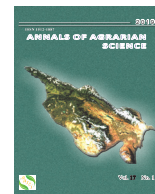




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### Parasitic mites of animals and fighting them

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#### ABSTRACT

The dissemination of ixodid mites has been studied throughout in four regions (Marneuli, Bolnisi, Gardabani, Dmanisi) of Kvemo Kartli (Georgia). 9 species of parasitic mites were identified from 5 families of Ixodidae. Periods of attack of parasites have been identified on agricultural animals. The highest number of pupa was observed in May-July, nymph - August-September (Marneuli, Gardabani, Bolnisi), and in the highlands (Dmanisi) imago is active from the second half of March until the end of May, pupa-from the end of July to the end of August, nymph-from the second half of March until the second half of May.

For the increase of fighting efficiency against the parasitic mites, on the basis of synthetic pyrethroids there was developed environmentally little dangerous preparation (conditional name “Giometrin”) with the prolonged acaricidal effect. Prolongation of acaricidal effect of “Giometrin” became possible in the result of combination of water-oil emulsion and synthetic piretroides. The simultaneous use of hydrophilic and organophilic surfactants in the formulation led to an increase in oil up to 80%.

By diluting the obtained preparation in the water, milk-like liquid is formed, that has longer acaricidal action (25-28 days), than the imported preparations (12-15 days) presented on the local market. It was confirmed, that after 96 hours of the treatment of hungry imago by the 0.015-0,02% solution of the preparation in lab conditions 100% mortality was identified, and in case of 0,0001-0,0075% of solution the mortality rate varied within 78.9-95.9%. In real conditions, in four farms, cattle were treated with of working solutions with concentration 0.0075-0.001-0.015-0.02%. Totally a 4-5 adult and adolescent cattle was treated with “Giometrin” in each farm. In daily monitoring, the viability of ticks and the number of dead ticks fallen from the skin of cattle were monitored. It has been confirmed, that imago forms totally die during 3-4 days, while the satiated mites die 4-5 days later after the treatment. The clinical condition of observed cattle was in norm, milk yield was not decreased. Conducted macro and micro morphological studies have shown that the spraying of 0,02% solution of the oil-emulsion preparation “Giometrin” does not cause changes in skin and internal organs. The reaction of the organism is within the norm. 1 hour later after spraying, weakly expressed capillary hyperemia and perivascular infiltration is detected in skin and regional lymph nodes. 6 hrs later after the spraying no deviation from the norm is indicated, it means that the change is not the result of the toxicity of the preparation or the cumulative effect of the skin. During the season for the guaranteed protection against the ticks 15 procedures are required in case of insecticide-acaricide preparations presented on veterinary market, while in case of treatment with the developed oil-emulsion preparation “Giometrin” with prolonged action only 7-8 times is enough. If we take into account the self-price of “Giometrin”, that is, 10-15% less compared to imported, preparations, overall developed preparation can reduce almost 2 times the expenses for the protection of animals from ectoparasites. Georgian Patent (GEP2011.5346B) has been adopted for the development of a concentrated oil-water emulsion concentrate with acaricide properties.

**Keywords:** Parasitic mites, Animals, Veterinary preparation, Ectoparasites, Ixodidae, Species.

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## Introduction

From many of the existing species of ectoparasites, mites from the family Ixodidae are the most harmful for the agricultural farming, which spread all Diseases caused by endogenous parasites. [1-4]. The dissemination of ixodid mites has been studied throughout four locations of Georgia (Marneuli, Bolnisi, Gardabani, Dmanisi) in Kvemo Kartli region, lowland, foothill and mountainous zones (fieldy, bushy, hilly meadows, riverside and forest-field areas).

From ticks of family Ixodidae there are identified 9 species of parasitic ticks (*Haem. punctata*, *Haem. sulcata*, *Haem. otophila*, *H. marginatum*, *H. anatolicum*, *B. calcaratus*, *Rh. sanguinalis*, *Rh. Bursa* and *I. ricinus*) from 5 genus (*Haemaphysalis*, *Hyalomma*, *Ixodes*, *Rhipicephalus*, *Boophilus*).

On pastures these species are crawling on the fur of cattle and stick to the surface of their skin. In host organism ticks inoculate saliva with anticoagulant action, which leads to both general and local toxicity. In general, allergic reactions occur when a metabolic disorder occurs in the body. In this case, the general reaction of the organism changes significantly, which is mainly due to the inhibition of the animal, decrease its productivity and the number of red blood cells in the blood. According to studies, mites for one feed can suck out from 0.5 to 3 ml. of blood from the cattle. Thus, the estimated total loss of blood can be 166 mg / per day on one adult animal [5]. Mites are transmitters of viral, bacterial, fungal and rickettsiose pathogens in animals [6,7,8]. In addition, ticks of this family are also spreaders of various human diseases (Encephalitis, Barrelos-Lyme disease, Fever, Tularemia, Brucellosis, etc.), [9-12].

Significant change in climatic conditions on the earth is gradually becoming noticeable due to global warming. Radiation activity of the sun increased. In some regions there is a decrease in the amount of precipitation, and in some cases, an increase. In comparison with previous years, the air temperature has increased by 1 - 2.1°C [13]. These climatic changes significantly affect the development of ticks. As well as other countries, Georgia is also focused on determining the area of distribution of various types of parasitic ticks in accordance with the geographical zones and seasons. Data of studies conducted in this direction are not available since 1958 (14).

Parasitic mites are widespread in Georgia. Consequently, cases of agricultural animal diseases caused by tick attacks are regular, which is a difficult task for farmers. The fight against them will not be a desirable result if the country does not take into consideration the bio-ecological, climatic and geographical factors of ticks in different zones of the countries. Treatment of diseases caused by attack of mites is associated with many difficulties. It is easier to carry out complex preventive measures against mites, which are implemented by regular treatment of cattle with effective and safe acaricidal preparations.

Measures against parasitic mites in Georgia are carried out by imported means. In recent years, insecticie-acaricidal preparations prepared on the basis of synthetic pyrethroids are widely used against parasitic mites [15]. Unlike previously used preparations, made from organochloric, organophosphoric and carbamic acids, they are less toxic to worm-blooded animals. They have poorly expressed allergic properties; do not have teratogenic, mutagenic and carcinogenic effects, as well as photosensibilizing properties.

The aim of the study was to develop an environmentally low-hazard preparation, with long-lasting acaricidal effect based on synthetic pyrethroides using natural compounds and the evaluation of its effectiveness. The low self-cost of the developed preparation for competition with other similar imported means was desirable.

## Computational Methods

Four raions - Marneuli, Bolnisi, Gardabani and Dmanisi were selected for the research in Kvemo Kartli region. 1628 cattle, 841 sheep and goats, and 27 dogs were clinically examined in these areas. 4032 units parasitic mites were totally collected, including: imago – 2916, nymph - 624, larva - 492. Parasites were collected from the animal skin, from animal farms, as from adjacent territories and pastures. The morphological examination of the collected ticks has been done through Galuzo and Zakhvatkin's tables [16] and using MBC-9 microscope. The temperature of the environment was determined by the thermograph, measuring soil temperature at 4-5 cm depth - using a thermo probe, and determination of moisture – using a psychrometer.

Systemic observation was carried out on cattle to determine the quantity of mites. Research

works were conducted both in laboratory and field conditions, in areas of the region. To determine the activity of ticks on livestock and pastures in different seasons of the year, tests were conducted for individual parasites (imago, nymph, and pupa) at different temperatures in warmth (at a temperature of 7-10-20-25°C) and in the cold (1-3-5°C using dry ice).

In order to determine the influence of the preparation “Gimetrin” on the reactivity of the animal organism, totally 9 clinically healthy rabbits were examined. After treatment of the animal’s skin with 0,02% solution of “Gimetrin”, the reaction of the animal and clinical signs were observed within 5 days after application of the preparation. In parallel, pathomorphological changes in the body were studied.

After 1, 6, 24 hours and 3 days of treatment the animals slaughter and autopsies were carried out. Were cut off in order materials from subcutaneous tissue, parenchymic organs and lymph nodes for pathomorphological examination. The primary fixation of pathomorphological material was carried out in a 10% solution of formalin, which was then transferred to a 15% solution. The material was embedded in paraffin, in the case of pathomorphological changes in organs and tissues, the coloring method with Sudan-3 was used to study fatty dystrophy and lipids.

## Results and Discussion

There are identified 9 species of parasitic mites (*Haem. punctata*, *Haem. sulcata*, *Haem. otophila*, *H. marginatum*, *H. anaticum*, *B. calcaratus*, *Rh. sanguinalis*, *Rh. Bursa* and *I. ricinus*) from 5 genus (*Haemaphysalis*, *Hyalomma*, *Ixodes*, *Rhipicephalus*, *Boophilus*) of family *Ixodidae*.

In the lowland territories of selected regions of Kvemo Kartli there are spread mites of species *B. Calcaratus*, *H. marginatum*, *Rh. Bursa*, *H.anaticum*, *Haem. punctata* and *haem. sulcata*, the dominants are *B. calcaratus*, *H. Marginatum* and *Rh. Bursa*.

*B.Calcaaratus* - species are widely spread throughout the country, especially in western Georgia. In the Kvemo Kartli zone, these species have been collected in large quantities in lowland areas that are abundantly covered with vegetation. High quality of tick activity was observed on the

territory of Mtkvari, Iori and Khrami.

Duration of life-cycle of these parasitic ticks in 4 regions of Kvemo Kartli lowlands is 56 days, in foothill - 80 days, in mountainous areas 92 days.

In foothill territories there are spread *Rh. Bursa*, *H.anaticum*, *I. ricinus*, *Haem. punctata*, *Haem. sulcata*, *B.Calaratus* and *Haem. otophila* species, dominants are *Rh. Bursa* and *H.anaticum*.

*Ryipicephalus bursa* – Parasit mainly of large and small cattle. It is distributed on non-irrigated pastures, shrubs and hills of all four regions. Even in case of high quantity of ticks on large cattle, in Gardabani region, only few individuals of imago parasites were found on sheep.

*Hyalomma anaticum* – This parasite is of a large size. In addition to the foothill zones, it is widespread in the lowland areas, where the territories are abundantly covered with a vegetation and more are observed in the bushy areas.

*H. Marginatum*, *I.ricinus*, *H. punctata* and *Rh. Bursa* are widespread in the mid-mountain zone, dominant is *H. Marginatum*.

*Hyalomma marginatum* - Adult parasite is large. Its distribution is observed throughout the whole territory of all four regions.

In mountainous areas genus of mites «*H.Otophila*» and «*Haem. Punctata*» are common out. Of these, the most common is «*Haem. Punctata*».

*Haem. Punctata* –is spread in lowlands, foothills and mountainous areas of all four regions. They are tiny mites. The main owner of adult mites is the large cattle.

A high price for high-quality imported preparations, represented on the Georgian veterinary market, is too expensive for most farmers engaged in cattle breeding. Accordingly, prophylactic processing of the animal’s body is not performed at all or partially carried out. As a result, there are often cases of various infectious and invasive diseases and the fall of livestock. Therefore, it was important to develop a low-cost and effective acaricidal preparation of local production, the use of which would be economically acceptable for farmers, engaged in cattle breeding, in order to protect livestock from parasitic mites.

It is not possible to produce cheaper local analogues of imported preparations, since there is no production of the necessary components in Georgia. Therefore, it was decided that the studies

were aimed at prolonging the acaricidal activity, in particular, on the development of the preparation, which will have a longer period of action than the imported (12-15 days). This became possible by the controlled isolation of molecules of the main active substances (evaporation). At the same time, the modern requirements for insecticide-acaricidal preparations necessary for veterinary practice were considered [17].

Prolongation of the acaricidal action of the developed preparation made possible by the combination of water-oil emulsion concentrate with the synthetic pyrethroids.

In case of increase of the oil (vaseline) content up to 80% in the composite system, two types of hydrophilic and organophilic surfactants were introduced into the system to avoid the stability decrease of the emulsion concentrate and phase separation during long-term storage. By diluting the resulting preparation in water (ratio 1: 100), a milky-like working solution is formed which has an acaricidal effect for 25-28 days. High oil content in the working solution ensures reliable fixation of the product on the treated surface and maintenance of acaricidal properties for a long time even in case of contact with water.

The effectiveness of the acaricidal preparation (conditional name «Giometrin») prepared on the basis of a microemulsion concentrate was performed

in laboratory conditions and in real environment.

Impact on the hungry imago *B. calcaratus* with different concentrations of preparations was carried out for 1 minute. Dead and paralyzed ticks were recorded through 12, 48, 72, 96, 120 and 144 hours. The results are presented in Table 1.

In real environment, the study of acaricidal properties of the preparations was continued in 4 farmings in Dmanisi region on large cattle with mites (20-25 mites were recorded on animals). On average 4-5 adult and young animals were treated by the preparation «Giometrin» in each farm. The processing of animals was carried out with 0,0075-0,001-0,015-0,02% of working solution containing cypermethryn.

Three periods of attack of the species *B. Calcaaratus* were registered on livestock in the pastures:

I. In early spring - from the second half of February to the first half of April (Gardabani, Marneuli and Bolnisi lowlands); From the beginning of March to the first half of May (Dmanisi),

II. In summer - from the beginning of June to the end of August (Gardabani, Marneuli and Bolnisi); From the second half of June to the end of August (Dmanisi).

III. In autumn - from the beginning of the september to the second half of October (Marneuli, Gardabani) in the foothills of Bolnisi. autumn attack period was not observed in Dmanisi.

## Treatment

P - Paralyzed D - Dead

**Table 1.** The results of the impact of «Giometrin» on hungry imagoes of *Boophylus calcaratus*

Concentration of Cypermethrin in the preparation	Quantity of ticks	Tick mortality %													
		12 hr		24 hr		48 hr		72 hr		96 hr		120 hr		144 hr	
		P	D	P	D	P	D	P	D	P	D	P	D	P	D
0,0001	20	100	-	85,7	14,3	65,5	34,5	43,9	56,1	21,1	78,9	4,3	95,7	-	100
0,001	20	63,1	26,9	41,9	58,1	29,1	80,9	16,3	83,7	9,4	90,6	1,2	98,8	-	100
0,0075	20	52,2	47,6	36,7	67,3	18,2	81,6	11,1	88,9	4,1	95,9	-	100	-	-
0,015	20	40,4	59,6	20,1	79,9	12,3	87,7	6,5	93,5	-	100	-	-	-	-
0,02	20	10,2	89,8	7,1	92,9	4,5	95,5	1,2	98,8	-	100	-	-	-	-
Control water	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Experiments in the laboratory and environmental conditions showed that the major host of parasitic ticks *B. Colcaaratus* is 95% of the livestock.

The massive attack of adult ticks of *Hyalomma anatolicum* on livestock throughout Gardabani and Marneuli districts, and in the lowlands of the Bolnisi region, was observed from mid-March to the end of May, nymph and pupa from the beginning of June to mid-July. Some mites are observed on the skin cover of bovine livestock in late period (late September), parasite was fixed on the skin cover of the animal in Bolnisi region from the end of March to the first half of April.

Imago «*Hyalomma marginatum*» are attacking cattle in early spring - at the end of February and reaches the maximum in the first half of May. Nymph and pupa is observed later from the second half of March. The major host of parasites are cattle and other animals.

In the laboratory conditions not hungry imago and nymph dies at -10 C (using dry ice) in 33-35 days.

When the mites (imago, nymph and pupa) are placed in water for 3 hours, death occurs in 18 days, and with a 2-day placement - the mites die on day 6.

First attack of adult mites of *Haem. Punctata* on animals is observed from early spring, at the end February to the end of April. Second attack in autumn, in August-September. The largest number of ticks is observed in May-July, nymph-August-September (Marneuli, Gardabani, Bolnisi). In the Dmanisi region imago is active from the second half of March until the end of May, pupa - from the end of July to the end of August, nymph - from August to September.

The quantity of mites on cattle reaches its maximum in May. From the end of May, the number of animal mites decreases and only one parasites is found on the surface of the host skin. In Dmanisi district the attack of ticks is observed later.

A study of the relationship between the environmental climate and the activity of parasite mites was confirmed that *Haem. Punctata*, *I. ricius*, *H. anatolicum*, *H. marginatum* attack agricultural animals after wintering, when the air temperature reaches 6-80 C and the soil temperature is - 70C. Species-*B. Colaratus* and *Rh. Bursa* attack animals when air temperature is 11-120C and the soil 90 C. These indicators make it easier to predict attack period.

According to the results of efficacy testing of preparation “Giometrin” in laboratory conditions presented in Table 1. it is confirmed, that 100% mortality was observed after 96 hours after the contamination of hungry imago with 0.015-0,02% of solution containing cypermethrin and in case of treatment with 0,0001-0,0075% solution after 96 hours the mortality varied within 78.9-95, 9%.

In real environment the preparation efficiency was evaluated according to the the status of mites after the processing of skin cover of cattle. In the daily monitoring mode, the viability of the ticks was observed, the number of dead and number of dropped from the cattle skin cover was recorded.

It has been established that imago forms of hungry mites completely die for 3-4 days, and non hungry full parasites - 4-5 days later. The clinical condition of the cattle that was in the scope of norm, milk yield was not reduced.

Visual Observation was performed on the skin of treated animals for 20 days to determine the residual acaricidal activity of the preparation. Within 15-18 days after processing, there were no ticks on skin cover of animal, whereas on on the skin of untreated animals, being together on the herd an average number of mites was 10-15 pcs/day.

Skin vessels, especially reticular layers are enlarged and filled with blood. This circulatory reaction was primarily concerned to capillaries, around which in a single cases weak infiltration of serous fluid and leucocytes (eosinophilic) was detected in a small areas. Histological structure of vessel wall was maintained. Fatty glands and ducts were well expressed. Totally was maintained the connective tissue of animal fur.

The nearest (regional) lymph nodes adjacent to the site of spraying the preparation were not enlarged in volume (with characteristic grayish-white color and consistency). According to histological research, the histological structure of the lymph node has been preserved; the primary and secondary follicles were well expressed (with well-maintained light zones in the secondary follicles). The follicles were filled with lymphocytes and reticular cells.

Hyperemia in lymphaden and medullary layer is kept, that mainly was expressed in venous capillaries. Anatomic parameters of the liver were

preserved and macro-morphological changes were not observed. Pathomorphological studies have shown weakly expressed blood reaction in the form of hyperemia in venous capillaries.

Micro- and macromorphological changes in the heart, lungs and kidneys were within the norm and no deviation from them was observed.

After 6 hours of spraying with the preparation previously expressed hyperemia and weak peripheral infiltration were not marked.

The clinical signs of animals were within the norm. Weakly expressed hyperemia in regional lymph nodes has returned to norm. Deviations from the norm of histological changes were not recorded in the investigated organs. A similar picture is expressed 24 hours after the use of the preparation.

According to the studies carried out on the preparation - «Giometrin», conducted in G. Natadze Sanitation, Hygiene and Medical Ecology Scientific-Research Institute of the Ministry of Labour, Health and Social Affairs of Georgia issued a hygienic and toxicological decision on its safety / harmless for humans and animals, which makes it possible to use a preparation for parasitic tick control.

After receiving such a conclusion, a large-scale test of the preparation was conducted in field conditions. Agricultural animals were treated in the mountainous and lowland regions of Kakheti, Samegrelo, Imereti, Shida Kartli and Kvemo Kartli. The original design of the mixer-stirrer was developed to provide large-scale field trials, and the finished product/preparation was packaged in 100 ml bottles.

Throughout the field trials, 585 souls of cattle was treated, including milker cows, adolescents

and calves. Continuous monitoring of the cattle was carried out within 30 days. The result was satisfactory, the contraindication had no place.

According to the results of the field exams, the use of «Giometrin» significantly decreases need processing repetition of cattle. During the season only 7-8 fold treatment is enough for the guaranteed protection of animals from mites, with developed emulsion preparation -»Giometrin» with prolonged insecticide-acaricidal action, instead of 15 fold. As a result, the quantity of used preparation is reduced by 40%. If we take into account the self-cost of the emulsion preparation, which is 10-15% less than the cost of imported preparations, the costs for protection of animals from ectoparasites, with preparation «Giometrin» (for which a Georgian patent GEP2011.5346B was obtained), will be reduced almost 2-fold, as shown in Table 2.

## Conclusion

A study of the bioecological, climatic and geographic features of ticks in the Kvemo Kartli zone established that in the phenology of ticks there are certain peculiarities considering species. The highest number of pupa was observed in May-July, nymph - August-September (Marneuli, Gardabani, Bolnisi), and in the highlands (Dmanisi) imago is active from the second half of March until the end of May, pupa-from the end of July to the end of August, nymph-from the second half of March until the second half of May.

By G. Natadze Sanitation, Hygiene and Medical Ecology Scientific-Research Institute of the Ministry of Labour, Health and Social Affairs of Georgia issued hygienic and toxicological decision on the safety /harmlessness for humans and animals

Preparation	Acaricidal activity, days	Repetition of processing in season	Preparation expense /per cattle ml	Price for expenced Preparation GEL
Pyrethroid	12-15	15	30	1,5
Giometrin	22-25	8-9	17	0,76

**Table 2.** *The effectiveness of «Giometrin» in comparison to imported acaricidal preparations*

of the developed preparation “Giometrin” with prolonged acaricidal effect.

Conducted macro and micromorphological studies have shown that the spraying of 0,02% solution of the oil-emulsion preparation “Giometrin” does not cause changes in skin and internal organs. The reaction of the organism is within the norm. 1 hour later after spraying, weakly expressed capillary hyperemia and perivascular infiltration are detected in skin and regional lymph nodes. 6 hrs later after the spraying no deviation from the norm is indicated, it means that the change is not the result of the toxicity of the preparation or the cumulative effect of the skin. Probably, it is associated with manipulations on rabbits, as a sign of a stressful reaction that does not go beyond the physiological limits of the organism.

“Giometrin” fully meets the modern requirements for veterinary preparations:

- Easy to use
- Safe for human and animal health;
- Does not have cumulative feature;
- It is characterized by a wide spectrum of action and the effect of prolonged acaricidal action;
- During storing there is no change in physical-chemical characteristics;
- Suitable for use during 3 years.

The compositional preparation “Giometrin” is an innovation for the Georgian veterinary market, therefore, an introductory work was carried out for farmers and the rural population, which showed the advantage of using “Giometrin” in comparison with foreign analogues.

Based on the above, the widespread use of the preparation “Giometrin” will significantly facilitate the improvement of cattle care in Georgia and increase the production of livestock products.

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