and llamas. An estimated 600 million poor people, including 150 million landless poor, own livestock, around the world (Delgado et al., 1999; Thornton et al., 2002).

Livestock in developing countries, like Pakistan, is posed to a variety of problems. Endo- and ecto-parasites are a big risk and act as a factor in decreasing animal productivity. Ticks are the most important ectoparasites of livestock in tropical and subtropical areas and are responsible for substantial economic losses.

Ticks not only cause direct effect on animals, in terms of nutrients and mineral deficiencies by sucking blood, but they are also responsible for the transmission of several dreaded protozoan, rickettsial and viral diseases (Soulsby, 1982; Yatoo et al., 2013). The harm done by tick bites and blood sucking has been reduced by control measures taken to check the diseases transmitted by them (Kumar et al., 1992). A single adult female tick may suck 0.5-2.0 ml of blood (Pegram and Chizyuka, 1990) and if single animal carries numerous ticks, a substantial loss of blood may occur. Heavy infections do occur in nature, it is more usual for animals carry a few hundred ticks. These produce what is generally known as "tick worry". Systematic control of ticks always results in improved weight gains and yields. Hyalomma ticks, as vectors of tropical theileriosis are widespread in North Africa, southern Europe, Middle East, Central Asia and China (Preston, 2001). In Pakistan, piroplasmosis, theileriasis and anaplasmosis, are common livestock diseases, which are transmitted by the ticks (Abdussalam, 1959).

Pakistan being a tropical country provides optimal climatic conditions for growth and multiplication of ticks. Ticks are widely distributed in Pakistan. They are transmitters of numerous etiologic agents that pose serious threats to both public health and livestock industry in the region.Ticks mainly transfer diseases through their saliva and their saliva is extracted by using several compounds. One of which is by applying pilocarpine, since isolated glands do not respond to pilocarpine but to dopamine, this might be the final effector molecule which is responsible for salivation. Dopamine gets destroyed in tick haemolymph. This is the reason, why pilocarpine is the most used agonist of tick salivation. Pilocarpine is a muscarinic cholinomimetic agent. It is obtained from the leaves of a tropical American shrub from the genus Pilocarpus. It is a non-selective muscarinic receptor agonist acting on the parasympathetic nervous system. It has several known

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effects. It contracts smooth muscle within the intestinal tract, affects blood pressure and inhibits lymphocyte function. In medicine, it is used for the therapy of xerostomia and glaucoma (Rosin, 1991).

Saliva of ticks play important role in research on tick-transmitted diseases, but its collection in sufficient quantity is a great problem. Pilocarpine stimulates the secretion of large amounts of saliva and sweat. Therefore, it is used for the collection of tick saliva by applying it to the tick's back. It has an effect up to 3 hours. It is known, that pilocarpine is also present in the collected tick saliva. A ten times bigger amount of pilocarpine was found in the saliva of Ixodes scapularis than in Amblyoma americanum(Gil et al., 2001). This might be caused by differences in the cuticle permeability. The concentration of this agent in tick saliva can be measured using a High performance liquid chromatography- Mass spectrometry method (Rang et al., 2003; Gil et al., 2001).

The present study was proposed to investigate the effect of pilocarpine in tick saliva collection in our local environmental conditions. The main objective of the study was to compare the tick saliva quantities and time line with and without pilocarpine which could facilitate the sample collection procedures.

A total of 200 ticks were collected from the surrounding area of Tandoallayar and Hyderabad and brought to the laboratory of the Department of Veterinary Parasitology, Sindh Agriculture University, Tandojam. The collected ticks were divided into 10 groups, each contains 20 ticks. Half of the ticks (5 groups, designated as P1 - P5) were used for pilocarpine application, whereas, the other half (O1 – O5) was used for without the use of pilocarpine (ordinary method). Saliva was extracted from them by applying pharmacological active agonists pilocarpine to their cuticle. Capillary tubes were attached to the mouthpart of the ticks to collect saliva. Average per tick saliva was calculated using the following formula that was used to estimate the differences among groups.

Average per tick saliva difference:Amount of saliva (with pilocarpine) – amount of saliva (without pilocarpine).

Table 1. Amount of saliva extracted with and without pilocarpine*

Group Code**	Per Group***	Per tick
P1	16.01	0.8005
P2	16.29	0.8145
P3	16.19	0.8095
P4	16.64	0.832
P5	16.12	0.806
Average	**16.25**	**0.81**
O1	10.93	0.5465
O2	10.49	0.5245
O3	11.23	0.5615
O4	10.77	0.5385
O5	10.72	0.536
Average	**10.83**	**0.54**

* µl, ** P= Pilocarpine; O= ordinary method (without pilocarpine), ***n=20

As shown in Table 1, the saliva production through ordinary method (without pilocarpine) was 0.54 µl per tick (10.83 µl per group) and the saliva production using pilocarpine was 0.81 µl per tick (16.25 µl per group). It further showed that there was a difference of 0.27 µl per tick (5,42 µl per group) between the saliva extracted by applying pilocarpine as compared to saliva extracted without the use of pilocarpine (Table 2).

Table 2. Differences in amount of tick saliva (in µl)*

Groups	Per Group**	Per tick
P1	5.08	0.254
P2	5.8	0.29
P3	4.96	0.248
P4	5.87	0.2935
P5	5.4	0.27
Average	5.422	0.2711

* Saliva obtained through pilocarpine – saliva obtained through ordinary method, **n=20

Tick saliva is a cocktail of several immunomodulatory and pharmacologically active compounds, including anticlotting, antiplatelet, vasodilatory, anticomplement, antineutrophil and kininases,etc (Titus et al., 2006). As an immunological response against these pharmological agents, hosts develop effective immunity against ticks. Effective anti-tick immunity with near complete suppression of tick feeding, moulting, and oviposition is primarily found in non-natural hosts following prior tick exposure and is directed against salivary antigens. Natural hosts develop only partial immunity that does not completely interrupt blood feeding, indicating the adaptation of ticks in the immunological/inflammatory background of their hosts (Ribeiro et al., 2004).

Classically, tick saliva collected by removing feeding ticks from their hosts and injecting or applying pharmacologically active agonists of salivation like pilocarpine, dopamine etc, to the tick cuticle. In response to pharmacological agents ticks produce saliva that collected into capillary tubes placed around the tick mouthparts. However, in little quantity, saliva could be collected without the application of these stimulatory agents. Pilocarpine is thought to act on neural ganglia, innervating the glands to produce the final effector molecule, possibly dopamine (Ribeiro et al., 2004).

Pilocarpine is often applied or injected into ticks to induce salivation, and the resulting saliva used to test for various pharmacological, biochemical and immunological activities. Due to the known effects of pilocarpine on smooth muscle and immune cells, appropriate controls are proposed and discussed for proper interpretation of results using this saliva preparation (Ribeiro et al., 2004).

Salivation is induced by pilocarpine. As a consequence, pilocarpine is taken up into the tick's body fluids and therefore also present in the secreted saliva. Pilocarpine has effects on the immune system. This effect is tested on the production of cytokines TNF-α and IFN-γ. Tick salivary glands and extraction of saliva by using pilocarpine have been studied by various groups worldwide, reflecting the huge importance of this organ in the transmission of various pathogens to humans and domestic animals. Undoubtedly, the great advances in molecular biology technologies will a have tremendous effect on our understanding of tick salivary gland physiology. There have been several expressed sequence tag (EST) projects on salivary glands of various tick species (Nene et al., 2002).

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Barker et al., (1973) compared the infrared heat, pilocarpine injection and electrical stimulation as methods for inducing oral secretion in ticks. Electrical shock and infrared heat caused higher percentages of tick secretions, and the greatest volumes of secretions were collected following infrared heat stimulation. This might be due to a more stimulatory effect of infrared heat on slivary glands as compared to electrical method and pilocarpine application. In our present study, the use of pilocarpine indicated the promising results as compared to ordinary method (without using any stimulatory agent). Hence, it could be concluded that, pilocarpine is a potential agent to produce large quantities of tick saliva.

REFERENCES

Abdussalam M (1959) Ticks inter relation to disease in Indo-Pakistan. Pak. J. Anim. Sci. 1(4): 14 - 17.

Barker W,Robert, Burris, Sauer E, Hair JR, Jakie A (1973). Composition of tick oral secretions obtained by three different collection methods. J. Med. Entomol. 10:(2). 198 - 201.

Delgado C, Rosegrant M, Steinfeld H, Ehui S, Courbois C (1999)."Livestock to 2020: The Next Food Revolution. IFPRI Food, Agriculture, and the Environment Discussion Paper 28. Washington, D.C. (USA): IFPRI.

Gil D, Spalding T, Kharlamb A, Skjaerbaek N, Uldam A, Trotter C, Li D, WoldeMussie E, Wheeler L, Brann M (2001). Exploring the potential for subtypeselective muscarinic agonists in glaucoma. Life Sci. 68, 2601 - 2604.

Kumar GA, Hussasin SI, Ahmad M, Shakoori AR (1992).Prevalence of ticks (Ixodidae) of buffaloes at Khairpur Mir's and its adjoining areas in the province of Sindh, Pakistan. Sindh University, Jamshor. Pak. Cong. Zool. 12: 455.

Nene V, Lee D, Quacken J, Bush, Skiton R, Mwaura S, Gardner MJ, Bishop R (2002). avGI, an index of gene transcribed in the salivary glands of ixodid tick Amblyomma variegantum. Int. J. Parasitol. 32: 1447 - 1456.

Rang HP, Dale MM, Ritter JM, Moore PK (2003). In: Pharmacology, 5th edition, Churchill Livingstone.

Rasul G and Akhtar AS (1975).Survey of hard ticks of livestock in Pakistan. Pak. J. Anim. Sci. 1 (4): 7 - 11.

Ribeiro JMC, Zeidner NS, Ledin K, Dolan MC, Mather TN (2004). How much pilocarpine contaminates pilocarpine-induced tick saliva? Med. Vet. Entomol. 18:(1) 20 - 24.

Rosin A (1991). Pilocarpine. A miotic of choice in the treatment of glaucoma has passed 110 years of use. Oftalmologia. 35: 53 - 55

Soulsby EJL (1982). Helminths, Arthropods and Protozoa of domesticated animals. 7th Edition. Baillier Tindall and Cassel Ltd.London.

Thornton P, Kruska R, Henninger N, Kristjanson P, Reid R, Atieno F, Odero A, Ndegwa T (2002). Mapping poverty and livestock in the developing world. Nairobi, Kenya: Int. Livestock Res. Inst.

Titus RG, Bishop JV, Mejia JS (2006). The immunomodulatory factors of arthropod saliva and the potential for these factors to serve as vaccine targets to prevent pathogen transmission. Parasite. Immunol, 28: 131 – 141.

Yatoo MI, Dimri U, Sharma MC (2013). Status of micro mineral deficiency in cattle in Kashmir valley. J. Anim. Health Prod. 1 (3): 24 - 28.

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