Research Article

Using Bee Propolis to Treat Parasitic Diseases in Saiga Calves in the Akboken Nursery: A Veterinary and Pathoanatomic Study

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ABSTRACT

The study aimed to identify the pathoanatomic aspects of piroplasmosis and monieziasis in saiga antelopes kept in a nursery and evaluate the effectiveness of bee propolis as a therapeutic and prophylactic agent. In May 2024, 100 saiga calves were removed from nature and placed in the Akboken nursery (Kazakhstan). A clinical examination, hematological and biochemical blood tests, a pathoanatomic autopsy of 10 corpses, and diagnosis by blood smears and coproovoscopy were performed. Azidin, hyperimmune serum, and propolis extract were used as part of the therapy. The studies were conducted for 7 weeks. The saiga calves were diagnosed with piroplasmosis and monieziasis. For the first time, characteristic pathomorphological changes of internal organs in these diseases were established. Treatment with propolis had a positive effect: the death rate decreased, the number of leukocytes increased, the concentration of total protein and globulin increased, and the level of triglycerides decreased. This pioneering research firmly establishes propolis as a novel, effective, and natural therapeutic intervention, vital for enhancing the health and survival of saiga calves challenged by parasitic diseases in nursery environments and offering significant promise for broader wildLife conservation strategies.

Keywords: Piroplasmosis, Pathoanatomic aspect, Monieziasis, Fauna, Invasion, Serum.

INTRODUCTION

The saiga antelope (*Saiga tatarica*) is a relict species and a surviving representative of the Pleistocene fauna. Despite its evolutionary resilience, the saiga antelope is now critically endangered, facing severe pressures that have caused dramatic population declines. In recent decades, its numbers have plummeted by approximately 95%—from about 1.2 million individuals in the mid-1970s to nearly 50.000 by the early 2000s—due to mass mortality events linked to climate anomalies and infectious diseases, extensive poaching and habitat degradation (Kock et al. 2018; Hanski et al. 2023; Ganbold et al. 2024; Myrzabayev et al. 2024). Although some populations have begun to recover as a result of coordinated conservation actions (Begilov et al. 2024), the species remains highly vulnerable, underscoring the need for effective long-term preservation measures. In this context, captive breeding programs and nurseries play an increasingly important role, enabling detailed studies of

saiga biology and physiology as well as the establishment of insurance populations for future reintroduction (Minoranskii & Dankov 2016; Cui et al. 2017).

However, managing saiga calves in nurseries poses serious health challenges, particularly from infectious and parasitic diseases. Field studies in the West Kazakhstan region reveal a high prevalence of coccidiosis and helminth infections in wild saigas, with *Eimeria elegans* detected in up to 88.57% of animals and *Moniezia expansa* found during both summer and autumn seasons. (Abdybekova et al. 2023; Kushaliyev et al. 2023). Additional surveys identified nine species of helminths and two protozoa in naturally deceased individuals (Kushaliyev et al. 2024), while PCR diagnostics confirm widespread infections with *Eimeria* spp. (Kidiraliyev et al. 2024). Parasite transmission is strongly influenced by environmental conditions and pasture overlap between wild saigas and domestic livestock, which facilitates cross-infection and shapes mixed helminth communities (Albery et al. 2018; De Winter et al. 2020; Kushaliyev et al. 2023).

Because reliable ante-mortem diagnostic tools for wildLife are limited, early cases often require necropsy combined with hematological and pathomorphological assessments to determine the cause of death.

Piroplasmosis has recently been recorded in saiga calves for the first time, transmitted by *Rhipicephalus bursa* ticks circulating among domestic ruminants and wild ungulates (Salkova et al. 2015; Tarav et al. 2017). Affected calves show depression, weakness, anorexia, tachycardia, jaundice, anemia, conjunctival hemorrhage, and occasionally hemoglobinuria and lameness (Sahu et al., 2020; Mahdy et al., 2023; Ijaz et al., 2024). Such infections can rapidLy escalate in nursery settings, where young animals with immature immune systems are particularly vulnerable to severe outcomes (Carreira et al. 2019; Beaumelle et al. 2021; Dallas & Warne 2023). In one nursery outbreak, saiga calves exhibited massive diarrhea, metabolic disorders, and high mortality within 1.5–2 months, underscoring the urgent need for effective therapeutic and preventive measures.

Conventional pharmaceutical treatments, such as antibiotics and anthelmintics, are often unsuitable for saiga calves due to their heightened sensitivity to many drugs. This sensitivity can disrupt the gut microbiome, impair nutrient absorption, and adversely affect immune development (Peltzer et al. 2017; Bahl et al. 2020; Miller et al. 2024). Additional challenges include increasing global resistance of parasites to commonly used anthelmintics (Muchiut et al. 2018; Höglund & Gustafsson 2023; Nielsen et al. 2023) and the practical difficulties of administering medication to semi-wild animals, which require restraint and may suffer stress-induced complications (Moreno Manas et al. 2019; McCallum et al. 2024). These issues highlight the need to identify alternative, safe, inexpensive, and biologically compatible treatments for parasitic diseases in wildLife (Ranasinghe et al., 2023). Therefore, this study aims to assess the effectiveness of bee propolis in improving hematological parameters and survival outcomes in saiga calves diagnosed with piroplasmosis and monieziasis under nursery conditions.

Bee propolis is one such promising candidate due to its well-documented antimicrobial, antiparasitic, anti-inflammatory and immunomodulatory effects (Beaumelle et al. 2021; Loukas & Maria 2023; Stevanović et al. 2024; Bava et al. 2025). Given saiga calves' high sensitivity to conventional drugs, propolis was selected as a therapeutic and prophylactic agent for piroplasmosis outbreaks complicated by monieziasis in nursery-reared animals.

MATERIALS AND METHODS

All procedures involving animals were conducted in accordance with the ethical standards of the Ministry of Ecology and Natural Resources of the Republic of Kazakhstan and were approved by the Animal Ethics Committee of Zhangir Khan West Kazakhstan Agrarian

and Technical University.

In May 2024, 100 heads of the Ural saiga calf population were removed from the natural environment with the permission of the Ministry of Ecology and Natural Resources of the Republic of Kazakhstan. The saiga calves were housed in the Akboken nursery, established in 2022 and located 20km from Uralsk on a 56-ha territory.

In 2022, the Akboken nursery was established at the Zhangir Khan West Kazakhstan Agrarian and Technical University (ZKATU), on the territory of the Uralsk Agricultural Experimental Station (USKhOS), Derkul rural district (20km from Uralsk), with a 56-ha territory. Before settling saiga calves in this area, epizootic monitoring was conducted for infectious and invasive animal diseases, and the territory was confirmed to be safe. However, saiga calves were removed from the West Kazakhstan region, which was affected by helminthiasis, including monieziasis and coenurosis.

With 100 heads of saiga calves removed from the natural environment in 2024, the main difficulty was feeding them during the preweaning period. Newborn saiga calves showed signs of massive diarrhea and metabolic disorders, which required appropriate therapeutic and preventive measures. In the preweaning period, saiga calf groups were formed with 15-20 heads, each in a separate pen fenced with a plastic mesh with a mesh size of 3×3cm and a height of 1.8m. This type of fencing is advisable during the preweaning period to avoid injury to young animals during growth and development.

The service staff fed saiga calves with milk according to the feeding schedule, which was standardized for each animal, and registered the quantity fed to each head. This allowed for monitoring the health of saigas and taking timely veterinary and sanitary measures.

The targeted organizational work included veterinary and organizational measures that ensured strict compliance with zoohygienic requirements. These requirements aimed to create optimal conditions for feeding and keeping saiga calves, considering their age and physiological condition. Veterinary care involved restraining animals, studying their clinical condition, and taking blood for hematological analysis and thermometry, as well as a systematic and prophylactic examination of the entire livestock.

These measures made it possible to promptly identify signs of animal diseases of various etiologies and take specific measures to prevent the full manifestation and spread of diseases. Saigas in the Akboken nursery were under strict veterinary supervision. Veterinary and preventive measures (vaccination, fortification, etc.) were carried out against infectious and helminthic diseases.

Saiga calves were fed according to the prescribed diet (Table 1), which shows the norms for whole milk, whole milk substitute (WMS), and Nestogen dry milk formula for up to one month. Feeding standards were determined mainly by the calves' physiological state, activity, and intensity of consumption of green feed, hay, later concentrates, etc.

Table 1: Feeding standards for saiga calves

Age	Quantity, mL	Times per day	Age	Quantity, mL	Times per day
1-3 days	50-100 (whole milk)	5	2-3 months	400 (Nestogen)	3
4-12 days	100-150 (whole milk)	4	3-3.5 months	450 (Nestogen)	3
13-43 days	250 (whole milk)	4	4 months	500 (Nestogen)	2
1-2 months	250-300 (WMS)	4	5 months	500 (Nestogen)	1

On the third day, the saiga calves were given 150mL of WMS, which caused diarrhea in several calves simultaneously. After that, the quantity and frequency of feeding were reduced. On the next day, they switched to cow milk. The body of saiga calves does not tolerate a change in the composition of milk and WMS.

On day 43, saiga calves received WMS for a month, and no stomach disorders were observed. From the first days of being fed with milk, the calves received up to 2 mL of fish oil, and from 1 month to three months, up to 5mL of fish oil once a day. The rest of the time, the saiga calves received a dry milk mixture called Nestogen, and grain (barley) was added to the diet of the saiga calves starting from the second month.

From the age of 4 months, we included feed chalk in their diet, which was given first with milk and then added to concentrates.

When feeding saiga calves, we followed the feeding standard, feeding regime, and the appropriate diet (Table 2).

Table 2: Calculation of the saiga diet

Feed name	Daily ration per head	Terms and calculations, kg		
		day	month	year
Green grass	1.9	10.7	325	649 (2 rolls)
Concentrated feed	0.8	1.5	50	600
Hay	1.39	8.2	251	3,000 (6 rolls)
Salt	unlimited	40kg per year		
Chalk	unlimited	40kg per year		

Many hours of observation of the feeding behavior of hand-reared newborn saigas allowed us to establish that the time interval after which a newborn saiga calf began to suck for the first time was at least 20 minutes.

The weight of the feed consumed depends on the season, the condition of the feed, the age and sex of the animals, and other physiological characteristics. The daily feed requirement for young saiga antelopes is 2.6kg of hay, 0.4kg of concentrates, and unlimited salt and water.

The technology of feeding young saigas during the preweaning period was developed; however, after 2.5 months, massive diarrhea and death among saiga calves suddenly occurred.

The pathoanatomic autopsy of the corpses was performed by the method of complete evisceration of organs using the Shora method; the protocol was compiled according to the scheme of autopsy developed by Gavrilov and Shishkov (1983).

The study of animal population immunity to hematological parameters (leukocytes, erythrocytes, platelets, basophils, eosinophils, neutrophils, lymphocytes, and monocytes) was carried out using a Micros-60 hematology analyzer (France). Biochemical analysis of blood serum (total protein, calcium, and phosphorus) was conducted on BioChem FC-200 (USA).

To determine the infection of saiga calves with monieziasis, we used postmortem complete helminthological autopsy and lifetime diagnosis by Fulleborn's method of coproovoscopic examination at the Research Institute (RI) of ZKATU. The number of helminth eggs in 1 g of feces was calculated using a VIGIS 19J counting chamber.

The animals in the Akboken nursery were microchipped, and blood sampling was carried out in separate vacutainers for hematological and biochemical analysis. A veterinary and sanitary measures plan was drawn up at the Akboken nursery.

Statistical Analysis

Continuous variables (hematological and biochemical indices) are presented as mean±SD together with 95% confidence intervals (CI) for each group. Distributional assumptions were assessed using Shapiro–Wilk tests and Q–Q plots; variance homogeneity was checked with Levene's test. Between-group differences (Propolis-treated vs Healthy reference) were evaluated with Welch's independent-samples t-tests; when assumptions were violated, the Mann–Whitney U test was prespecified. Multiple comparisons across blood indices were controlled using the Holm–Bonferroni procedure. Effect sizes are reported as Cohen's d (with 95% CI). Two-sided $\alpha = 0.05$. Analyses were performed in R 4.3 (stats, rstatix).

RESULTS

Based on the conducted clinical examination of saiga calves, signs of toxic dyspepsia were established. An autopsy of 10 saiga calves' corpses was performed, an autopsy protocol was drawn up, and individual pathoanatomic aspects of the disease were identified (Fig. 1). To exclude an infectious disease (salmonellosis), the pathomaterial was delivered to the branch of the Republican Veterinary Bacteriological Laboratory in Uralsk. Salmonellosis was excluded.

When making the diagnosis, large, pear-shaped, rounded, and amoeboid-shaped piroplasms located at an acute angle to each other were found in erythrocytes in the blood smears of saiga calves.

This was the first time we found piroplasmosis in saigas. In saiga calves, the disease manifested itself with signs of depression, weakness, anorexia, increased heart rate and respiration, lagging behind their groups, and lying down often. Anemia and jaundice of the mucous membranes were also observed. Some saiga calves showed limping, hemorrhagic conjunctivitis, and hemoglobinuria.

Based on the conducted clinical examination of saiga calves, signs of toxic dyspepsia were established. An autopsy of 10 saiga calf corpses was performed, and autopsy protocol 1 was drawn up; certain pathoanatomic aspects of the disease were identified (Fig. 1). To exclude an infectious disease (salmonellosis) the pathomaterial was delivered to the branch of the Republican Veterinary Bacteriological Laboratory in Uralsk, and salmonellosis was excluded.

Protocol 1
Pathoanatomic autopsy of a saiga calf that died in the akboken nursery

Autopsies of 10 saiga calf corpses were performed in the dissecting room of the Veterinary Faculty of ZKATU, from July 22 to July 29, 2024, during the daytime starting at 10 a.m. The autopsy was performed by Professor K.Zh. Kushaliev, Doctor of Medical Sciences, in the presence of researcher A.R. Kozhaev and doctoral student B.G. Satybaev.
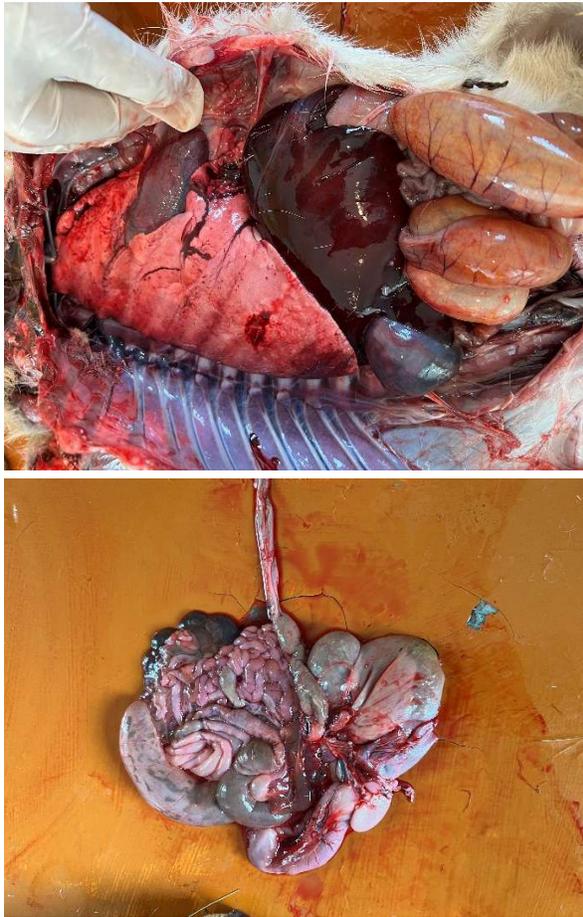
Fig. 1: Pathoanatomic changes in the internal organs of saiga calves; a) serous-hemorrhagic enteritis b) gas accumulation and jaundice of the large intestine.

Medical history

According to the nursery employee, R.G. Ispaev, saiga calves died suddenly within 3 to 4 hours for 7 days, with signs of impaired coordination of movement, muscle trembling, diarrhea, and foamy fluid discharge from the nasal cavity. There were frequent signs of bowel movements, tonic seizures, and reddish urine (hemoglobinuria). After a slight run, the animals experienced shortness of breath; some saiga calves demonstrated limping, conjunctivitis, and jaundice of the eyeballs. They would often lie down and stand in one place for a long time, with moaning, intense thirst, and circular movements. Liquid feces stained the tail and hind limbs. Sick saiga calves cannot get up; they throw their heads back, and they die from exhaustion and toxicosis.

Because the pathoanatomic signs in the internal organs of 10 saiga corpses were the same, we present one autopsy protocol of a saiga calf corpse as an example.

External inspection

Identification signs indicated the corpse of a female saiga calf, aged 3.2 months, gray, skinny, well-fed, of correct build, with a body weight of 9kg, individual 99. Cadaveric changes showed the corpse was cold and emaciated, and the abdomen was slightly swollen. Rigor mortis was mild, and cadaveric hypostasis was observed on the right side. In the disease-specific part, visible mucous membranes, such as the conjunctiva of the right eye, were swollen and anemic, yellow, while the left eye was pale, and the cornea was completely yellowish. Foamy liquid was released from the nasal cavity. The hair on the buttocks and tail was slightly stained with liquid feces. There was an accumulation of serous-hemorrhagic infiltrate in the subcutaneous and intermuscular connective tissue, and no fatty tissue existed. Hooves were without visible changes. The superficial lymph nodes (pre-scapular and superficial cervical ones) were swollen, and a grayish fluid drained from the incision. Bones, tendons, and joints were without visible changes.

Internal inspection

The abdominal cavity contained about 150mL of reddish-yellowish liquid. The organs' positions were anatomically correct, and the peritoneum is moist, smooth, shiny, and light red.

The pleural cavity contained about 100mL of yellowish-red liquid (cadaveric transudate). The pleura is smooth, shiny, and bluish-yellow. Due to the expansion of the right section, the heart was rounded and oval. The epicardium was smooth and shiny, with dotted, striped hemorrhages. The heart muscle was reddish-gray in places with a yellowish tinge and flabby. The endocardium was smooth and shiny, and the heart valves were elastic, with striped hemorrhages in places. The blood in the cavities of the heart was unclotted. The spleen was enlarged and softened, full-blooded, dark reddish, and had little scraping. The larynx, trachea, pharynx, esophagus, and their mucous membranes were swollen, reddish and bluish-tinged. The lungs were grayish-red in color, with a doughy consistency, and bloody fluid was draining from the incision surface. The bronchi were bluish-red and contained a small amount of foamy liquid. Bronchial and mediastinal lymph nodes were without visible changes.

The liver was enlarged, and its edges were rounded, full-blooded, dense, and unevenly colored, alternating with areas of grayish-yellow. The gallbladder was slightly distended and filled with dilute, light-yellow bile. The kidneys were both dense, and the section did not show the boundary between the cortical and medullary layers. The bladder was empty; the mucous membrane was pale pink, smooth, moist, and shiny.

The rumen was slightly filled with a 150g yellowish-greenish feed mass, and the mucous membrane was light brownish. The net contained a small liquid feed mass, and the mucous membrane was light-yellow. The omasum contained a small amount of dry feed mass. The mucous membrane and the leaflets of the omasum were unchanged. The abomasum was semi-empty; the mucous membrane was shiny, grayish-red, edematous, dotted with small spot hemorrhages, and covered with mucus. The small intestine, including the duodenum, jejunum, and ileum, was moderately filled with dense grayish-brown feed mass, and the mucous membrane was swollen, edematous, dark red, and covered with mucus. In some areas, the small intestine was swollen due to gas overflow. Moniezia was found, with the strobili torn off (1-2 Moniezia in every saiga calf).

The contents of the large intestine were yellowish-brown, and the mucous membrane was pale gray, with a pinkish tinge, and shiny. The mesenteric lymph nodes were slightly enlarged, swollen, grayish-red in color, and

riddLed with hemorrhages. Regarding the brain, the vessels of the meninges were moderately filled with blood, the brain matter was flabby, and the cerebral ventricles contained a small amount of clear fluid.

Pathoanatomic diagnosis:
- Acute serous-hemorrhagic enteritis.
- Acute serous-hemorrhagic inflammation of mesenteric lymph nodes.
- Serous-hemorrhagic enteritis and 1-2 Moniezia in every saiga calf.
- Gas accumulation and jaundice of the large intestine.
- Congestive liver disease.
- Acute congestive pulmonary hyperemia.
- Dilation of the right heart.

Based on anamnestic data, clinical signs, and the results of a pathoanatomic autopsy, it should be concluded that the (female) saiga calf died with signs of toxic dyspepsia from piroplasmosis complicated by a parasitic infection (monieziasis).

Laboratory results: The blood was watery, and the number of erythrocytes in it had reduced to 4 million per $1mm^3$. The percentage of hemoglobin had also decreased. Anisocytosis, poikilocytosis, and basophilic granularity in erythrocytes are noted.

Thus, the pathomorphological aspects of saiga calves with piroplasmosis complicated by monieziasis were observed mainly at 2-3 months. The corpse was emaciated, the visible mucous membranes were anemic, the muscles were pale and hydrous, the subcutaneous tissue was infiltrated and, in some places, strongly edematous. The small intestine was hyperemic, with dotted and striped hemorrhages, an abundance of thick mucus, and swollen and enlarged mesenteric and mesenteric lymph nodes. The main pathologies were characterized by damage to the mucous membranes, lungs, liver, and kidneys, as well as swelling, redness, and accumulation of exudate in the abdominal cavity in the form of ascites. Based on the autopsy results, a quarantine was established in the Akboken nursery, and veterinary and sanitary measures were organized with disinfection work in the pens. The deaths stopped in 14 days.

Therapeutic and preventive measures

The saiga was a timid and cautious animal. In captivity, the resistance was noted to be lower. Unstable resistance to stress was the initial factor of a disease, and the general state of health and the results of hematological and biochemical studies were assessed. To treat piroplasmosis in saiga calves, azide was used intramuscularly in a 7% solution at a dose of 1mL per 20kg of animal weight. Antitoxic hyperimmune serum against toxic dyspepsia in a 2g/m dose was used for weakened saiga calves. To raise the overall immune status, propolis extract was given orally at a dose of 3g/saiga; the death rate among saiga calves stopped within 3-5 days, and an excellent effect was established. The treatment lasted 21 days, and blood and fecal samples were taken weekly for 7 weeks. Blood samples were taken from 20 healthy and 30 saiga calves that received propolis for hematological and blood serum for biochemical studies. Blood counts of livestock animals are shown in Table 3 and 4.

According to hematological parameters, propolis increased the total number of leukocytes, but there were no significant differences in other blood parameters. During the outbreak we documented the first identification of piroplasmosis in saiga calves at the Akboken nursery. Clinically, affected calves presented with depression, weakness, anorexia, tachycardia and tachypnea, frequent recumbency and lagging behind the herd; anemia with mucosal jaundice was common, and some animals showed limping, hemorrhagic conjunctivitis, and hemoglobinuria. Bacteriological examination at the Republican Veterinary Bacteriological Laboratory (Uralsk) excluded salmonellosis. Thin blood smears revealed large pear-shaped, rounded and amoeboid piroplasms within erythrocytes arranged at acute angles, confirming piroplasmosis. Necropsy of 10 calves showed a consistent pathoanatomic pattern dominated by acute serous-hemorrhagic enteritis with abundant mucus and petechiae, enlargement and edema of mesenteric lymph nodes with hemorrhages, congestive hepatopathy with uneven gray–yellow areas, pulmonary hyperemia, dilation of the right heart, small volumes of reddish–yellow ascitic and pleural fluid, and the presence of Moniezia (typically 1–2 per calf) (Fig. 1).

Hematological and biochemical profiles differed between healthy reference calves (n=20) and calves receiving propolis as part of the treatment protocol (n=30). Propolis-treated calves had markedLy higher leukocyte counts (30.3±1.43 vs 21.1±0.76 ×10⁹/L; mean difference 9.2; 95% CI 8.59–9.81; Welch's t≈29.5; P<0.001; Cohen's d=7.60) and elevated ESR (1.1±0.08 vs 0.7±0.04 mm/h; difference 0.40; 95% CI 0.37–0.43; t≈23.3; P<0.001; d=5.96). Hemoglobin was modestly higher in the treated group (220.5±5.76 vs 214.4±8.78g/L; difference 6.10; 95% CI 1.73–10.47; t≈2.74; P=0.010; d=0.86), whereas erythrocytes, neutrophils and lymphocyte proportion did not differ materially after multiplicity control (all adjusted P>0.05). In the serum chemistry, total protein and globulins were increased with treatment (80.3±2.4 vs 75.3±2.2g/L; difference 5.0; 95% CI 3.71–6.29; t≈7.59; P<0.001; d=2.15; and 59.05±4.6 vs 43.01±4.6g/L; difference 16.04; 95% CI 13.44–18.64; t≈12.08; P<0.001; d=3.49, respectively), while triglycerides did not differ significantly (99.76±22.3 vs 101.71±21.4 mg/dL; difference −1.95; 95% CI −14.26 to 10.36; t≈−0.31; P=0.76; d=−0.09). Full estimates with 95% CIs are provided in Table 3–4.

Following initiation of the azidin + hyperimmune serum + oral propolis protocol and quarantine with pen disinfection, mortality fell rapidLy, ceasing within 3–5 days, and no deaths were recorded after day 14 of quarantine. Kaplan–Meier survival with log-rank statistics is shown in Fig. 2.

DISCUSSION

The current study delineated specific pathoanatomic aspects and evaluated the therapeutic potential of bee propolis in saiga calves affected by piroplasmosis and monieziasis, conditions first recorded in saiga calves in this nursery setting. This investigation into disease manifestation and treatment efficacy in a critically endangered species like the saiga (*Saiga tatarica*)

Table 3: Hematological parameters of blood in saiga calves that received propolis extract

Indicators	Erythrocytes, (×1012/L)	Leukocytes (×109/L)	Hemoglobin/L	Erythrocyte sedimentation rate (ESR) mm/h	Basophils	Eosinophils (×109/L)	Neutrophils (×109/L)	Lymphocytes %	Monocytes (×109/L)
Healthy saiga calves n=20	7.6±0.41	21.1±0.76	214.4±8.78	0.7±0.04	0.09±0.04	4.3 ±0.22	3.6 ±0.32	33.8±1.20	8.3±0.43
Propolis-treated saiga calves n=30	7.8±0.49	30.3±1.43	220.5±5.76	1.1±0.08	0.10±0.04	5.2±0.31	3.7±0.20	34.1±2.11	9.5±1.87
Difference (95% CI)	+0.20 (−0.06 to +0.46)	+9.20 (+8.57 to+9.83)	+6.10 (+1.73 to +10.47)	+0.40 (+0.35 to +0.45)	+0.01 (−0.02 to +0.04)	+0.90 (+0.75 to +1.05)	+0.10 (−0.04 to +0.25)	+0.30 (−0.74 to +1.34)	+1.20 (+0.43 to +1.98)
Welch t (df)	1.56 (≈45.4)	29.53 (≈46.1)	2.74 (≈29.9)	23.33 (≈45.1)	0.83 (≈45.1)	12.52 (≈42.0)	1.41 (≈35.6)	0.62 (≈33.4)	3.19 (≈33.6)
P	0.125	<0.001	0.010	<0.001	0.410	<0.001	0.167	0.541	0.0018
P_adj (Holm)	0.502	<0.001	0.052	<0.001	0.902	<0.001	0.583	0.974	0.011
Cohen's d (Hedges' g)	0.43 (0.43)	7.91 (7.80)	0.86 (0.84)	5.96 (5.88)	0.25 (0.25)	3.61 (3.56)	0.39 (0.39)	0.17 (0.17)	0.92 (0.90)

Data are mean±SD; difference = Propolis − Healthy with 95% CI (Welch). Welch's two-sample t-test; Holm–Bonferroni correction applied within table; effect sizes as Cohen's d (Hedges' g).

Table 4: Biochemical parameters of blood in saiga calves treated with propolis extract

Indicators	Total protein, g/L	Albumins, g/L	Globulins, g/L	Protein ratio	Glucose, mg/dL	Cholesterol, mg/dL	Triglycerides, mg/dL	Fe, mg/dL
Healthy saiga calves n=20	75.3±2.2	52.7±1.2	43.01±4.6	1.6±0.1	77.9±4.3	149.2±13.3	101.71±21.4	93.2±5.86
Propolis-treated saiga calves n=30	80.3±2.4	53.7±1.4	59.05±4.6	1.9±0.8	78.1±4.3	150.9±12.1	99.76±22.3	93.4±5.88
Difference (95% CI)	+5.00 (+3.71 to+6.29)	+1.00 (+0.25 to+1.75)	+16.04 (+13.36 to+18.72)	+0.30 (−0.00 to+0.60)	+0.20 (−2.27 to+2.67)	+1.70 (−5.82 to+9.22)	−1.95 (−14.26 to+10.36)	+0.20 (−3.22 to +3.62)
Welch t (df)	7.59 (≈43.3)	2.70 (≈44.9)	12.08 (≈40.9)	1.99 (≈28.4)	0.16 (≈37.3)	0.46 (≈36.8)	−0.31 (≈42.0)	0.12 (≈41.0)
P	<0.001	0.0098	<0.001	0.051	0.873	0.649	0.758	0.907
P_adj (Holm)	<0.001	0.059	<0.001	0.256	1.000	1.000	1.000	1.000
Cohen's d (Hedges' g)	2.15 (2.12)	0.76 (0.74)	3.49 (3.43)	0.66 (0.64)	0.05 (0.05)	0.14 (0.14)	−0.09 (−0.09)	0.03 (0.03)

Data are mean±SD; difference = Propolis − Healthy with 95% CI (Welch). Welch's two-sample t-test; Holm–Bonferroni correction applied within table; effect sizes as Cohen's d (Hedges' g).

is particularly relevant, given the significant health challenges faced by saiga populations both in the wild and in captive breeding programs (Minoranskii & Dankov 2016 Cui et al. 2017; Liu et al. 2019; Mullineaux et al. 2024).

The pathoanatomic findings in saiga calves, including acute serous-hemorrhagic enteritis, presence of *Moniezia* in the intestine, congestive liver disease, acute congestive pulmonary hyperemia, and dilation of the right heart, align with the systemic impact typically associated with severe parasitic infections in young ruminants (Cabaret et al. 2019; Abdelhamid 2021; Awadin et al. 2021). The discovery of piroplasmosis in saiga calves, characterized by large, pear-shaped or amoeboid piroplasms within erythrocytes, represents a significant finding. Clinical signs observed—depression, weakness, anorexia, increased heart rate and respiration, anemia, jaundice of mucous membranes, limping, hemorrhagic conjunctivitis, and hemoglobinuria—are highly indicative of a severe hemoparasitic infection like piroplasmosis (Abdel-Hamied et al. 2020; Gurjar et al. 2023). These signs reflect the widespread destruction of erythrocytes and subsequent organ damage, including hepatic and renal compromise, as reported in other ruminant species and canids with piroplasmosis (El-Diasty et al. 2017; Zygner et al. 2021). The severe anemia (reduced erythrocytes and hemoglobin, anisocytosis, poikilocytosis, basophilic granularity) observed in the saiga calves is a hallmark of piroplasmosis, where parasites multiply within red blood cells, leading to their lysis (Bilwal et al. 2017).

Concurrent monieziasis, indicated by the presence of *Moniezia* and serous-hemorrhagic enteritis, likely exacerbated the clinical picture. While *Moniezia* is sometimes considered less pathogenic in adult animals, heavy infestations in young animals can lead to digestive disturbances, diarrhea, reduced weight gain, and general ill health, contributing to the overall morbidity and mortality (Cabaret et al. 2019; Abdelhamid 2021; Awadin et al. 2021). The observed damage to the intestinal mucosa and mesenteric lymph nodes points to a significant gastrointestinal insult, consistent with helminthic infections (Kondakova et al. 2020; Meryem et al. 2024). These pathological changes collectively suggest a severe disease complex, particularly dangerous for immunologically naive young saiga calves removed from their natural environment and subjected to nursery conditions, which can predispose them to disease outbreaks (Liu et al. 2019).

The study demonstrated a positive therapeutic effect of propolis extract, evidenced by a decreased death rate and improved hematological and biochemical parameters in treated saiga calves, including increased leukocytes, total protein, and globulin, and decreased triglycerides. This observed efficacy of propolis in ameliorating the disease burden is consistent with a growing body of literature highlighting its diverse pharmacological properties (Abu-Seida 2023).

Propolis, a natural resinous product collected by honeybees, possesses a complex chemical composition, primarily flavonoids, phenolic acids, and their esters, which are responsible for its broad spectrum of biological activities (Kabiloglu et al. 2023; Ahmadi et al. 2023). Its antiparasitic action against protozoa and helminths has been attributed to these compounds, which can interfere with parasite metabolism, growth, and reproduction (Abu-Seida 2023; Hegazı et al. 2023). For instance, propolis has been shown to reduce infection intensity in giardiasis and trypanosomiasis and demonstrates *in vitro* activity against various parasites, including *Trypanosoma cruzi*, *Trichomonas vaginalis*, *Fasciola gigantica* and *Cryptosporidium* (Abu-Seida 2023; Hegazı et al. 2023). The specific mechanism against piroplasms may involve direct parasiticidal effects or disruption of their erythrocytic lifecycle. Against *Moniezia*, propolis compounds could exert anthelmintic effects, potentially by disrupting the parasite's tegument or metabolic pathways, as suggested for other helminths (Soltan & Patra, 2020; de Melo Garcia et al. 2022).

Beyond direct antiparasitic effects, propolis is well-recognized for its potent immunomodulatory and anti-inflammatory (de Melo et al. 2020; Kabiloglu et al. 2023). The observed increase in leukocytes, total protein, and globulin in treated saiga calves strongly suggests an enhancement of the host's immune response. Propolis can stimulate various components of the immune system, including macrophage activation, lymphocyte proliferation, and the production of cytokines and antibodies (Buñay et al. 2018). This immunomodulatory effect is crucial in combating parasitic infections, as a robust immune response can help clear pathogens and mitigate disease severity. For example, propolis supplementation has been shown to increase immunoglobulin A levels in lambs and immunoglobulin G concentrations in calves, improving overall immune status and resistance to infections (Soltan & Patra 2020; Ahmadi et al. 2023).

Furthermore, the anti-inflammatory and antioxidant properties of propolis likely played a significant role in reducing disease pathology. Inflammation is a key feature of both piroplasmosis, due to erythrocyte lysis and host immune response, and monieziasis, due to intestinal irritation. Propolis can suppress inflammatory mediators and scavenge free radicals, thereby protecting tissues from oxidative damage induced by infection (de Melo et al. 2020; Buñay et al. 2021). This protective effect may have contributed to the observed improvements in organ function and overall health status. The decrease in triglycerides and the increase in total protein and globulin are indicative of improved metabolic function and immune status, which are essential for recovery from severe parasitic diseases (Januškevičius et al. 2018; Saichenko et al. 2021).

The findings highlight the significant vulnerability of saiga calves to parasitic diseases in captive settings and underscore the necessity for effective therapeutic and prophylactic strategies. The identification of piroplasmosis in saiga calves, coupled with the detailed pathological descriptions, contributes valuable knowledge to saiga health management. The demonstrated efficacy of propolis, likely mediated through its combined antiparasitic, immunomodulatory, and anti-inflammatory mechanisms, positions it as a promising natural alternative or adjunct therapy in veterinary medicine, particularly for sensitive young animals where conventional treatments may have adverse effects or limited efficacy due to resistance (Ávila et al. 2020; Nawaz et al. 2022; Shang et al. 2022). Further research could explore the precise molecular mechanisms

of propolis action against saiga-specific parasites and optimize its formulation and administration for wildlife conservation efforts.

Our findings show large, consistent effects on leukocytes and ESR and substantial increases in total protein and globulins (Cohen's d ≈ 2.1–7.6), aligning with the immunomodulatory properties attributed to propolis. The lack of material differences in erythrocytes and most cellular lineages after multiplicity control suggests a targeted response rather than broad hematologic toxicity. Clinically, the rapid cessation of mortality during quarantine complements the laboratory profile and supports the practical value of the azidin + hyperimmune serum + oral propolis protocol in nursery settings.

Conclusion

Using bee propolis for piroplasmosis and monieziasis of saiga calves in a nursery gave an excellent therapeutic effect. In the long term, propolis's variety of medicinal properties, availability of raw materials, simplicity of manufacturing preparations, and harmlessness lead to the interest of researchers and veterinary practitioners. Even with a mixed infection of the diseases of various systems and organs that we cited, in which propolis was used with positive results, we are convinced that propolis will rightfully take its place among the means of treating animal diseases of both infectious and non-infectious etiology.

DECLARATIONS

Conflict of Interest: The authors declare no competing interests.

Data Availability: Data available upon request from the corresponding author.

Ethics Statement: All animal procedures were approved by the Animal Care Committee at Zhangir Khan West Kazakhstan Agrarian and Technical University, Approval No. 012, dated 11-07-2025. Clinical observations and treatments were conducted as part of routine veterinary care at the Akboken nursery; no experimental infections were performed. Necropsies were performed on animals that died of natural causes during the outbreak. All procedures complied with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (1986, ETS No. 123) and the national Law of the Republic of Kazakhstan "On Responsible Treatment of Animals" (No. 97-VII LRK, 2021), as well as relevant institutional guidelines. Where applicable, reporting follows the ARRIVE recommendations.

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