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An investigating the antiparasitic effect of sulfur hot springs on sheep flocks using the minimal lethality method

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Abstract

For many years, animals have contracted secondary illnesses from their skin such as parasites and fungi, leading to a higher death toll. With the diverse nature of our country, there is a volcanic hot spring containing sulfur and other substances that can aid in managing and eliminating various parasites and fungal skin conditions. First, external parasites (ticks) were collected from 20 tick-infected sheep in Dehloran city (Ilam province, Iran) from different flocks randomly. Subsequently, 1000 ml of water was collected from two sulfur springs in Dehloran and Mehran cities in Ilam province. Then, 10 dilutions were created ranging from 10% to 100%. The laboratory plates held ticks in 11 test tubes; one tube containing distilled water as a control and 10 tubes containing various concentrations of the substance. They were taken away for 20 minutes and inspected with a stereomicroscope. There was a positive effect observed when increasing the concentration from 70% to 100%. At 100% concentration, the movement of ticks was completely stopped. Considering the low cost and availability of this method for areas that have such springs, it can be concluded that this method can be used in herds that are far from access to anti-parasitic drugs.

Introduction

Each year, the animal husbandry industry faces significant harm from external parasites, specifically two key families of ticks known as Ixodidae and Argasidae, which are responsible for transmitting diseases to livestock. It comes after fungal, bacterial, and viral infections and poses a potential threat to this sector (Evans and Edgerton, 2002). Parasites not only endanger livestock but also pose a significant threat to human health. They are contaminating (Daszak *et al*., 2000; Cunningham *et al*., 2017). Because of the significant impact of these illnesses in zoonoses, the failure to effectively treat them can lead to spreading and result in not only financial harm to the farmer but also irreversible consequences for others (Epstein, 2001). However, pursuing new, affordable, and safe treatment options through research and development is an integral part of the scientific process and a responsibility of scientists.

The varied and rich geographical features of our country and the presence of valuable natural resources allow researchers to explore and advance through traditional methods that coexist with the cultural identity of the inhabitants of a specific region.

Whenever possible, it is important to implement these solutions in a way that aligns with the scientific aspects of the situation. Various ointments and mixtures of multiple ingredients are available globally for treating skin dermatitis and skin parasites, containing common elements like zinc, sulfur oxide, permethrin, and metronidazole, known for their high level of efficacy (Platonova, 2016).

The garlic plant is another substance rich in sulfur and has demonstrated anti-parasitic properties (Krstin *et al*., 2018). Various conventional techniques, like employing *Nicotiana tabacum* and *Azadirachta indica* leaves, were used as aqueous extract on animals to shield them from skin parasites (Zaman *et al*., 2012). Beginning in 1944, they discovered sulfur's anti-parasitic properties and incorporated it into ointments (Strakosch, 1943). Overall, there are two categories of ticks responsible for zoonotic and livestock illnesses such as Ixodidae and Argazide ticks, which encompass 183 species worldwide across four genera (Guglielmone *et al*., 2006). These parasites have the ability to transmit pathogens to a wide range of organisms, leading to various issues (Ramazan *et al*., 2020).

Sulfur is effective in treating parasitic infections like Chagas, among other similar ones. The primary drugs employed for this condition contain a key component that utilizes sulfur compounds to treat skin parasites effectively. Similar situations should follow this pattern (Keirans and Durden, 2001). Sulfur compounds combined with fluorine can also serve as remedies for treating *Toxoplasma*, the parasite responsible for toxoplasmosis (Rubio-Hernandez *et al*., 2022). Inexpensive conventional treatment options may serve as a suitable choice for a large group of animals, effectively removing parasites at an affordable price and consistently (Szajnman *et al*., 2017). Moreover, historically across the globe, essential oils were extracted from *Tagetes minuta* plants to fight ticks in livestock (Galaka *et al*., 2017). This problem highlights the necessity of exploring innovative methods to combat skin parasites, prompting researchers in this field to also investigate and upgrade traditional methods with newer, safer alternatives.

Theileraria, a parasite transmitted through bites, can lead to economic losses and diseases in ruminants by infecting white and red blood cells, causing a cancer-like disease in erythrocytes of the host (Bishop *et al*., 2004; Tavassoli *et al*., 2011). This illness shows no symptoms until acute signs arise in the ruminant animal, who is also a carrier (Nchu *et al*., 2012).

The soft mite (Arachnida) specifically targets vertebrates (Rai *et al*., 2016). This organism harbors numerous disease-causing agents like bacteria, intracellular parasites, and viruses (Hanafi-Bojd *et al*., 2021). These organisms can spread different infections like NRLP-12, Crimean-Congo hemorrhagic fever, Lyme disease, babesiosis, encephalitis, and anaplasmosis in animals (Janbakhsh, 1956; Ahmed *et al*., 2007). This study examined the sulfur hot springs in the cities of Dehloran and Mehran in Ilam province, considering the geographical context and input from old ranchers, along with scientific texts. The focus was on the anti-parasitic properties of sulfur, particularly in treating leg band parasites, with detailed results outlined in the article.

Materials and Methods

In this study, 20 sheep with tick infestations were initially detected and separated from the main herd by being moved to two smaller flocks of 50 sheep each in Dehloran city. In the following stage, they were eliminated in a conventional manner without causing any harm to this tick from various locations. One hundred random ticks were collected from the animals' bodies and were prepared to be sent to the pathobiology clinic at Ilam University. Next, a range of concentrations, starting from 10% and going up to 100%, was created using the standard dilution method, with the concentration of the water from these springs being considered as 100%. The test tubes were filled with varying concentrations ranging from 10% to 100% in 11 tubes, with 5 ml each, and one tube received 5 ml of distilled water as a control sample before being placed on the rack. The condition of ticks was verified using a magnifying glass, and the condition of 70 ticks was confirmed before they were extracted with tweezers and six ticks were placed in the water in each tube. The test tubes were submerged at the bottom. Following a 20-minute period, the substance from every test tube was removed using filter paper, and the ticks from each concentration were isolated and relocated to distinct Petri dishes along with the filter paper. It was immersed in 5 ml of pure water, then observed through a stereomicroscope, with designated time intervals assessed using individual chronometers for each group.

Results

Based on the passage at hand, the ticks were inspected with a stereomicroscope and it was seen that the higher the level of our active ingredient, the slower the pulsating leg movements of this external parasite became, with a complete halt at 70% concentration. The leg pulsations have ceased. Following the investigation, the filter paper containing the parasite was left in indirect sunlight for 30 minutes. It was then examined in a petri dish and under a magnifying glass microscope, but no movement was observed in concentrations above 70%. However, when the concentrations decreased, the parasites moved faster from the test tubes the longer they stay after this period, transitioning from 30% to 10% concentration. The entire motion of ticks was documented as normal. The ticks that passed this experiment were killed by alcohol.

The rate of ticks returning to their normal state increased as the concentration increased from 30% to 60%. Ticks in 30% concentration took 23 minutes to normalize, while those in 40% concentration took 35 minutes, and 50% recovered in 44 minutes and 60% in 108 minutes. A substantial time gap between two levels of 50 and 60% can suggest that the potency of the active ingredient in volcanic and sulfuric hot springs may exceed this concentration. Nevertheless, the ticks found in 70% of the grains were not visible under the stereomicroscope following five hours. To confirm, they were examined with the stereomicroscope once more 24 hours later, and the test result remained the same after another 24 hours (Tables 1, 2 and 3).

Table 1. The results of various concentrations of test substance based on drying.

Dilution of the test item	The time required to return to normal
control solution (distilled water)	As soon as it dries
10% (per 5 ml of solution, 0.5 ml of active substance)	As soon as it dries
20% (per 5 ml of solution, 1 ml of active substance)	As soon as it dries

Table 2. The results of various concentrations of test substance based on time.

Dilution of the test item	The time required to return to normal
30% (per 5 ml of solution, 1.5 ml of active substance)	23 minutes
40% (per 5 ml of solution, 2 ml of active substance)	35 minutes
50% (per 5 ml of solution, 2.5 ml of active substance)	44 minutes
60% (per 5 ml of solution, 3 ml of active substance)	108 minutes

Table 3. The results of various concentrations of test substance based on death.

Dilution of the test item	The time required to return to normal
70% (per 5 ml of solution, 3.5 ml of active substance)	Complete death of ticks
80% (per 5 ml of solution, 4 ml of active substance)	Complete death of ticks
90% (per 5 ml of solution, 4.5 ml of active substance)	Complete death of ticks
100% (5 ml effective substance)	Complete death of ticks

Discussion

A study carried out in 2016 examined the antimicrobial characteristics of sulfur nanoparticles, confirming their efficacy in treating resistant microbial infections (Rai *et al*., 2016). In a study conducted in 2021, thorough research was carried out on the biological assessment of sulfur-containing acids as anti-parasitic agents, finding these compounds to be effective against parasitic agents (Hanafi-Bojd *et al*., 2021). During research carried out in 2007, the effectiveness of sulfur-containing natural solutions in treating fungal and parasitic infections in fish was examined and validated (Ahmed *et al*., 2007). Sulfur is a common skin antiparasitic in Ilam province, where it is popular because of sulfur hot springs. The stable isotopes discovered in the springs include deuterium, oxygen-18, and sulfur-34 based on identification markers (Alimoradi *et al*., 2021). The presence of sulfur compounds in the water suggests that sulfur may be responsible for the skin antiparasitic effect in these animals, as sulfur bisphosphonate can potentially disrupt the cellular respiration of these parasites (Urbina *et al*., 1999). Sulfur compounds are commonly utilized in various skin conditions nowadays. In a study comparing the impact of 10% sulfur ointment and Ivermectin on scabies in 2015, it was found that the sulfur ointment was as effective as Ivermectin in the fourth week of treatment (Alipour and Goldust, 2015). Additionally, creating and producing sulfur-containing compounds can serve as an effective anti-parasitic solution for aquatic animals and play a vital role in controlling microbial growth in breeding environments (Tavassoli *et al*., 2011; Nchu *et al*., 2012; Yi *et al*., 2017; Anokhina *et al*., 2021). Moreover, besides their skin anti-parasitic properties, sulfur compounds can also help prevent other parasites in farmed chickens, including coccidiosis (Block and Steiner, 1986). In certain situations, resistance at the molecular level to acaricides can occur with various classes of compounds like acetylcholinesterase inhibitors. This enzyme impacts the parasite's nervous system, leading to its paralysis and death. However, ticks, particularly saline ones, are more resistant to the toxin. Their resistance and insensitivity to it prompted a reevaluation (Harrington *et al*., 2004; Githaka *et al*., 2022).

The molecular mechanism of acaricide resistance is understood for multiple compound classes, at least in certain circumstances (Anokhina *et al*., 2021). Organophosphorus agents (Ops) inhibit acetylcholinesterase (AChE), an essential enzyme for the nervous system to work properly. Since 1950, ticks in 40 countries have developed resistance to over 30 OPs and carbamates, with target site insensitivity identified as the primary mechanism of resistance (Janbakhsh, 1956). With the rise of parasite resistance in Africa, the demand for a safe treatment option to eradicate ticks and other parasites is growing rapidly (Abbas *et al*., 2014). As a result, straightforward and accessible treatments may be an effective solution.

In conclusion, based on the findings of this research, it is possible to state that the sulfuric hot springs found in various locations across the country like Dehloran and Mehran cities are an inherent and continuous resource. Individuals utilized anti-parasitic medication not only to save money for farmers but also as a remedy for various skin parasites and dermatitis. Since many farmers are located far from medical and veterinary services, it serves as a substitute treatment option. Sulfur solution can be applied topically to pets with ticks or skin parasites, offering a cost-effective and safe treatment option.

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Conflict of Interest

All authors declare that they have no conflicts of interest.

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