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Study of the Distribution of Salmonella Infection in Poultry Farms in the Northern Region of Kazakhstan

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Abstract

Background and Aim. Avian salmonellosis represents a serious public health concern, as infected birds and contaminated poultry products serve as major sources of transmission. This study aimed to perform serological analyses and isolate *Salmonella enterica* from samples collected from poultry farms in northern Kazakhstan.

Materials and Methods. A total of 334 chicken serum samples and 285 biological and pathological samples were collected from poultry farms located in the Akmola, Kostanay, and Karaganda regions. The serum samples were analyzed using the indirect enzyme-linked immunosorbent assay (ELISA). To isolate *Salmonella* strains, the samples were cultured on differential diagnostic media, and resulting isolates were identified using biochemical and molecular genetic methods. The antimicrobial resistance of the isolated strains was determined by the disk diffusion method.

Results. Antibodies specific to Salmonella antigens were detected in serum samples from chickens at one poultry farm. Nine *Salmonella* isolates were recovered from pathological material. The isolates were identified as *Salmonella* enterica subsp. enterica, and the following serotypes were determined: *S. enteritidis, S. paratyphi, S. moscow, S. infantis,* and *S. mbandaka.* Antimicrobial susceptibility testing revealed sensitivity to amikacin, ceftriaxone, gentamicin, amoxicillin, and ciprofloxacin. However, the isolates exhibited multidrug resistance to several antibiotic classes, including rifamycins, macrolides, glycopeptides, cephalosporins, tetracyclines, lincosamides, aminoglycosides, nitrofurans, and penicillins.

Conclusion. The results of this study confirm the circulation of Salmonella at certain poultry farms in the northern region of Kazakhstan and provide insight into the serotypes of the strains and their antimicrobial resistance profiles. These findings may be used to support the development of effective antimicrobial therapy strategies in poultry farming.

Keywords: avian salmonellosis; antibody titers; isolates; identification; antimicrobial resistance.

Introduction

Poultry farming is currently one of the most economically profitable sectors of animal husbandry in the Republic of Kazakhstan, providing the country with valuable poultry products. However, infectious diseases, particularly salmonellosis, can hinder the development of this industry. Salmonellosis causes

significant damage to poultry production and is one of the most common causes of foodborne diseases in humans [1]. A high level of *Salmonella* infection in poultry has been reported in many countries around the world, including Kazakhstan [2, 3, 4, 5]. The main source of infection is infected birds, which excrete large quantities of the pathogen via feces and eggs. Transmission can occur via the digestive tract (feed, water), transovarially (to embryos), as well as by airborne and ocular routes. Adult birds often experience asymptomatic infection and act as carriers, with the pathogen primarily localized in the ovaries [6, 7]. The principal causative agent of chicken salmonellosis is *Salmonella pullorum/gallinarum*; however, chickens are frequently infected with *Salmonella* enteritidis and other serovars that do not cause clinical signs or mortality, complicating the assessment of the infection status of a poultry farm [8]. Reports suggest that salmonellosis outbreaks have demonstrated an increased frequency of Salmonella isolation from domestic poultry, including chickens [9]. According to WHO experts, the absence of clinical signs in birds and the difficulty in identifying the carrier birds pose a constant risk of environmental and food contamination [10]. The maintenance and spread of infection are also facilitated by birds from private household farms [11] and wild migratory birds. Changes in feeding behavior and migration patterns associated with climate change have increased interactions between wild bird and urban environments. Salmonella infection has been documented in approximately 140 wild bird species, highlighting their role in the long-distance spread of the pathogen [12].

According to statistical data, there has been a decline in the number of reported cases of salmonellosis in Kazakhstan [13]. However, studies conducted at individual poultry farms confirm the continued presence of *Salmonella* [5, 14, 15]. Additionally, research into antimicrobial resistance of Salmonella bacteria is currently highly relevant due to several factors. Firstly, *Salmonella* remains one of the major pathogens causing foodborne poisoning and infectious diseases in humans. Secondly, the continuous emergence of new *Salmonella* strains that are resistant to antimicrobial agents poses serious challenges for both veterinary medicine and public health, as standard treatment regimens may prove ineffective [16].

This study aimed to investigate samples collected from poultry farms in the Akmola, Kostanay, and Karaganda regions of the Republic of Kazakhstan, through isolation and identification of *Salmonella* strains and evaluating their microbial resistance profiles.

Materials and Methods

Ethical approvals

All research procedures were approved by the Ethics Committee of S. Seifullin Kazakh Agrotechnical Research University (Protocol No. 2, dated November 1, 2023) and were conducted in accordance with biosafety regulations and ethical standards for animal care and use.

The study material included blood serum collected from chickens of various ages, as well as other biological and pathological samples (feed, surface swabs from equipment and tools, as well as the organs of deceased birds and gastrointestinal contents). Samples were collected from poultry farms located in the Kostanay and Akmola regions of Kazakhstan. Sampling was conducted in accordance with the “Rules for Sampling and Biological Material Collection” (Order of the Ministry of Agriculture of the Republic of Kazakhstan, dated April 30, 2015, No. 7-1/393).

Biological material samples were placed in transport medium tubes (Swab, Tokyo, Japan). Blood samples from chickens and chicks were collected from the subclavian vein into Vacutainers and processed to obtain serum. Pathological material samples (parenchymal organs from deceased birds) were collected in sterile disposable containers and kept in an icebox. Sampling was supervised by the poultry farm veterinarians, and all the samples were delivered to the Kazakhstan-China Laboratory for Biosafety at Saken Seifullin Kazakh Agrotechnical Research University in strict compliance with the “cold chain” protocol.

The research protocols were approved by the Ethics Committee of Saken Seifullin Kazakh Agrotechnical Research University (Protocol No. 1, dated November 15, 2023). All procedures were conducted in accordance with biosafety regulations and the ethical standards for animal care and use.

For the serological examination, a commercial enzyme-linked immunosorbent assay (ELISA) kit (ID Screen Avian Salmonella Indirect, Montpellier, France) was used to quantitatively assess the presence of antibodies against Salmonella (groups B and D) in chicken and turkey sera.

Microbiological and bacteriological studies were performed in accordance with GOST 31659-2012: "Food Products. Methods for the Detection of *Salmonella* spp." [17]. The following culture media were used for pathogen isolation: buffered peptone water (LLC "Scientific and Production Center Biokompas-S," Russia), Rappaport-Vassiliadis soybean broth (LLC "Scientific and Production Center Biokompas-S," Russia), bismuth sulfite agar, Ploskirev medium, and Endo medium (Federal State Scientific Center for Applied Microbiology and Biotechnology, Russia).

Colony morphology was documented after incubation in a thermostat for 24-48 h. It is well known that on Ploskirev medium, *Salmonella* colonies appear as colorless, round colonies with black centers. On Endo medium, *Salmonella* form round, colorless or slightly pink colonies. On bismuth sulfite agar, Salmonella produces black colonies with a characteristic metallic sheen or greenish colonies encircled by a dark green border.

For rapid detection of Salmonella, Compact Dry SL indicator tests (Tokyo, Japan) were employed with a ready-to-use selective dry medium for *Salmonella*. Solid samples were pretreated by adding sterile peptone water in a 1:9 ratio and then homogenized for 1 min. For liquid samples, peptone water was added in the same 1:9 ratios. The liquid sample was filtered through a membrane filter and incubated in a thermostat for 20-24 h at 35-37 °C. Using a sterile pipette, 0.1 mL of the enriched sample was applied to the surface of the Compact Dry SL plate and incubated at 41-43 °C for 20-24 h. The results were evaluated visually according to the manufacturer's instructions.

The following materials were used for biochemical identification: Hiss medium with sucrose and mannitol (LLC "Scientific and Production Center Biokompas-S," Russia), Mueller-Hinton agar (HiMedia Laboratories, India), lead paper, oxalate paper, OF-test (Erba Lachema, Czech Republic), a reagent kit for the Voges-Proskauer reaction (Micro-VOGES-PROSKAUER-NICF, Russia), and reagents for the catalase and oxidase activity tests (Erba Lachema, Czech Republic). Biochemical identification included determining the ability to ferment glucose, lactose, mannitol, and sucrose; to produce hydrogen sulfide and indole; and the assessment of catalase and oxidase activity.

Isolates were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) with Bruker Realtime Classification software (Bruker Daltonics, Billerica, Massachusetts, USA), where a score of ≥2.0 was considered reliable.

Bacterial cultures were identified at species level by analyzing the nucleotide sequence of the 16S rRNA fragment and by intact cell mass spectrometry. The 16S rRNA fragment was amplified and further sequenced according to a previously described protocol [18].

Antibiotic susceptibility was determined using the disk diffusion method in accordance with MUK 4.2.1890-04: "Determination of Microorganism Sensitivity to Antibacterial Drugs". The method was conducted on Mueller–Hinton agar, and 33 antibiotic disks were used for the testing (NICF, Russia) [19]. Interpretation of the results was based on EUCAST criteria (versions 8.0, 2018 and 9.0, 2019). Statistical data analysis was performed using Microsoft Excel 2010, applying the student's t-test at a significance level of $\alpha<0.05$.

Results and Discussion

The samples selected for the study included feces, surface swabs from equipment and tools, blood, and parenchymal organs from deceased birds (liver, kidneys, pancreas) (Figure 1).

A B C

Figure 1 – Sampling of material from the poultry farm
(A– surface swabs from equipment, B – organ sampling, C – blood collection)

Screening for salmonellosis in chickens at a poultry farm in the Kostanay region using the ELISA revealed that among 100 birds of different ages, young chickens (56 and 153 days old) tested negative. Specific antibodies against *Salmonella* antigens were detected in 33 samples from adult birds (340 and 431 days old) (Figure 2).

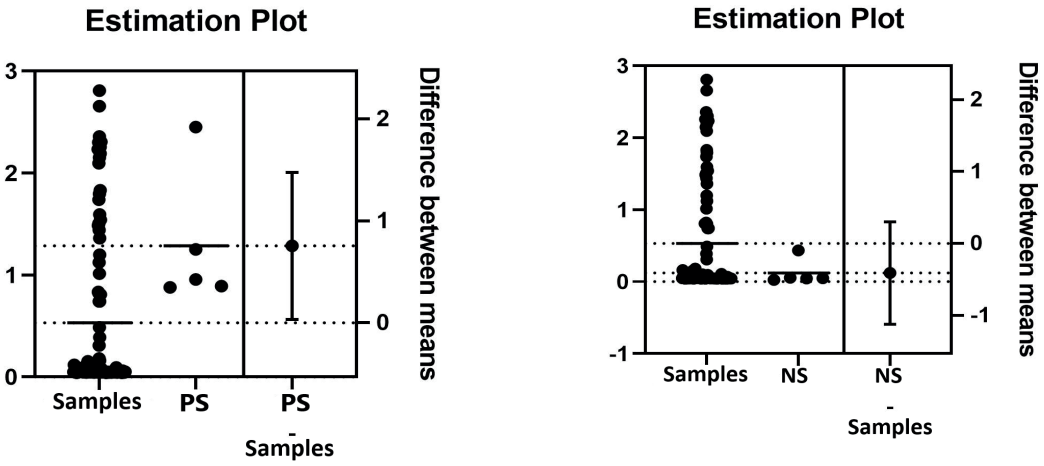

Figure 2 – Comparative analysis of tested chicken serum samples with sera from healthy and infected birds
(PS – positive samples, NS – negative samples)

As depicted in Figure 2, the optical density (OD) values of the ELISA results comprising sera from birds, as well as from positive and negative controls, displayed a fairly normal distribution. Consequently, it confirmed the homogeneity of the samples and allowed for the application of parametric statistical methods. Comparative analysis of the OD values between the studied groups was performed using the student's t-test. The use of this test allowed for an objective determination of the significance of the observed differences and confirmed that the experimental data accurately reflected the actual relationship between the clinical condition of the birds and the level of specific antibodies detected by the ELISA method.

Similarly, serum samples collected from two different poultry houses at poultry farms "M" and "A" in the Akmola region, as well as serum samples from poultry farm "K" in the Karaganda region, were examined. No specific antibodies against the *Salmonella* genus were detected in the bird serum samples. The analysis of the ELISA results for the blood serum samples from various poultry farms is presented in Table 1.

Table 1 – Results of blood serum sample testing from different poultry farms

No.	Poultry farm	Number of samples	Bird age (days)	Positive	Negative
1	Kostanay region. Poultry farm "A"	12	56	0	12
		30	153	0	30
		28	340	14	14
		30	431	19	11
2	Akmola region. Poultry farm "M"	72	Adult bird	0	72
3	Akmola region. Poultry farm "A"	126	(broilers) 50 days	0	126
4	Karaganda region Poultry farm "K"	36	Adult bird	0	36
Total		334		33	301

Investigation of the serum samples from chickens at several poultry farms revealed a high seroprevalence rate in adult birds at a poultry farm in the Kostanay region. Specific antibodies to Salmonella antigens were detected in 17.55% of the tested samples. No antibodies specific to Salmonella bacteria were detected at any of the other poultry farms.

Simultaneously, microbiological studies were conducted to detect Salmonella bacteria in the samples collected from the poultry farms (Figure 3). For rapid detection, the material samples were applied to the chromogenic test substrates in a Compact Dry SL *Salmonella* kit (Tokyo, Japan).

Figure 3 – Results of sample testing for the presence of *Salmonella* on Compact Dry plates (1, 2 – negative results, 3 – positive result, 4 – control)

The analysis results showed that the presumptive presence of Salmonella was detected in 12 samples collected from a poultry farm in the Kostanay region. After inoculation onto differential diagnostic media and incubation for 24 and 48 h, the colonies were examined, and bacterial colonies typical of the genus *Salmonella* were selected (Figure 4).

A B C

Figure 4 – Growth characteristics of *Salmonella* bacteria on Ploskirev medium (A), Endo medium (B), and bismuth sulfite agar (C)

The samples from poultry farm “A” in the Kostanay region and poultry farm “M” in the Akmola region showed growth of typical *Salmonella* colonies, which were further confirmed using Gram staining and microscopic examination. The microscopic examination confirmed Gram-negative rod-shaped bacteria measuring 3-7 μm in length and 0.30.7 μm in width.

Because birds serve as natural reservoirs for *Salmonella* and *E. coli,* they are commonly found on poultry farms, and production conditions may facilitate their spread, leading to the contamination of poultry products. On simple media such as nutrient agar and nutrient broth, *Salmonella* and *E. coli* can exhibit similar growth patterns, making it difficult to distinguish them based solely on colony morphology. To differentiate these microorganisms, biochemical tests are warranted. Table 2 presents the comparative analysis of the biochemical properties of *Salmonella* and *Escherichia* bacteria.

Table 2 – Comparison of the biochemical properties of bacteria of the genera *Salmonella* and *Escherichia*

A genus of bacteria belonging to the family Enterobacteriaceae	Products		Fermentation of sugars				catalase	oxidase
	Hydrogen sulfide	Indole	lactose	glucose	Sucrose	mannitol		
Salmonella	+	−	−	+	−	+	+	−
Escherichia	−	+	+	+	−	+	+	−

Biochemical identification revealed that the isolated cultures could produce hydrogen sulfide, ferment glucose and mannitol, and exhibit a positive catalase reaction: indicating characteristic features of the genus *Salmonella*.

To confirm the affiliation of the isolates with *Salmonella* bacteria, identification was performed using MALDI-TOF MS (Figure 5).

Organism names which are in blue and underlined are linked to the matching hint table below.

Analyte Name	Analyte ID	Organism (best match)	Score Value	Organism (second best match)	Score Value
A8 (+) (B)	A8	Salmonella sp	1.96	Salmonella sp	1.941
A9 (++) (C)	A9	Salmonella sp	2.143	Salmonella sp	2.141
B8 (-) (C)	B8	no peaks found	< 0	no peaks found	< 0
B9 (++) (A)	B9	Niallia circulans	2.255	Niallia circulans	2.037
C8 (-) (C)	C8	not reliable identification	1.697	not reliable identification	1.681
C9 (+) (B)	C9	Niallia circulans	1.889	Niallia circulans	1.826
D8 (++) (A)	D8	Salmonella sp	2.152	Salmonella sp	2.14
D9 (+) (B)	D9	Niallia circulans	1.84	Niallia circulans	1.727
E8 (++) (A)	E8	Salmonella sp	2.272	Salmonella sp	2.264
E9 (+) (B)	E9	Niallia circulans	1.822	Niallia circulans	1.76
F8 (-) (C)	F8	not reliable identification	1.543	not reliable identification	1.542
F9 (++) (A)	F9	Niallia circulans	2.051	Niallia circulans	2.034
G8 (++) (A)	G8	Salmonella sp	2.265	Salmonella sp	2.225
G9 (+) (B)	G9	Niallia circulans	1.926	Niallia circulans	1.766
H8 (++) (C)	H8	Salmonella sp	2.237	Salmonella sp	2.225
H9 (++) (A)	H9	Niallia circulans	2.004	Niallia circulans	1.927

Figure 5 – Results of bacterial isolate identification using mass spectrometry

As a result of ion detection and comparison of their mass, structure, and abundance with the reference database integrated into the mass spectrometer, nine cases demonstrated their affiliation with the bacteria of genus *Salmonella*. These data are consistent with the results obtained using Compact Dry SL *Salmonella* kit. Genotyping of the isolated strains determined their taxonomy as *Salmonella enterica,* subspecies *enterica* (subspecies I) and identification of the serotype of each isolate (Table 3).

Table 3 – Characteristics of the isolated bacterial strains

Isolate number	№ 973-2	№ 1259	№ 1800	№1141	№ 969-2	№ 966	№ 477-M1	№ 478-M2	№ 479-M3
Predicted identification	*Salmonella enterica subspecies enterica (subspecies I)*	*Salmonella enterica subspecies enterica (subspecies I)*	*Salmonella enterica subspecies enterica (subspecies I)*	*Salmonella enterica subspecies enterica (subspecies I)*	*Salmonella enterica subspecies enterica (subspecies I)*	*Salmonella enterica subspecies enterica (subspecies I)*	*Salmonella enterica subspecies enterica (subspecies I)*	*Salmonella enterica subspecies enterica (subspecies I)*	*Salmonella enterica subspecies enterica (subspecies I)*
Serotype	*S.enteritidis*	*S.paratyphy C*	*S.enteritidis*	*S.moscow*	*S.enteritidis*	*S.enteritidis*	*S. infantis*	*S.infantis*	*S.mbandaka*

As shown in Table 3, all isolates belonged to Salmonella enterica subspecies enterica (subspecies I) but represented different serotypes.

After studying the biochemical properties of the bacterial isolates and confirming their taxonomic classification as *Salmonella*, antibiotic susceptibility was tested via the disk diffusion method. The results were evaluated after 24 h and found antimicrobial resistance of the nine *Salmonella* isolates obtained from poultry farm samples in the Republic of Kazakhstan (Figure 6).

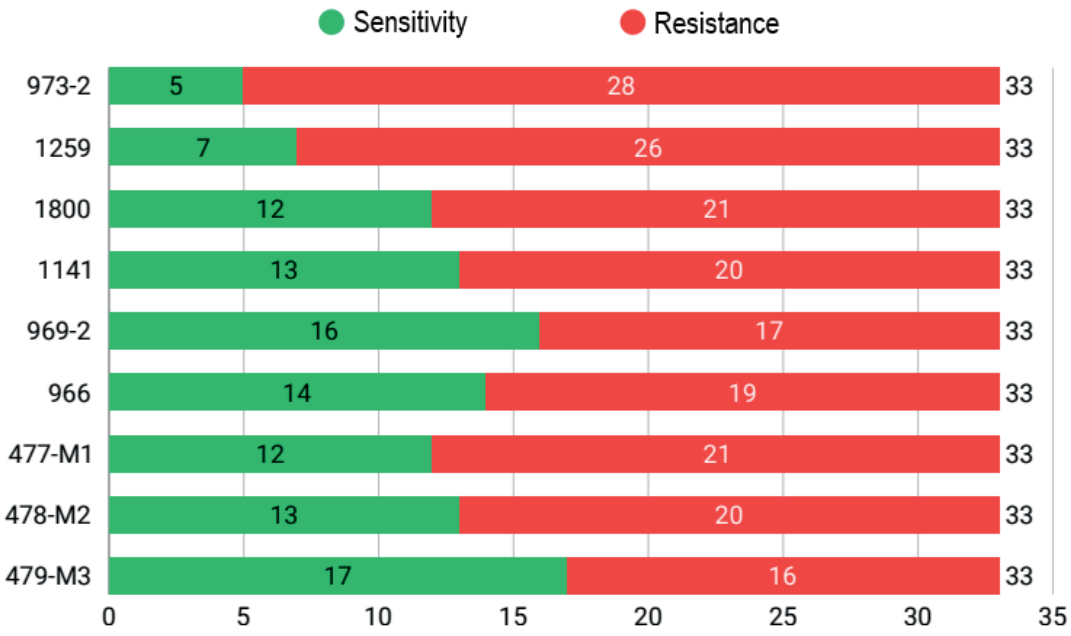

Figure 6 – Determination of the antimicrobial susceptibility of isolated Salmonella strains

The figure illustrates the sensitivity and resistance profiles of nine *Salmonella* strains to 33 tested antimicrobial agents. The Y-axis represents the strain identifiers, while the X-axis indicates the number of antimicrobial agents for which efficacy was assessed. Green bars indicate the number of drugs to which each strain was sensitive, whereas the red bars represent the number of drugs to which resistance was detected. As shown in the chart, the highest level of susceptibility was observed in strain 479-M3 (17 out of 33 agents), whereas the lowest susceptibility was recorded in strain 973-2 (5 out of 33 agents).

Analysis of the antimicrobial susceptibility results revealed cases of multidrug resistance among the isolated *Salmonella* strains. The isolates demonstrated sensitivity to several antibiotics, including amikacin, ceftriaxone, gentamicin, amoxicillin, and ciprofloxacin. However, resistance was recorded to multiple classes of antibiotics, including rifamycins (rifampicin), macrolides (azithromycin, erythromycin), glycopeptides (vancomycin), cephalosporins (cephadroxil, cefuroxime, cefaclor, cephalothin, cefazolin), tetracyclines (doxycycline), lincosamides (clindamycin), aminoglycosides (kanamycin, streptomycin), nitrofurans (nitrofurantoin), and penicillins (piperacillin).

Differences in susceptibility among isolates of different serotypes were noteworthy. *Salmonella infantis* exhibited a particularly high level of resistance, including resistance to antibiotics that were effective against most other serovars. In contrast, *Salmonella mbandaka* showed sensitivity to a wide range of antimicrobial agents, including amoxicillin/clavulanic acid and co-trimoxazole.

The increasing resistance of bacterial strains to antimicrobial agents often necessitates the use of higher drug dosages. Therefore, based on the results of antimicrobial susceptibility testing, it is recommended that antibiotics to which the studied strains demonstrated the highest sensitivity be preferentially used in practical applications.

Salmonella is a major cause of foodborne disease outbreaks in many countries. In the European Union, the number of reported cases of salmonellosis reached nearly 88,000 in 2019, but decreased to 57,000 in 2020, representing the lowest level recorded since 2007 [20]. In Asian countries, as well as in North and South America and Africa, non-typhoidal salmonellosis remains one of the leading causes of foodborne zoonoses [21, 22, 23].

In veterinary laboratories, the diagnosis of salmonellosis is performed using bacteriological methods along with modern tests, such as ELISA and PCR [24]. Although ELISA has long been used to detect specific antibodies in poultry serum, the method continues to be refined. For example, Ma et al. (2018) demonstrated that the outer membrane protein, PagC, is present in all common Salmonella serovars, with a sequence similarity of 98%, and used purified recombinant PagC protein to test chicken serum samples. The ELISA results using rPagC, compared with agglutination tests, showed 80.6% agreement with agglutination tests when analyzing 252 clinical chicken serum samples, suggesting that indirect ELISA based on PagC antibodies may serve as a convenient and novel diagnostic method for salmonellosis [25].

Other researchers have also employed recombinant proteins to develop new variants of ELISA. For example, Gao et al. (2023) utilized recombinant SifA protein for the early diagnosis of salmonellosis in poultry [26]. Additionally, an indirect ELISA based on recombinant IpaJ protein has been proposed as a novel method for the specific detection of S. pullorum infection, which may facilitate the eradication of this pathogen in poultry farming [27]. In another study, *Yeh* et al. used an automated capillary ELISA to quantitatively determine antibodies in chicken serum against recombinant proteins of *Salmonella enterica* serotype Heidelberg [28].

Nevertheless, bacteriological methods remain the primary approach for isolating and characterizing the pathogen. For instance, in Romania, 112 isolates were obtained from raw poultry meat between 2011 and 2021 [29]. That study determined the serovar characteristics of *Salmonella*, their susceptibility to antimicrobial agents, and presence of antimicrobial resistance genes. The most common serotypes were *Salmonella enterica* serovars Enteritidis and Typhimurium (56% and 25%, respectively), and most isolates were resistant to at least three antimicrobial agents, indicating the presence of multidrug-resistant *Salmonella* serovars in poultry meat products [29].

According to *Drauch* et al. (2022), *Salmonella infantis* is currently the most prevalent serovar in broilers within the European Union. Previous studies have shown that *Salmonella* is detected less frequently in the feces of laying hens than in that of broilers; therefore, fast-growing broilers pose the greatest risk of transmitting the infection to humans [30].

In Iraq, 300 samples of poultry products and human feces were examined for the presence of *Salmonella enterica.* The pathogen was detected in 8.66% of poultry samples and in 4.6% of human samples. Genetic mutations associated with alterations in molecular characteristics and development of multidrug resistance were identified in *S. enterica isolates* [31].

The resistance of *Salmonella* strains represents a serious challenge in controlling avian salmonellosis, and numerous studies have reported multidrug resistance (MDR) among *Salmonella* isolates. For example, in Ethiopia, 32.7% of strains tested in one study showed resistance to streptomycin (75%) and ampicillin (59.4%) [32]. In Sudan, an antimicrobial resistance study of 64 Salmonella isolates revealed frequent resistance to ampicillin and cephalexin [33]. In Vietnam, a meta-analysis of publications from 2013 to 2020 showed that bacterial isolates, including Salmonella, obtained from pigs and poultry exhibited multidrug resistance, emphasizing the need to restrict antibiotic use in farm animals [34].

Our findings are consistent with previously published studies on avian salmonellosis in Kazakhstan, where *Salmonella* spp. isolates were recovered from retail outlets and poultry farms in the northern regions of the country. Most of the isolates were identified as *S. enteritidis*, with 64.3% demonstrating resistance to three or more classes of antimicrobial agents, indicating the widespread occurrence of multidrug resistance among poultry-associated *Salmonella* strains [35, 36].

Taken together, these results indicate the widespread distribution of *Salmonella enterica* and the presence of multidrug resistance, which is likely attributable to the indiscriminate use of antimicrobial agents in farm animals [37, 33].

Conclusion

Examination of chicken serum samples from several poultry farms in northern Kazakhstan using the ELISA method revealed the presence of antibodies specific to the *Salmonella* pathogen. Subsequently, nine bacterial isolates, presumptively identified as *Salmonella* were recovered, from samples collected at these farms, primarily from parenchymatous organs of deceased birds. Biochemical identification demonstrated that the isolates were capable of producing hydrogen sulfide, fermenting glucose and mannitol, and exhibiting a positive catalase reaction, features characteristic of bacteria belonging to the genus *Salmonella.*

Identification using MALDI-MS and genotyping confirmed that the bacteria belonged to Salmonella enterica subsp. enterica and revealed the following serotypes: *S. enteritidis, S. paratyphi, S. moscow, S. infantis,* and *S. mbandaka.* Investigation of antimicrobial resistance in the isolated strains revealed their sensitivity to several antibiotics (amikacin, ceftriaxone, gentamicin, amoxicillin, ciprofloxacin), but their resistance to multiple classes of antibiotics, including rifamycins, macrolides, glycopeptides, cephalosporins, tetracyclines, lincosamides, aminoglycosides, nitrofurans, and penicillins, confirming multidrug resistance among the isolates.

These findings are of concern, as the isolates were recovered from poultry farm environments that may serve as sources of contamination for poultry products. Therefore, careful selection of effective antimicrobial agents by veterinary specialists is essential. Differences in antimicrobial susceptibility among *Salmonella* strains at the serotype level should also be considered when assessing the effectiveness of antibiotic therapy. Furthermore, comprehensive surveillance studies across all regions of the country are needed to identify circulating *Salmonella* strains and characterize their resistance profiles.

Authors' Contribution

SB, ZhA and GD: designed and supervised the study, conducted a comprehensive literature search, analyzed the collected data and drafted the manuscript. DSh and EB: performed statistical analysis and contributed to drafting the manuscript. AS: conducted the final revision and proofreading. All authors have read, reviewed, and approved the final manuscript.

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UDC 619:616.5-002:636.7 **Research article**

Diagnostic Approaches and Comparative Effectiveness of Modern Methods for Treating Atopic Dermatitis in Dogs

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Abstract

Background and Aim. Atopic dermatitis (AD) is a common allergic skin disorder in dogs and is often difficult to diagnose due to overlapping clinical signs with other dermatological conditions. This study aimed to assess the prevalence, clinical and epidemiological features, diagnostic approaches, and comparative effectiveness of modern anti-pruritic therapies in a clinical setting.

Materials and Methods. The study was conducted between 2022 and 2024 at the "VetAlliance" veterinary clinic in Kostanay, Kazakhstan. Out of 482 dogs presenting with dermatological complaints, 53 dogs with chronic pruritus (>6 weeks), meeting ≥5 Favrot criteria, and free from parasitic, infectious, or endocrine diseases were included. Diagnostic evaluation included history-taking via standardized questionnaires, clinical examination, CADLI and PVAS scoring, Favrot criteria assessment, cytology, and serological testing for allergen-specific IgE in 18 dogs. Dogs were allocated to three therapeutic groups: monoclonal antibodies (Lokivetmab/Cytopoint), JAK inhibitor (Oclacitinib/Apoquel), and prednisolone. Clinical signs were assessed on days 0, 14, 28, and 56. Statistical analysis included descriptive statistics, $\chi 2$ tests, ANOVA or Kruskal–Wallis tests, and odds ratios, with significance set at $p < 0.05$.

Results. AD was confirmed in 53 of 482 dogs (~11%), predominantly in females (58.5%) aged 3-4 years. French Bulldogs and West Highland White Terriers were the most frequently affected purebred dogs. Secondary infections with *Staphylococcus* spp. and *Malassezia* spp. were detected in 43.4% and 15.1% of cases respectively. All three treatments significantly reduced pruritus and skin lesions. Lokivetmab and prednisolone provided more rapid initial improvement, while comparable clinical efficacy among all treatments was observed by day 30.

Conclusion. Canine AD is a multifactorial allergic disease requiring a comprehensive diagnostic approach, including history, clinical evaluation, PVAS/CADLI scoring, Favrot criteria assessment, and cytological analysis. Treatment should be individualized according to disease severity and patient characteristics, utilizing modern anti–pruritic agents–monoclonal antibodies, JAK inhibitors, or prednisolone–combined with regular monitoring, risk factor management, and owner education to ensure long-term disease control.

Keywords: atopic dermatitis; dogs; itching; diagnosis; treatment.

Introduction

In clinical veterinary practice, dermatological diseases in dogs are common and often challenging to diagnose due to the similarity of clinical signs across different conditions. Accurate diagnosis requires not only an understanding of disease pathogenesis but also the appropriate selection of diagnostic methods for effective differentiation. One such disease, whose clinical signs may be similar to other skin diseases, is atopic dermatitis in dogs. Atopic dermatitis is a common skin disease that is diagnosed in an average of 10-15% of dogs [1, 2]. It is a genetically predisposed inflammatory skin disease accompanied by itching [3]. The pathogenesis and clinical signs of the disease are associated with a type I hypersensitivity reaction to environmental allergens and skin barrier dysfunction [4, 5].

The diagnosis of atopic dermatitis is complicated by the fact that other skin diseases in dogs can have symptoms similar to AD. Therefore, if atopic dermatitis is suspected, it is necessary to carefully collect the patient's medical history, conduct a clinical examination, and differentiate it from other skin diseases with similar manifestations [6]. Allergy tests (intradermal and serological tests) are often performed to determine which allergens should be avoided and which should be included in allergen-specific immunotherapy [1, 7, 8].

To reduce itching and clinical manifestations, each patient is individually selected for supportive therapy, which may include glucocorticoids (systemic or topical), cyclosporine, lokivetmab, and oclacitinib. Antihistamines, supplements with essential fatty acids (systemic or topical), therapeutic baths, and other methods are also used [9].

There is still debate about which diagnostic method is the most accurate and which treatment regimen to choose so that the animal can lead a full life. Therefore, the topic of atopic dermatitis remains relevant worldwide, and further scientific research is needed to successfully control the skin condition of patients suffering from atopic dermatitis.

Research objective: to comprehensively assess the prevalence, clinical and epidemiological characteristics, and diagnostic approaches to identifying atopic dermatitis in dogs in a veterinary clinic, as well as to evaluate the effectiveness of various anti-pruritic treatment regimens used in clinical practice.

Materials and Methods

Ethical approval

This study was conducted in strict accordance with generally accepted standards and rules for the treatment of experimental and clinical animals, as reflected in current international recommendations on bioethics and humane treatment. All procedures involving dogs were performed only after obtaining permission from the local animal ethics committee (similar to IACUC), which reviewed the study protocol and confirmed its compliance with requirements for minimizing stress and discomfort in animals. Biological material was collected by veterinary specialists and only with the prior informed consent of the animal owners. The owners were provided with full information about the purposes, methods and potential risks of the procedure, after which they voluntarily confirmed their pets' participation. All actions were carried out in accordance with humane treatment standards and measures aimed at ensuring the safety and well-being of the dogs. The information obtained during the study was used exclusively for research purposes. The confidentiality of the animals and their owners was fully preserved: personal information was not disclosed or used outside the scope of this project.

The study was carried out from 2022 to 2024 at the “VetAlliance” veterinary clinic (Kostanay). During this period, 482 dogs with various dermatological problems were admitted to the clinic, of which n=53 animals with clinical symptoms of atopic dermatitis were selected:

- chronic itching lasting more than 6 weeks;
- compliance with ≥5 Favrot criteria;
- primary itching in the absence of parasitic diseases (scotch test, cytology);
- exclusion of infectious and endocrine diseases mimicking AD;
- absence of systemic GCS <30 days and oclacitinib/cytopoint <60 days prior to the study.

To clarify the anamnestic data, each owner filled out a standardized questionnaire containing information about: breed, age, and sex; seasonality of exacerbations; duration of itching; type of feeding; housing conditions; preventive treatment for parasites; last bathing; concomitant diseases; previously used antipruritic therapy.

Based on the questionnaire data, the animals were divided into groups according to the following criteria:

- Breed: French Bulldog, West Highland White Terrier, Yorkshire Terrier, American Akita, Bichon Frise, other breeds, mixed breeds.
- Age: 1–2, 2–3, 3–4, 4–6, 6–8, 8–10 years.
- Sex: males and females.
- Living conditions: indoors/outdoors or mainly outdoors.
- Type of food: premium commercial food, economy-class food, homemade food, mixed type. These data were used to analyze risk factors.

The clinical examination of dogs was performed by visual inspection under standard lighting conditions, which included a general examination of the animal from the front, rear, and side, and a detailed examination on the table: muzzle, periorbital area, ears, neck, chest and pelvic limbs, back, abdomen, groin and armpit areas, and tail.

The following methods were used to assess the severity of lesions: CADLI (Canine Atopic Dermatitis Lesion Index) [10]; PVAS scale (subjective assessment of itching severity by the owner) [11, 12]; Favrot criteria (to confirm the diagnosis of AD) [13]. The following were recorded: erythema, excoriations, erosions, alopecia, lichenification, hyperpigmentation, prevalence, and localization.

Cytology was performed on all dogs to detect secondary bacterial and fungal infections. Samples were taken from the skin by applying a microscope slide to the affected areas and from the ear canal using a sterile swab. The samples obtained were stained with Diff-Quik and then examined under a microscope at 100×magnification (immersion system). The following were identified: cocci, rods, neutrophils, degenerative forms, yeast-like fungi (Malassezia).

For comparison of therapeutic regimens, dogs were divided into three therapeutic groups: Group 1 received Lokivetmab (Cytopoint) - monoclonal antibodies; Group 2 received Oclacitinib (Apoquel); Group 3 received Prednisolone (Prednicortone).

The effectiveness of the drugs was assessed at control points: day 0, day 14, day 28, and day 56.

The following were measured: PVAS dynamics; CADLI dynamics; rate of clinical improvement; presence/absence of side effects.

Statistical data processing was performed using the following methods: descriptive statistics (M±SD, %, n); χ^2 – comparison of categorical characteristics (breed, housing conditions); ANOVA / Kruskal–Wallis – comparison of CADLI and PVAS indicators between therapy groups; – OR (Odds Ratio) - assessment of risk factors for AD development; significance threshold $p < 0.05$. 2.9.

Results and Discussion

Between 2022 and 2024, the owners of 482 dogs sought treatment for skin problems at the VetAlliance veterinary clinic (Kostanay). After comprehensive diagnosis, atopic dermatitis was confirmed in 53 animals (Figure 1). The remaining cases were distributed as follows: parasitic diseases – 116 dogs, food allergies – 154, skin diseases of other origins – 159.

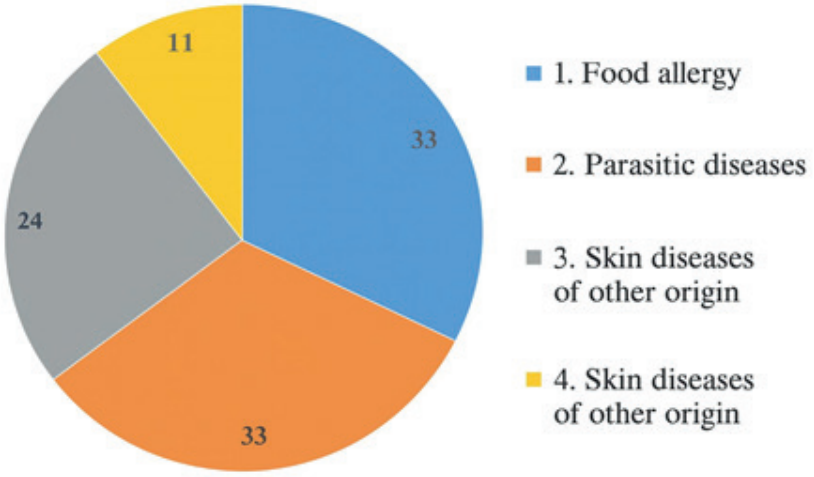

Figure 1 – Distribution of causes of dermatoses in dogs

Sex analysis showed that among animals with atopic dermatitis, females predominated – 31 (58.5%), males – 22 (41.5%) (Figure 2).

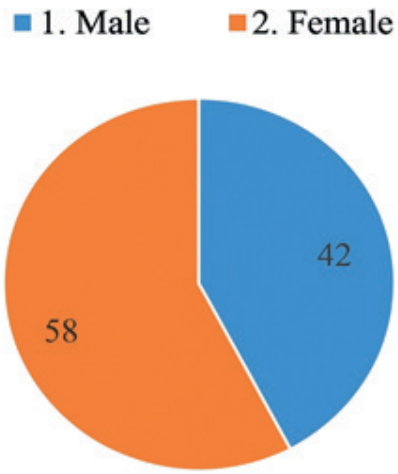

Figure 2 – Incidence of atopic dermatitis in dogs by gender

In the study group of 53 dogs, there were 51 purebreds and 2 mixed breeds. Among purebred animals, the largest numbers were French Bulldogs (17%), West Highland White Terriers (15.1%), Yorkshire Terriers and American Akitas (7.5% each), and Bichon Frises (5.7%). Other breeds accounted for 43.4% of the animals studied (1-2 dogs per breed) (Figure 3). Statistical analysis did not reveal a significant association between breed and AD incidence ($p>0.05$).

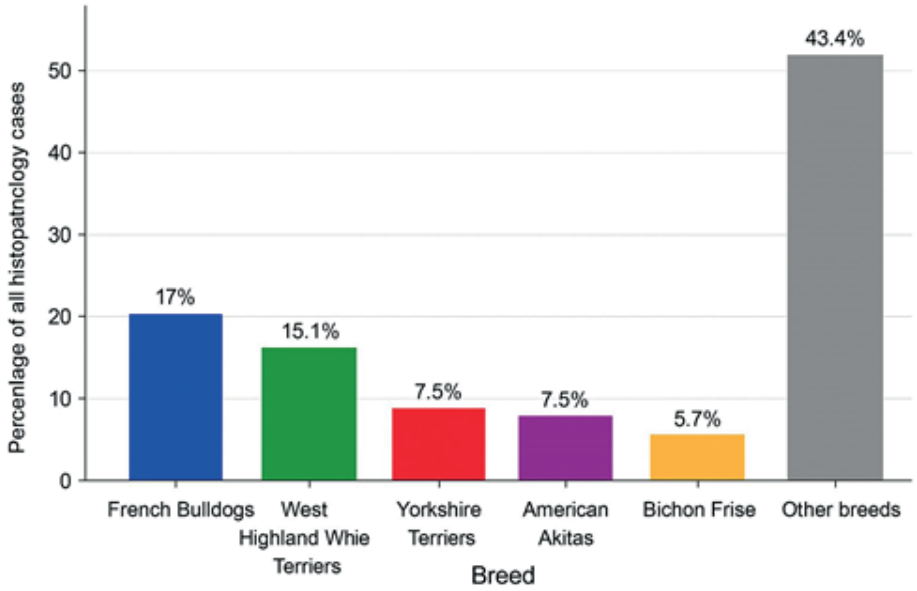

Figure 3 – Incidence of atopic dermatitis in dogs of different breeds

The age of the dogs studied ranged from 1 to 10 years. The dogs were divided into six age groups. The highest number of AD cases was recorded in dogs aged 3-4 years (n=16), 2-3 years (n=13), and 4-6 years (n=8). Animals aged 1-2 years (n=6), 6-8 years (n=5), and 8-10 years (n=5) were less frequently affected (Figure 4).

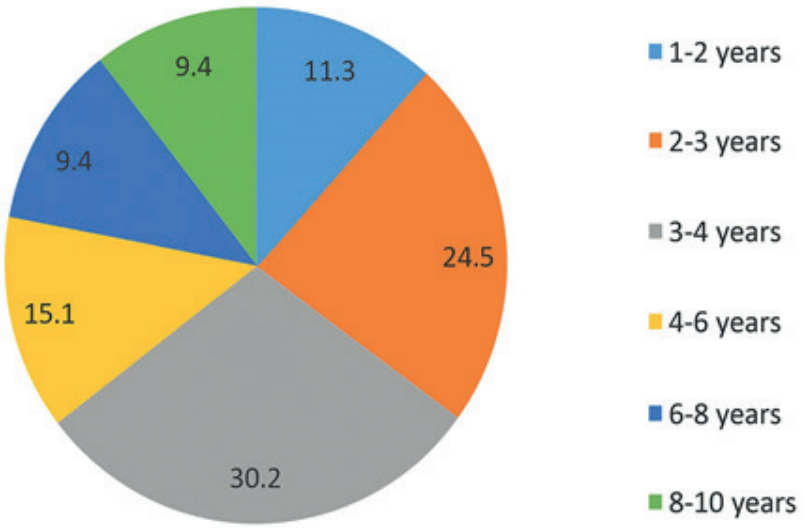

Figure 4 – Incidence of atopic dermatitis in dogs by age

An analysis of breed and age revealed the following:

- All mixed breeds (n=2) were between 1 and 2 years old (100%).
- French Bulldogs were more frequently affected at 4-6 years old (33.3%), less often at 3-4 years old (22.2%) and 8-10 years old (12.5%).
- West Highland White Terriers: 2-3 years old (50%), 3-4 years old (12.5%).
- Yorkshire Terriers : 3-4 years old (50%).
- American Akitas : 4-6 years old (50%), 8-10 years (25%).
- Bichon Frises: 2-3 years (66.7%).
- Dogs of other breeds: mainly 3-4 years (43.5%) (Figure 5).

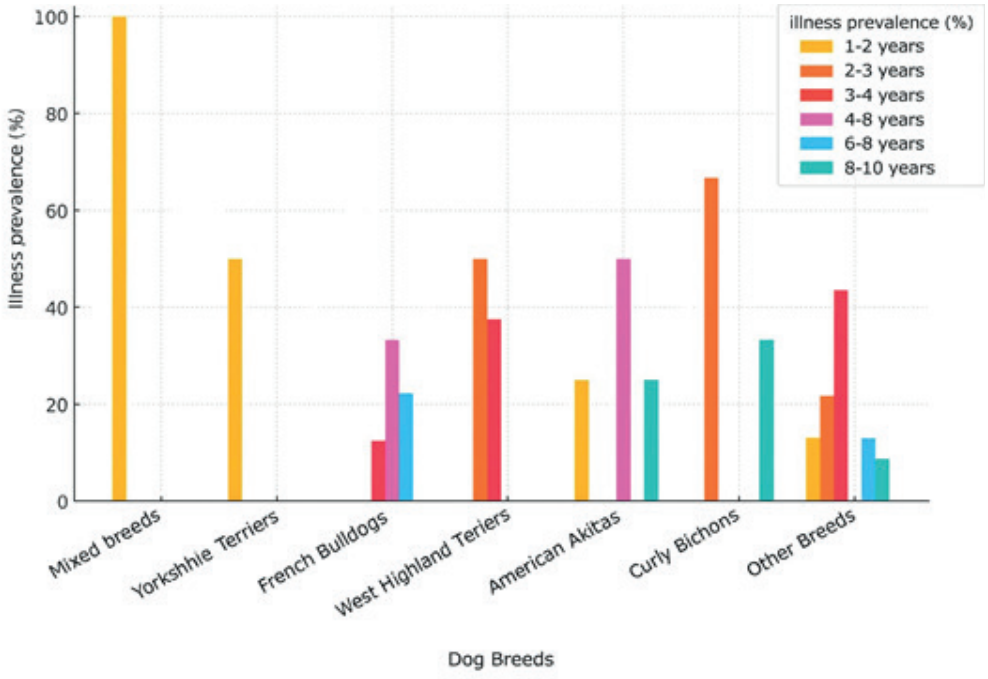

Figure 5 – Distribution of atopic dermatitis incidence by breed and age of dogs

Indoor and outdoor dogs were most frequently affected by atopic dermatitis (n=46). A small proportion of dogs (n=7) with atopic dermatitis were kept outdoors only (Figure 6).

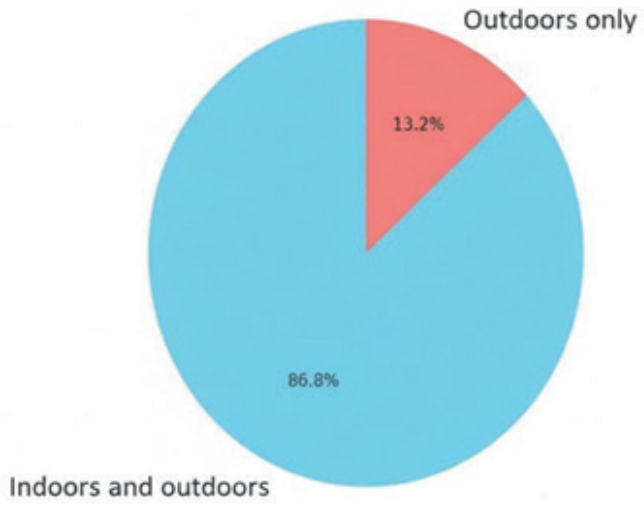

Figure 6 – Conditions for keeping dogs suffering from atopic dermatitis

The coats of French Bulldogs (100%), West Highland White Terriers (100%), Yorkshire Terriers (100%), and Bichon Frisés (100%) were kept both indoors and outdoors (Figure 7). Half of the American Akita (50%) and mixed breed (50%) dogs were kept outdoors only. Most other dog breeds (82.6%) were kept both indoors and outdoors, and only a small proportion (17.4%) were kept exclusively outdoors.

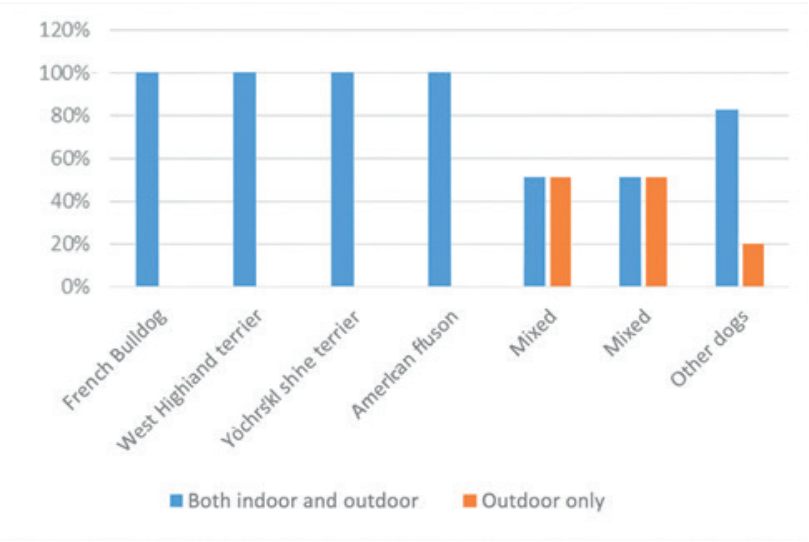

Figure 7 – Conditions for keeping dogs of different breeds

The results of the owners' responses to the questionnaire showed that most dogs (n=22) are fed a mixed diet (dry commercial food, canned food, treats, human food, etc.) (Figure 8). The smallest number of dogs (n=7) were fed homemade food (cooked or raw chicken, beef, pork, etc.). Fifteen dogs were fed commercial (premium) food, and nine dogs were fed commercial (economy class) food.

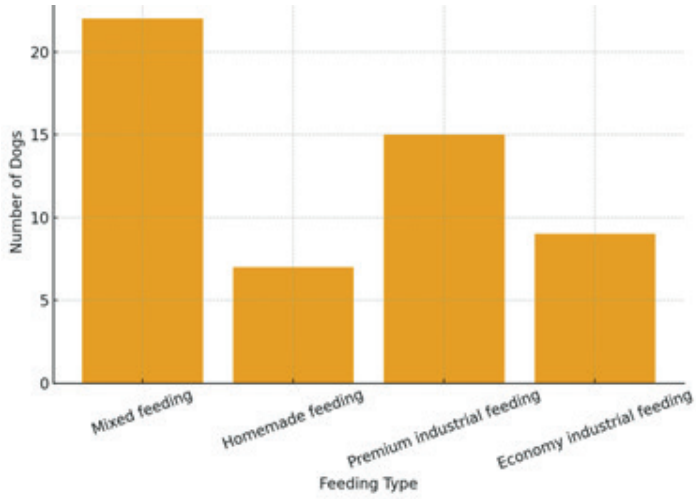

Figure 8 – Types of feeding for dogs suffering from atopic dermatitis

Thus, 100% of Yorkshire Terriers and their crossbreeds were fed a mixed diet. Half of the American Akita dogs were fed commercial (premium) and homemade food. Most French Bulldogs (44.4%) were fed a mixed diet, 33.3% were fed commercial (premium) food, and 22.2% were fed commercial (economy class) food. Most (50%) West Highland White Terriers were fed commercial (economy) food, while 12.5% were fed commercial (premium) food and 12.5% were fed homemade food. 33.3% of Bichon Frises were fed commercial (premium) food, commercial (economy class) food, and mixed food. Most other breeds of dogs (39.1%) were fed mixed food, and the smallest proportion (8.7%) were fed industrial (economy class) food. 34.8% of dogs of other breeds were fed industrial (premium) food, and 17.4% were fed homemade food.

When assessing the seasonality of clinical signs, it was found that in most dogs (n=34), clinical signs worsened in the spring and summer (Figure 9). The smallest number of dogs (n=6) had acute clinical signs throughout all seasons of the year, and the autumn-winter period was characterised by exacerbation in 13 of the dogs studied.

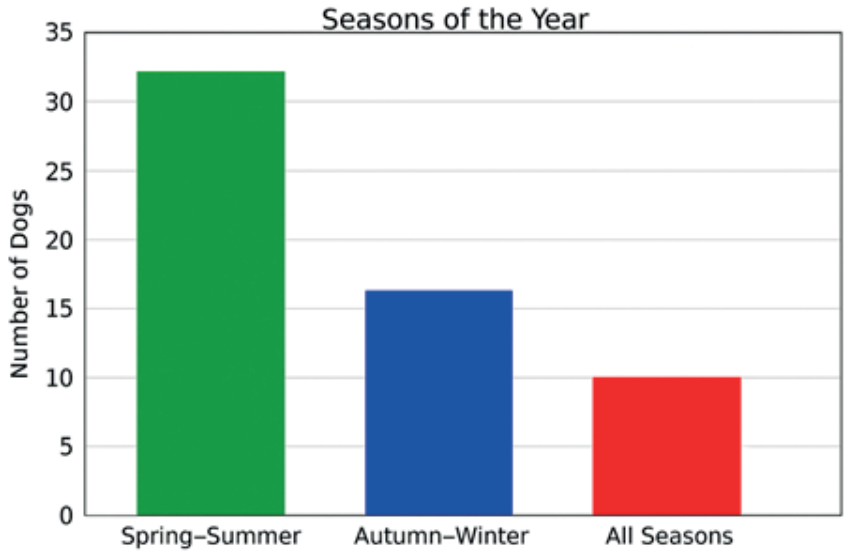

Figure 9 – Periods of exacerbation of clinical signs in dogs suffering from atopic dermatitis

Medical history collection, assessment of clinical sign severity (using the CADLI scale), application of Favrot criteria, and cytological examination of the skin or ears were performed for all dogs in the study (n=53). Serological testing for specific IgE to allergens was performed in 18 dogs in the study (Figure 10).

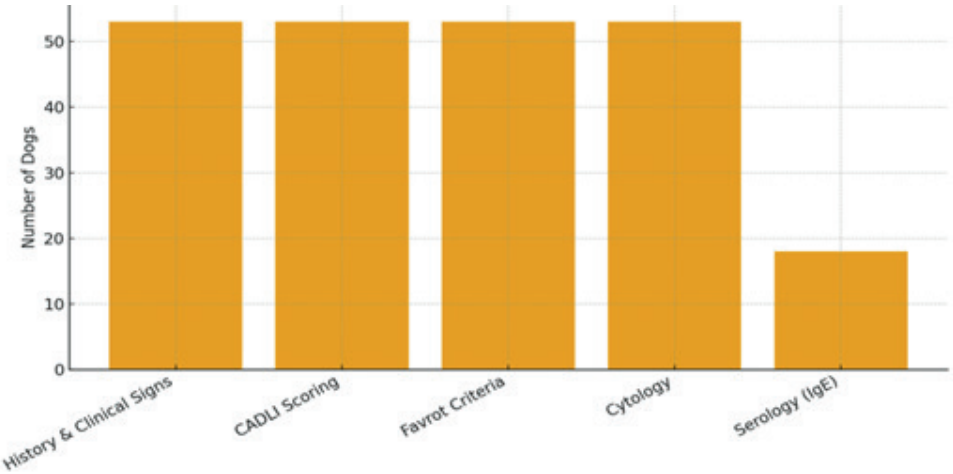

Figure 10 – Methods for diagnosing atopic dermatitis

As an adjunct to the diagnosis of atopic dermatitis in dogs, the Favrot criteria were applied to all subjects. For most subjects (n=29), 5 signs coincided. 6 signs coincided in 19 dogs, and 7 signs coincided in 5 dogs (Figure 11).

Cytological examination of the ears and skin was performed on all dogs examined (n=53) to determine the presence of secondary infection. The results of the study showed that 43.4% of dogs suffered from a secondary infection with *Staphylococcus* spp., 15.1% of dogs suffered from an infection with *Malassezia* spp., and 41.5% of the dogs examined had no pathogens detected in the cytological samples (Figure 11).

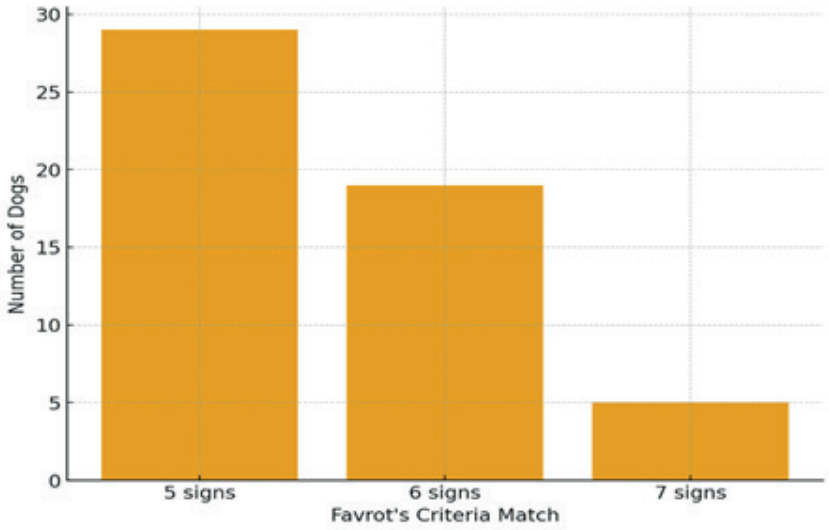

Figure 11 – Distribution of dogs by number of Favrot criteria met

When assessing the localization of clinical signs on the CADLI scale, it was found that the largest number of dogs examined had lesions on the front limbs (96.2%) and ears (71.7%) (Figure 12, 13, 14). The least frequent lesions were located in the groin (20.8%) and head (24.5%). More than half of the dogs studied (56.6%) had lesions on their hind limbs. Lesions were also found in dogs (n=53) in the ventral chest area (39.6%), ventral abdomen (35.8%) and armpits (32.1%).

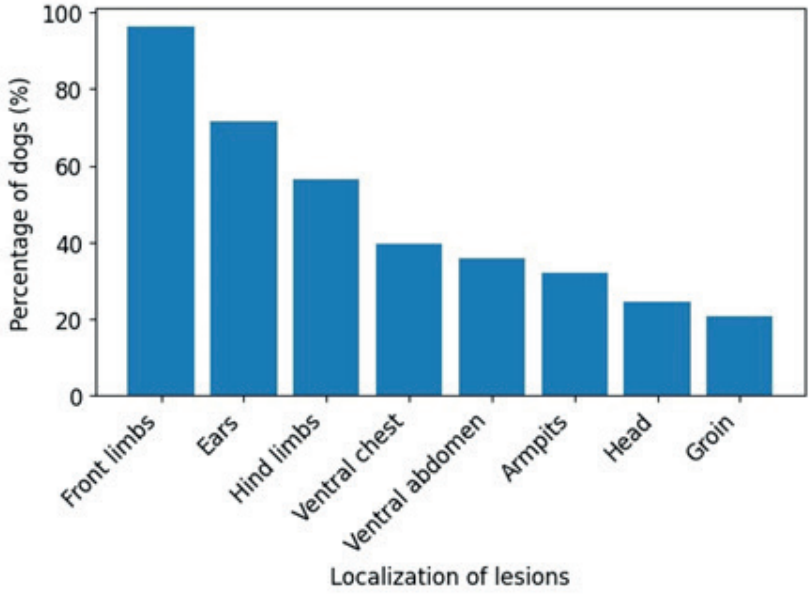

Figure 12 – CADLI scale assessment of clinical signs

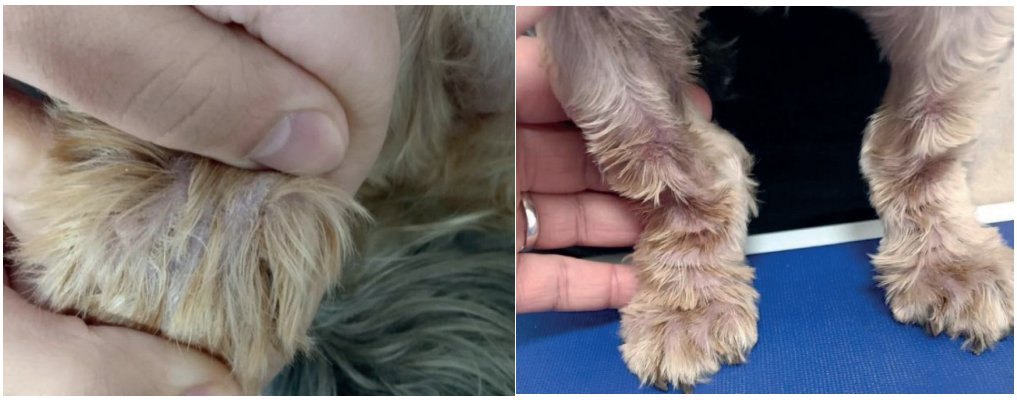

Figure 13 – Left: photograph of the front limbs taken during the initial visit; right: photograph taken during a follow-up visit 30 days later. The lesions are located on the front limbs

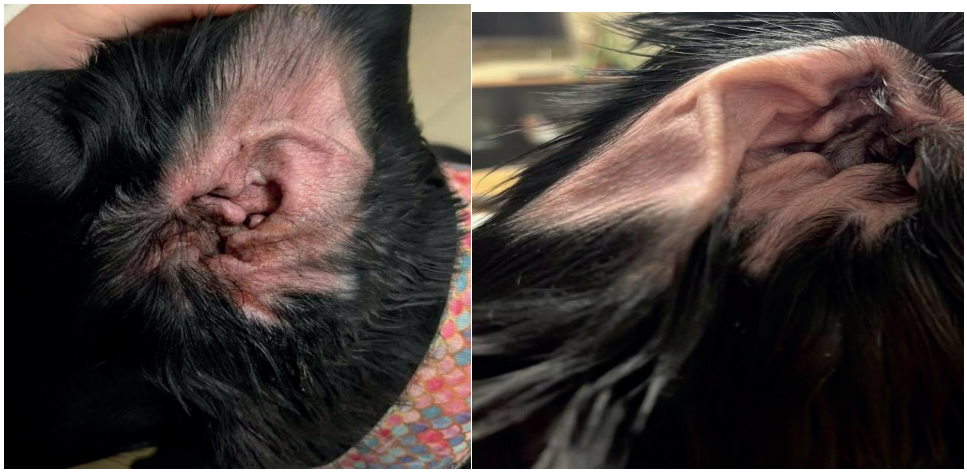

Figure 14 – Left: appearance of the ear during the initial visit; right:
during the follow-up visit 30 days later.
Lesions are localised in the ears

The CADLI scale was used to assess the severity of clinical signs. After analysing the data (Table 1), it was found that the average total score of the dogs examined during the first visit was 17.38±8.25 points. The minimum score was 6 and the maximum was 36.

When analysing the data between individual groups, the following was revealed: In group I, the average score was 21.65±9.66. The minimum value was 6, and the maximum was 36. In group II, the average score was 18.17±7.27. The minimum value was 7, and the maximum was 32. In group III, the average score was 12.56±4.89. The minimum value was 6, and the maximum was 21. Statistical analysis showed a significant correlation between the average scores in groups I, II, and III ($p<0.001$).

Table 1 – Severity of clinical signs at the initial visit according to the CADLI scale (in points)

Group	Average score (M±SD)	Minimum score	Maximum score
All dogs	17.38±8.25	6	36
I	21.65±9.66	6	36
II	18.17±7.27	7	32
III	12.56±4.89	6	21

After assessing the effectiveness of the therapies used at a follow-up examination 10 days later, it was found that clinical signs decreased by 55.2% in group I, 44.1% in group II, and 58.5% in group III (Figure 15). Statistical analysis showed that clinical signs decreased significantly between groups I and II, as well as between groups II and III ($p < 0.05$).

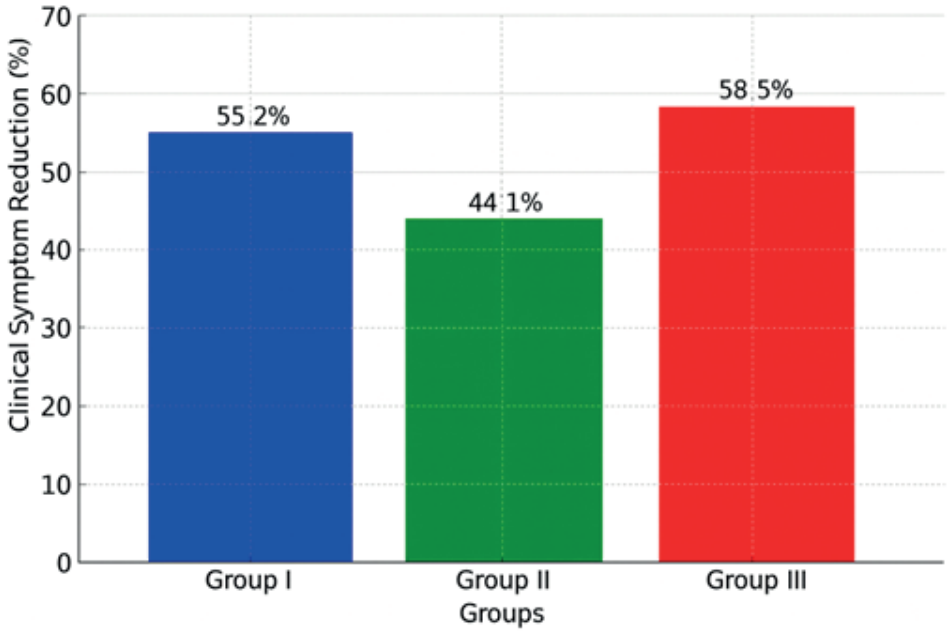

Figure 15 – Change in clinical signs during a follow-up visit 10 days later

Twenty days after the initial visit, it was found that clinical signs decreased by 73.8% in group I, by 62.7% in group II, and by 86.2% in group III (Figure 16). Statistical analysis showed that clinical signs decreased significantly between groups II and III ($p<0.05$).

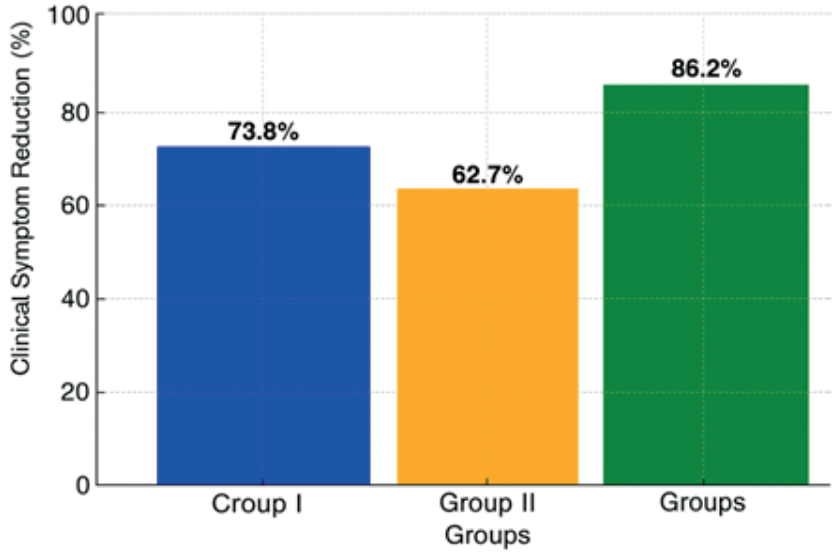

Figure 16 – Change in clinical signs at the follow-up visit after 20 days

At the follow-up examination after 30 days, assessing the reduction in clinical signs, it was found that the clinical signs in group I decreased by 82.2%, in group II by 71.4%, and in group III by 95.1% (Figure 17). Statistical analysis showed that clinical signs decreased significantly between groups I and III, as well as between groups II and III ($p<0.05$).

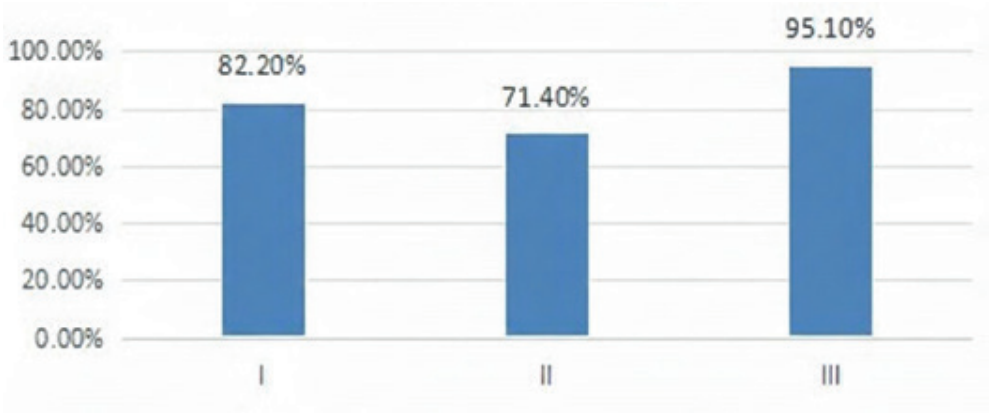

Figure 17 – Change in clinical signs at the 30-day follow-up visit

Thus, atopic dermatitis was more commonly detected in females, mainly aged 3-4 years. Among purebred dogs, French Bulldogs and West Highland White Terriers were at the highest risk, while mixed-breed dogs developed the disease at an earlier age (1-2 years).

The results of the present study confirm that atopic dermatitis (AD) remains one of the most frequently diagnosed allergic dermatoses in dogs, accounting for a substantial proportion of dermatological cases among clinical veterinary patients. In our cohort, AD was confirmed in 53 out of 482 dogs (≈11%), which corresponds with published data on the prevalence of Canine Atopic Dermatitis (CAD), estimated at up to 10% in the general dog population and associated with increasing risk factors such as environmental pollution, indoor housing, and the genetic predisposition of various breeds [31.6% according to international AD market data] [14].

Consistent with the literature, clinical signs of CAD most commonly manifest in early adulthood: the majority of patients show pruritus and skin lesions by 1-3 years of age, although disease onset can occur up to 7 years or later depending on individual characteristics and breed susceptibility [14, 15]. In our study, the highest number of cases was observed in the age groups 3-4 years (30%), 2-3 years (24.5%), and 4-6 years (15.1%), which aligns with literature reports of peak clinical manifestation of CAD in young to middle-aged dogs [15].

Gender differences in CAD prevalence in dogs are inconsistently described in the literature, and most authors do not find a consistent association between sex and CAD risk [16]. In our sample, females predominated (58.5%); however, this does not contradict existing studies, considering population variability and the influence of other risk factors such as breed predisposition and housing conditions.

Among the risk factors included in our survey, most affected dogs were kept both indoors and outdoors. Such combined housing conditions may increase exposure to a broad spectrum of airborne allergens (pollen, dust mites, fungal spores), which is recognized in the literature as a factor exacerbating the clinical manifestations of CAD [14].

Breed-specific risk factors are also supported by several studies. For instance, recent publications frequently report French Bulldogs among breeds with high susceptibility to allergic dermatitis, along with other companion-type breeds [16, 17]. In our cohort, the most common purebred cases were French Bulldogs and West Highland White Terriers, which corresponds with previous data on breed-related susceptibility.

Cytological examination confirmed a high prevalence of secondary infections with *Staphylococcus* spp. and *Malassezia* spp., which is consistent with known pathogenic mechanisms of CAD, where skin barrier dysfunction creates favorable conditions for bacterial and fungal overgrowth [18, 19]. Recent publications emphasize the importance of identifying and managing secondary infections in the comprehensive treatment of atopic dermatitis, as these infections independently exacerbate pruritus and inflammation and may reduce the effectiveness of basic therapy [19].

Regarding therapeutic approaches, our results show that all three compared regimens Lokivetmab (Cytopoint), Oclacitinib (Apoquel), and Prednisolone lead to significant reduction in clinical signs of CAD according to PVAS and CADLI scales. This is consistent with current clinical data: JAK

inhibitors, such as oclacitinib, rapidly reduce pruritus and can be comparable in short-term efficacy to glucocorticoids [17]. Monoclonal antibodies, such as lokivetmab, provide effective and sustained reduction of IL-31–mediated pruritus with a convenient administration schedule every 4-8 weeks, as confirmed by international therapeutic reviews and contemporary veterinary market analyses [15].

It is important to note that prednisolone remains an effective option for controlling acute symptoms; however, prolonged use requires caution due to the risk of side effects and potential relapses after discontinuation, as emphasized in domestic veterinary guidelines [17].

Thus, the obtained results support the current understanding of CAD as a multifactorial disease requiring a comprehensive approach to diagnosis and therapy. Comparing our data with recent literature highlights the importance of considering age and breed factors and confirms the clinical efficacy of modern anti-pruritic and anti-inflammatory drugs in veterinary dermatology practice.

Conclusion

Canine atopic dermatitis is a prevalent allergic skin disease, most frequently affecting female dogs aged 3-4 years and breeds such as French Bulldogs and West Highland White Terriers. Accurate diagnosis requires a comprehensive approach combining history taking, clinical examination, PVAS and CADLI scoring, Favrot criteria assessment, and cytological evaluation to identify secondary infections. Therapeutic strategies should be individualized according to disease severity and patient characteristics, employing modern anti-pruritic agents such as monoclonal antibodies (Cytopoint), JAK inhibitors (Apoquel), or prednisolone, along with regular clinical monitoring, risk factor management, and owner education to achieve long-term disease control.

Author Contributions

ZA: Writing – original draft, Investigation, Software, Data curation, Formal Analysis, Project administration, Visualization. AZh: Writing – original draft, Writing – review & editing, Investigation, Methodology, Formal Analysis, Visualization. RR: Writing – review & editing, Methodology; Writing – original draft, review & editing, Conceptualization, Data curation, Supervision. KS: Writing – original draft, Data curation, Formal Analysis. MKh: Writing – review & editing, Methodology. DZh: Writing – original draft, Investigation, Visualization.

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UDC 616-002.5 **Research article**

Immunoactive Proteins of *Mycobacterium bovis*: From Molecular Mechanisms to Biomarkers and Vaccines

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Abstract

Background and Aim. *Mycobacterium bovis* is a zoonotic member of the *Mycobacterium tuberculosis* complex and the causative agent of bovine tuberculosis, posing significant challenges to veterinary health, wildlife management and public health. Despite high genomic similarity to *M. tuberculosis*, M. bovis exhibits distinct host–pathogen interaction strategies that influence immune recognition, persistence and disease progression.

This review aims to systematize and critically synthesize current knowledge on immunoactive proteins of *M. bovis* and to elucidate their roles in modulation of innate and adaptive immune responses, with particular emphasis on mechanisms of immune evasion, diagnostic relevance and vaccine potential.

Materials and Methods. This narrative review synthesizes peer-reviewed literature retrieved from international scientific databases. Studies addressing molecular genetics, proteomics, tran-scriptomics, host immune signaling pathways, and experimental infection models of *M. bovis* were critically evaluated. Special attention was given to proteins involved in phagocytosis, autophagy, Toll-like receptor (TLR) signaling and cytokine regulation, as well as to comparative studies with M. tuberculosis.

Results. The analysis indicates that *M. bovis* actively modulates macrophage defense mechanisms through multiple molecular axes, including inhibition of phagosome–lysosome fusion, selective activation of PINK1–Parkin-dependent mitophagy and suppression of xenophagy. Key immunoactive proteins, such as ESAT-6, CFP-10, MPB70/80/83, PE/PPE proteins and lipoproteins, play central roles in shaping both inflammatory and regulatory immune responses. Activation of cytosolic DNA sensors, particularly Interferon-Inducible Protein 204 (IFI204), and downstream IFN-β signaling is more pronounced in *M. bovis* infection and contributes to species-specific immune responses. Differential expression and secretion of these proteins underpin their value as diagnostic biomarkers and potential vaccine antigens.

Conclusion. Immunoactive proteins of *M. bovis* form a complex molecular network that enables immune modulation, intracellular persistence and host adaptation. Their functional significance extends beyond virulence, positioning them as promising targets for Differentiating Infected from Vaccinated

Animals (DIVA)-compatible diagnostics and second-generation vaccines. An integrated understanding of these mechanisms is essential for improving control strategies for bovine tubercu-losis and reducing the risk of zoonotic transmission.

Keywords: Ag85; diagnostics; ESX-1; immunomodulation; MPB70/80/83; PE/PPE.

Introduction

M. bovis causes tuberculosis in domestic and wild animals and can also infect humans, making it a dangerous zoonotic infection. It is a member of a group of bacteria grouped under the *M. tuberculosis* complex and is well adapted to infect different animals. This poses significant challenges to both agriculture and public health [1, 2]. Its genetic material is almost identical to that *of M. tuberculosis* DNA (more than 99.95%). However, the *M. bovis* genome lacks certain regions and contains single nucleotide substitutions, as well as additional genes absent from the *M. tuberculosis* core genome, contributing to functional variability and immune adaptation. [3, 4, 5]. The most notable differences between *M. bovis* and other bacteria in the complex concern the genes that are responsible for the structure of the cell wall and for the proteins secreted to the outside. It is at these sites that the greatest diversity is found [6, 7, 8, 9].

Immunoactive proteins play a key role in how M. bovis interacts with the immune system and causes disease. Particularly important are those proteins that the bacterium secretes through the specialized ESX-1 system, such as ESAT-6 and CFP-10. Also important are lipoproteins and antigens from the MPB70, MPB80 and MPB83 families. These proteins help the bacteria to survive inside the body and influence the development of infection [10, 11, 12].

Due to the work of ESX-1 system proteins, when the immune system cells such as macrophages and dendritic cells are infected, the body reacts more strongly by activating bacterial DNA recognition, increasing the production of the protective agent IFN-β and triggering autophagy [13, 14, 15]. However, *M. bovis* is able to defend itself against these reactions by inducing mitophagy (destruction of mitochondria), which helps it to avoid other cellular defense mechanisms such as xe-nophagy. This allows the bacterium to persist longer inside cells and develop new, more dangerous forms [16, 17, 18, 19]. It has also been found that differences between strains (e.g., amino acid sub-stitution in the ESAT-6 protein) can affect the strength of the innate immune response and even the outcome of the disease [20].

Because of its molecular features, *M. bovis* poses a serious problem: the infection is difficult to detect, treat and develop an effective vaccine against it. MPB70, MPB80 and MPB83 are strongly immunogenic and serve as valuable biomarkers for detecting infection and distinguishing infected from vaccinated hosts. [21, 22, 23, 24]. On the other hand, even among different strains of this bacterium, large differences are found, making it difficult to find stable and accurate targets for diagnosis and treatment. Therefore, to develop effective solutions, comprehensive methods are needed, including the study of genes, proteins, metabolites, and other biological components simultaneously [25, 26, 27, 28].

Against the backdrop of globalization and increasing bacterial resistance to drugs, the im-portance of *M. bovis* in veterinary medicine continues to grow. In some regions, zoonotic tuberculo-sis is still widespread. This creates serious economic problems for farmers, hinders infection control in wildlife and increases the risk of human infection. To effectively manage this infection, a deeper understanding of how *M. bovis* immunoactive proteins influence disease progression, how they interact with the immune system, and how they can be used to identify infected and immunized animals is needed.

This review discusses the major *M. bovis* proteins, their role in protecting the bacterium from immune responses, in disease development, and their application in diagnosis, infection control, and the development of new vaccines [29, 30].

Materials and Methods

Literature search strategy. The present study was conducted as a structured narrative review of published data focusing on immunoactive proteins of *M. bovis* and their role in host–pathogen interactions. A comprehensive literature search was performed using international scientific databases, including PubMed, Scopus, Web of Science, and Google Scholar. The literature review covered publications from 1998 to 2025.

Search queries included combinations of the following keywords and their derivatives: *M. bovis,* immunoactive proteins, ESX-1, ESAT-6, CFP-10, MPB70, MPB83, Ag85 complex, PE/PPE proteins,

lipoproteins, autophagy, mitophagy, xenophagy, IFI204, TLR signaling, biomarkers, vaccines, and zoonotic tuberculosis.

Inclusion and exclusion criteria. Peer-reviewed original research articles, reviews, and experimental studies written in English were included if they addressed:

(i) molecular and cellular mechanisms of *M. bovis* interaction with the host immune system;

(ii) functional characterization of immunoactive proteins;

(iii) comparative analyses between *M. bovis* and *M. tuberculosis*;

(iv) diagnostic, prognostic or vaccine-related applications of *M. bovis* antigens.

Publications focusing exclusively on non-tuberculous mycobacteria or lacking immunological or molecular relevance were excluded.

Data extraction and analysis. Relevant data were extracted manually and organized according to protein families and functional pathways, including ESX-1–associated secreted proteins, Ag85 complex, MPB70/80/83 antigens, PE/PPE proteins, and lipoproteins. Particular attention was paid to experimental evidence describing modulation of phagocytosis, autophagy, mitophagy, Toll-like receptor signaling, and cytokine responses.

Comparative transcriptomic, proteomic and secretomic studies were analyzed to identify species-specific expression patterns and regulatory mechanisms distinguishing *M. bovis* from other members of the *M. tuberculosis* complex. Emphasis was placed on studies using macrophage infection models, animal models, and multi-omics approaches.

Synthesis and interpretation. The collected data were qualitatively synthesized to identify recurring molecular mechanisms, convergent immune evasion strategies, and consistent diagnostic or vaccine-related targets. No meta-analysis or quantitative statistical evaluation was performed, due to the heterogeneity of experimental designs across the included studies. The final interpretation integrates molecular, immunological and applied aspects to provide a comprehensive framework for understanding the role of immunoactive proteins in *M. bovis* pathogenesis, diagnosis and control.

Results and Discussion

The results of the literature analysis are presented below in a thematic manner, integrating molecular, cellular and immunological data to elucidate the biological basis of *M. bovis* pathogenicity and its interaction with the host immune system. The discussion begins with fundamental aspects of *M. bovis* biology and pathogenesis as a framework for understanding subsequent immune evasion mechanisms.

Biology and pathogenesis of M. bovis

M. bovis is one of the major members of the *M. tuberculosis* complex (MTBC). Although its genetic material is almost identical to the genome of *M. tuberculosis* (more than 99.95% similarity), these bacteria differ in which hosts they infect, how they develop the disease, and how they cope with the body's immune defenses [1]. The main difference between *M. bovis* is not the presence of unique genes, but rather how exactly the genes responsible for virulence and interaction with the immune system work. Whole genome studies show that the biggest differences between the bacteria relate to the structure of the cell wall and the proteins that the bacterium secretes outward. It is these proteins that determine how the bacterium will interact with the animal's immune system [7].

Pangenomic analysis (the study of all genes of different strains) showed that *M. bovis* has both a common set (approximately 2700 common genes) and a huge additional set of over 3800 genes that are only found in individual strains. This suggests that the *M. bovis* genome remains “open”, meaning it can change, but it does not have a strictly unique set of proteins common to all strains. When scientists grouped these genes by function, it turned out that many of them are partic-ularly associated with the regulation of other genes, fat metabolism, and secretory systems, especial-ly the so-called ESX (or Type VII) systems [2]. In *M. bovis*, for example, the ESX-1 system works more actively during infection of bovine cells than in *M. tuberculosis*, causing a stronger innate im-munity response [31]. Comparison of gene and protein activities in *M. bovis* and *M. tuberculosis* showed that *M. bovis* induces a stronger cellular defense response, in particular, pathways recogniz-ing foreign DNA are activated and IFN-β protein production is increased [32]. These differences are not due to the emergence of completely new proteins, but rather to the way in which already known antigens, such as ESAT-6, CFP-10, TB27.4, and

MPB70/80/83, are regulated. For example, *M. bovis* has a mutation in the Rv0444c gene, which affects the regulation of another gene (SigK), and this explains why it produces some proteins more strongly [33, 34].

M. bovis is able to subtly influence the host cells' defense mechanisms, especially the work of macrophages, the immune cells that normally engulf and destroy bacteria. One such mechanism is autophagy, which is the process by which a cell "digests" unwanted or dangerous elements. *M. bovis* has learned to control this process so that it does not die inside the cell. Specifically, the bacterium triggers mitophagy the destruction of mitochondria – thus interfering with another defense response, xenophagy, which is designed to kill invading bacteria. It alters the PINK1-PRKN pathway to avoid being seen and destroyed [19]. Also, during infection, IFI204 protein activity is enhanced, which triggers another form of autophagy accompanied by the release of the defense signaling protein IFN-β. These processes are part of the bacterium's strategy that allows it to hide from innate immunity [13].

M. bovis most often affects farm animals, especially cows, and this is due to both the characteristics of the bacterium itself and the way the immune system of these animals responds to infection [35]. However, *M. bovis* can infect not only cattle but also a wide variety of animal species, including domestic and wild animals, making it difficult to control the infection and eliminate it completely. In addition, although *M. bovis* is less likely to cause tuberculosis in humans compared to *M. tuberculosis*, such cases pose a serious risk. Especially because this pathogen is resistant to one of the main drugs, pyrazinamide, which complicates treatment [36].

The peculiarities of how *M. bovis* causes disease are not related to the appearance of some new, unique proteins, but to the way the work of already known genes is regulated. This allows the bacterium to change its properties, adapt to different conditions, cause different immune reactions, and infect many species, including humans.

Immune response and evasion mechanisms

Following *M. bovis* infection, both innate and adaptive immune responses are activated; however, the pathogen has evolved effective mechanisms to evade these defenses and establish persistent intracellular infection. To understand how it all starts, we need to look at exactly how the bacterium interacts with the innate immune system, specifically how it is engulfed by the macrophage (this is the cell that captures and destroys foreign microorganisms). Below is a diagram showing the basic steps of how a macrophage "eats" *M. bovi*s, as well as how the bacterium itself interferes with this process and escapes destruction.

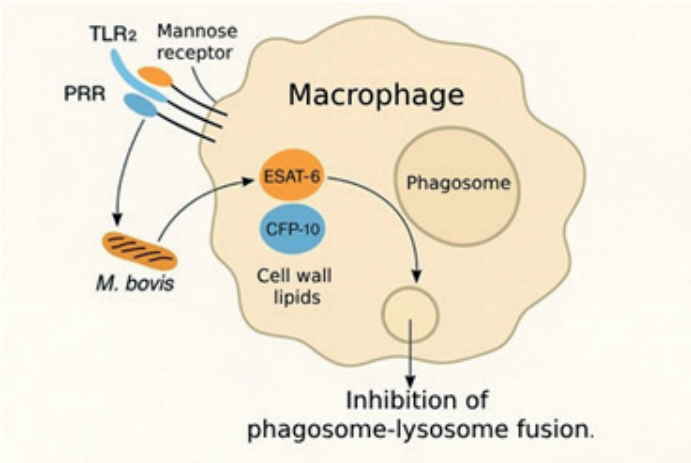

Figure 1 – Intracellular pathway of *M. bovis* phagocytosis in the macrophage, showing points of pathogen intervention, including inhibition of phagosome-lysosome fusion, suppression of oxygen burst, and DNA release into the cytosol

Recent studies have increasingly demonstrated how *M. bovis* differs from other closely related bacteria in the M. tuberculosis complex and what molecular tricks it uses to survive in the body. One interesting example is how this bacterium "tricks" immune cells, especially macrophages. One of these three important mechanisms of how *M. bovis* controls the cellular processes of mitophagy and xenophagy is explained below.

How *M. bovis* switches the macrophage to mitophagy and prevents it from destroying bacteria. *M. bovis* is adept at interfering with the defense systems of the macrophage, a cell that normally should engulf and digest foreign invaders. As a result of mitophagy activation, the cell begins to spend resources not on fighting the bacteria, but on "cleaning up the broken parts". As a result, the xenophagy system is weakened and the bacterium remains alive inside the macrophage. This deception is obtained through the involvement of one important signaling protein, p-TANK-binding kinase 1 (TBK1), which shifts the cell's attention from microbes to mitochondria. Scientists have proven that if mitophagy is blocked (e.g., by drugs or genetic interventions that inhibit the PINK1 protein), the macrophage begins to actively destroy *M. bovis* again. [16].

Figure 2 shows how exactly *M. bovis* interferes with the cell's defense processes, forcing it to focus on mitophagy rather than on destroying the bacterium itself.

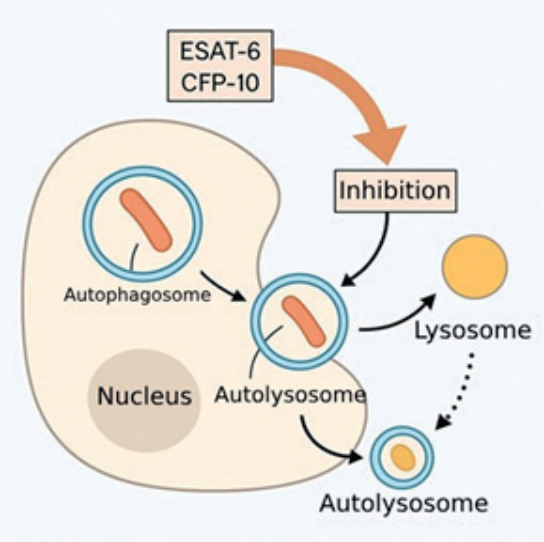

Figure 2 – Selective activation of mitophagy and suppression of *M. bovis* xenophagy through the PINK1-Parkin pathway and p-TBK1 regulation. This "cheating" of autophagy ensures the survival of the mycobacterium in the macrophage

Role of IFI204 in IFN-B activation and autophagy. One of the important elements of macrophage defense against *M. bovis* infection is the IFI204 protein, which works as a "sensor" of foreign DNA inside the cell. When the bacterium enters a macrophage, it is trapped in a special capsule called a phagosome. But if this capsule is destroyed, the bacterial DNA is released into the cell fluid (cytosol), where it is detected by IFI204. This triggers two defense processes at once. The first pathway: IFI204 activates the proteins stimulator of interferon genes (STING) and TBK1, which in turn trigger another protein IRF3. This leads to the production of interferon IFN-β, a signaling molecule that helps the cell fight infection. The second pathway: it starts the activation of autophagy, a process in which the cell "packs" unwanted or harmful elements (including bacteria) into bubbles and recycles them. At this point, the level of special proteins, such as LC3, increases and autophagosomes are formed, sort of like "garbage cans" inside the cell [13]. If the IFI204 gene is turned off in the macrophage (e.g., in a knockout experiment), IFN-β production drops severely and autophagy becomes weaker. As a result, the bacterium can more easily multiply inside the cell. In addition, *M. bovis* itself is adept at interfering with these defense pathways. It produces special microRNAs, such as miR-199a, that block the TBK1 protein. This impairs both IFN-β production and autophagy, which helps the bacterium to survive inside the macrophage even longer [37].

Differences in pathogen recognition between *M. bovis* and other MTBC members. Although *M. bovis* and *M. tuberculosis* have almost the same genetic information, the immune system recognizes them differently and this affects how the body responds to infection. *M. bovis* has been found to elicit a stronger immune response at the cellular level. In particular, macrophages, which engulf and destroy pathogens, react more actively to foreign DNA in the cytoplasm when infected with *M. bovis,* which triggers enhanced production of type I interferon (IFN-β) [7, 38]. Toll-like receptors, especially TLR2,

located on the surface of macrophages, also play an important role in the primary recognition of mycobacteria. These receptors are the first to come into contact with the pathogen and trigger a chain of signals aimed at activating the innate immune response. All these differences emphasize that, despite genetic similarities, *M. bovis* and *M. tuberculosis* interact differently with the immune system, which has implications for diagnosis, treatment and vaccine development (Figure 3).

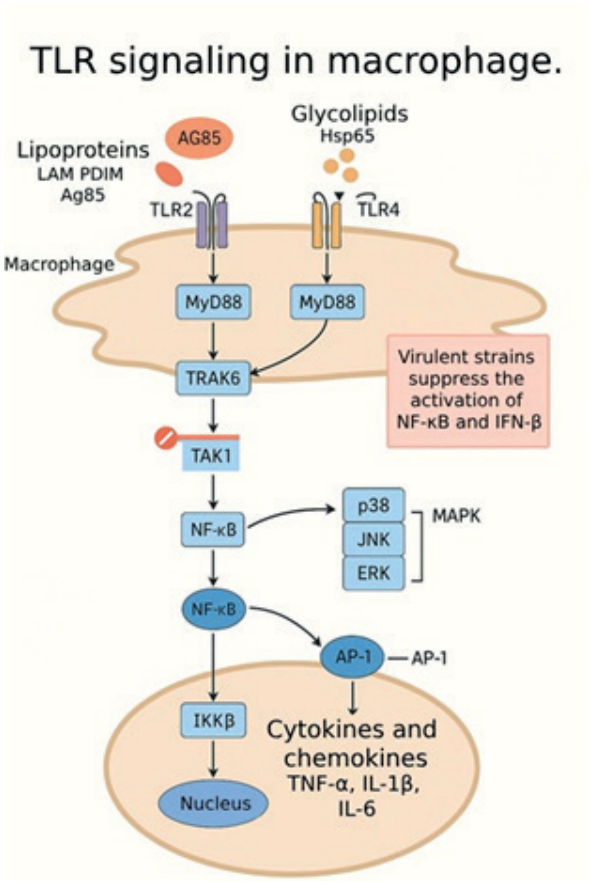

Figure 3 – Signaling pathway of innate immune response activation via TLR2 during *M.bovis* infection. Participation of adaptor proteins MyD88, NF-κB and production of proinflammatory cytokines is shown

One of the key features of *M. bovis* is the enhanced operation of the ESX-1 system, which secretes special proteins such as ESAT-6 and CFP-10. These proteins help bacterial molecules penetrate the host cell faster, which in turn activates the immune defense, especially the pathway associated with the IFI204, STING and interferon IFN-β proteins. This response is stronger in *M. bovis* than in *M. tuberculosis* infection. With regard to adaptive immunity, *M. bovis* induces active production of defense substances such as IFN-γ and IL-22, which are involved in the fight against infection. However, the strength and specificity of this response can vary greatly depending on the particular strain of the bacterium and which proteins it secretes [10, 39]. In addition, even small changes in the structure of key proteins (e.g., replacement of one amino acid residue in the ESAT-6 protein) can either enhance the immune system response or, on the contrary, make the pathogen less visible to the organism [15].

Classification of immunoactive proteins. ESX-1 secreted proteins (ESAT-6, CFP-10)

The secreted proteins of the ESX-1 system, primarily ESAT-6 and CFP-10, are important molecules by which *M. bovis* interacts with the host immune system and induces disease development. These proteins are encoded in a region of the genome known as difference region 1 (RD1), which is present in virulent strains of *M. bovis* but absent in the attenuated BCG vaccine strain. These proteins are secreted through a specialized type VII secretion system.

Increased expression of esx-1 in *M. bovis* and implications for the immune response. Comparison of gene and protein activities showed that *M. bovis* produces more components of the ESX-1 system, and

thus more ESAT-6 and CFP-10 proteins, than *M. tuberculosis*, especially in bovine macrophages. This leads to more severe damage to the phagosome membrane and active release of bacterial DNA into the cell cytosol. As a result, the mechanisms of innate immune response are activated, the IFI204 sensor is activated, the STING-TBK1 signaling pathway is triggered, and the synthesis of type I interferon (IFN-β) begins. Thus, *M. bovis* causes a stronger launch of defense mechanisms related to DNA recognition in the cytosol, stimulates the production of inflammatory molecules and forms an immune response with pronounced antiviral characteristics in cattle macrophages. This, in turn, affects the development and course of infection compared to infection caused by *M. tuberculosis* [38, 40].

Genetic differences in ESAT-6 and their influence on the immune response. It is significant that the ESAT-6 protein, which is part of the ESX-1 system, may differ slightly in its structure in different strains of M. bovis. For example, in some virulent strains, a single amino acid substitution, the socalled T63A mutation, which is located at the end of the ESAT-6 molecule, has been found. Studies have shown that this substitution enhances the ability of the bacterium to disrupt the phagosome membrane, activate the production of interferon IFN-β and trigger an inflammatory response in the macrophage. As a result, the immune system begins to produce inflammatory signaling agents more actively, making the response stronger compared to strains lacking this mutation [41, 42]. These differences between the protein variants are important because they may explain why different animals or humans experience infection differently, and why not all tests and vaccines work equally well.

The importance of ESAT-6 and CFP-10 in diagnosis. The ESAT-6 and CFP-10 proteins elicit a particularly strong immune system response, and it is this property that is utilized in modern TB tests such as IGRA (interferon release assays) and skin tests [43]. These tests are used to detect M. bovis infection in cows, deer and other animals. Importantly, these proteins are not present in the BCG vaccine strain, so that infected animals can be distinguished from simply vaccinated animals – this is called the DIVA-strategy. Tests containing ESAT-6 and CFP-10 peptides (e.g., Bovigam) show high accuracy because they induce a specific response only in infected individuals [44, 45]. In addition, these proteins are considered as possible components of future vaccines because they induce the desired type of immune response, a cellular response involving Th1-lymphocytes, which is essential to combat mycobacterial infections [46].

Ag85 complex (Ag85A, Ag85B, Ag85C)

The Ag85 complex, comprising three proteins, Ag85A, Ag85B, and Ag85C, is one of the most studied in mycobacteria such as *M. bovis* and *M. tuberculosis*. These proteins play an important role both in the development of infection and in the activation of immune defenses.

Role in cell wall construction. All three proteins of the Ag85 complex are enzymes involved in the synthesis of a special part of the mycobacterial cell envelope, mycolic acids. They work as transporters of these acids, combining them with other components to form a stable and dense cell wall. One important product of this process is the cord factor (TDM), which helps the bacterium survive in the host. If the work of Ag85C protein is blocked, the bacterium will not be able to build its shell normally, it will start to accumulate intermediates, and this will prevent its growth and reproduction [47].

During infection, especially in conditions of oxygen deficiency or increased acidity (which occurs in the body during chronic inflammation), it is Ag85B that begins to be actively produced, which indicates its important role in the survival of the bacterium during prolonged infection [48].

Impact on immune response and prospects for vaccines. The proteins of the Ag85 complex elicit a strong immune system response. They are particularly well recognized by the body's defense cells and trigger the so-called Th1-type immune response – with the production of substances such as IFN-γ, IL-2 and TNF-α, which help to control and suppress mycobacteria. In experiments with mice and cattle, it has been shown that a particularly active protective immunity is formed in response to Ag85B [49, 50]. Due to this fact, the proteins of the Ag85 complex began to be used in the creation of new vaccines. For example, vaccines based on modified virus (MVA85A) or BCG vaccines with increased Ag85B production were developed. These vaccines provided stronger protection compared to conventional BCG, as evidenced by both less damage in animal tissues and higher levels of protective substances such as IL-17 [51]. In addition, the combination of Ag85 with other proteins, such as ESAT-6, yields an even broader and stronger immune response. Therefore, Ag85 is now considered not only as a basis for new vaccines, but also as an important marker in diagnostics and can be used in tests that measure the level of protective cytokines (e.g., IFN-γ) to detect infection [27].

The proteins MPB70, MPB80 and MPB83 are among the most important antigens of the bacterium *M. bovis* for the immune system. These proteins are not only actively produced in the body, but also well recognized by the immune system and are of great importance in the diagnosis of tuberculosis in animals. MPB70 and MPB80 are soluble proteins that are secreted into the external environment and are almost not found in other mycobacteria such as *M. tuberculosis*. In contrast, MPB83 is a protein attached to the surface of the mycobacterium, making it particularly accessible to immune cells [52, 12]. These three proteins are produced in high amounts in M. bovis due to mutations in a regulatory gene (anti-SigK), which enhances the function of the SigK protein responsible for turning on these antigens. The BCG vaccine strain and *M. tuberculosis* lack such mutations, so their levels of these proteins are much lower [53, 11].

In terms of immune response, MPB70 and MPB80 enter the external environment and activate immunity at a distance, whereas MPB83 acts on the surface of the bacterium itself, directly contacting the body's defense cells. This difference is important in the development of tests and vaccines [54, 55].

These proteins are particularly valuable for diagnostic purposes because vaccinated animals (e.g., those that have received BCG) do not have a false reaction to them. Therefore, MPB70, MPB80 and MPB83 are used in various diagnostic tests, such as ELISA and interferongamma tests, allowing infected animals to be accurately distinguished from vaccinated animals. This makes them an excellent choice for implementing the socalled DIVA strategy. Interestingly, even though these proteins are quite similar, they differ in their structure at the sites where immune cells attach to them (called epitopes). For example, MPB70 and MPB83 have sites that differ even in individual amino acids. This is important in the development of accurate tests and vaccines: these proteins can be combined to improve the recognition of infection and reduce the risk of false results [54]. MPB83 is considered to be a particularly promising marker; antibodies to it appear at the earliest stages of infection, so it is convenient to use for early diagnosis. Also, the level of MPB70 protein can be useful for assessing the stage of the disease. In general, the high activity of these proteins and their immune uniqueness make them essential components in the control of tuberculosis in ani-mals [55].

PE/PPE proteins, components of phagocytosis and autophagy suppression

PE/PPE proteins and other special proteins secreted by M. bovis play an important role in how this bacterium avoids destruction by the immune system and continues to survive within the body.

PE/PPE proteins are a large group of proteins (there are over 160 in *M. tuberculosis*, and almost as many in *M. bovis*), many of which are found on the surface of the bacterium or secreted outward through the specialized ESX-5 system. It used to be thought that these proteins were simply different in structure and therefore difficult for the immune system to recognize. But it is now known that they do much more than that. They interfere with the immune system cells' ability to recognize the bacterium, affect the operation of key signaling pathways (e.g. TLR2, NF-κB and MAPK), can both enhance and suppress inflammation, and are involved in maintaining cell wall structure and nutrient transfer [56, 57, 58].

Some PE/PPE proteins (e.g., PE_PGRS20 and PE_PGRS47) are particularly important for bacterial survival within macrophages. These proteins interfere with the initiation of autophagy and block Rab1A, a protein that triggers autophagy. If these proteins are removed, the bacterium becomes vulnerable, as cells begin to destroy it more efficiently [59].

In addition to PE/PPE proteins, *M. bovis* has other defense tools. One of them is the PknG protein. It prevents the macrophage from connecting the phagosome to the lysosome, i.e. it actually blocks the “digestion” of the bacterium. Without this protein, the bacterium cannot survive inside the cell. Another protein, Zmp1, inhibits the inflammatory response by reducing the production of IL-1β signaling protein and simultaneously interferes with the formation of the phagolysosome as a key element for killing the bacterium [56, 57].

Thus, M. bovis utilizes a complex system of PE/PPE proteins and effectors such as PknG and Zmp1 to “fool” the immune system. It camouflages itself, interferes with its own processing inside cells, and regulates inflammation so that it doesn't cause too strong a response. All of this allows it to persist in the body for a long time. Disrupting these proteins makes the bacterium less dangerous, which makes them interesting targets for creating new drugs or vaccines aimed at disrupting immune evasion mechanisms [60].

Lipoproteins (LprG, LpqH, etc.), TLR2 stimulators

Mycobacterial lipoproteins such as LprG, LpqH, LprA, LppX, et al. – are special proteins found on the surface of microbes and are linked to fatty molecules. These proteins play an important role in how the body recognizes infection. They interact with special "sensors" on immune cells such as macrophages and dendritic cells. These sensors are called TLR2 receptors and allow the immune system to quickly detect mycobacteria and initiate a defense response [61, 62].

1.Immunomodulation and TLR2 signaling. Mycobacterial lipoproteins are recognized by special receptors on the surface of immune cells, TLR2 together with TLR1 or TLR6. This triggers a chain of signals inside the cell, including important molecules such as NF-κB and AP-1, resulting in the release of inflammatory substances such as TNF-α, IL-6, IL-12 and IL-1β. One of the most active proteins, LpqH, also promotes the production of interferon-γ and helps the cell to better present antigens to other elements of the immune system [63]. In order for lipoproteins to trigger such a reaction, they need a special fatty "tag" at their origin. If the enzymes responsible for such a tag do not work, lipoproteins will not be able to turn on the immune response effectively. In different strains o*f M. bovis* and *M. tuberculosis*, these "tags" may differ slightly, which affects the strength of the immune response [64].

2.Involvement in intracellular signaling, inflammation and antibacterial mechanisms. Lipoproteins not only trigger inflammation but also activate defense mechanisms in cells. For example, the same LpqH turns on a signaling chain that, through calcium and other proteins, activates the production of catheplicidin, a substance that destroys mycobacteria. This process is also linked to vitamin D and autophagy, a mechanism by which the cell "eats" harmful microbes [65].

Some lipoproteins, such as LprG, help mycobacteria to survive. They are involved in fat transport and strengthen the cell wall of the mycobacterium, which prevents the fusion of the phagosome with the lysosome and thus prevents the bacterium from dying. If LprG is removed, the mycobacterium is more easily destroyed inside the cell and the immune system recognizes it better [66].

Interestingly, if there are too many lipoproteins on the surface of the mycobacterium (as in some particularly dangerous forms, such as *M. abscessus*), this can cause too much inflammation, which itself can damage tissue. That is, lipoproteins can both help to fight infection and aggravate the course of the disease [67].

3.Diagnostic and vaccine potential. Mycobacterial lipoproteins, especially the LpqH protein, are active in inducing a strong immune response, in particular promoting the production of interferon-γ and triggering autophagy, the process by which a cell destroys harmful bacteria. Due to these properties, lipoproteins have attracted the attention of scientists as potential candidates for the creation of new vaccines against tuberculosis, as well as for the development of more accurate diagnostic methods [64]. These proteins play an important role in linking the two levels of immune defense: innate and adaptive. This helps the body to not just react to infection, but to turn on more effective control mechanisms that can prevent the disease from becoming chronic.

Lipoproteins produced by mycobacteria such as *M. bovis* and other closely related species serve a dual function. On the one hand, they trigger the innate immune response through TLR2 receptors, induce inflammation and activate defense mechanisms in cells, such as autophagy and the production of specific antimicrobial substances. On the other hand, these proteins can specifically regulate these same processes at different stages to "tune in" to the host immune system. Mycobacteria are able to change the amount and structure of their lipoproteins to achieve the right balance not to irritate the immune system too much to avoid its complete destruction, but also not to remain completely undetected. This fine-tuning helps them survive in the body for a long time, which is important for their ability to cause chronic forms of disease [67].

Comparative and integrative 'omics' data

Analysis and comparison of complex 'omics' data (transcriptomics, proteomics and secretomics) between *M. bovis* and *M. tuberculosis* has helped to reveal why these closely related bacteria cause tuberculosis with different features in animals and humans. Studies have shown how they interact differently with the body and the immune system.

Transcriptomics and proteomics: unique expression profiles. Although the genomes of *M. bovis* and *M. tuberculosis* are nearly identical, differences in gene activity (i.e., how strongly certain genes work) lead to marked differences in their behavior [5]. In *M. bovis*, genes responsible for virulence – especially

those that control the ESX-1 secretion system – are more actively turned on in macrophages (the body's defense cells) in cows. This includes the ESAT-6, CFP-10 proteins, and the espR and phoP regulatory genes. ESX-1 proteins are secreted in higher amounts as shown by mass spectrometry techniques [68].

Also, the production of two important lipoproteins, MPB70 and MPB83, is strongly increased in *M. bovis*. This is due to mutations in the rskA regulator, which controls the sigma factor SigK. Because of this, overproduction of these proteins is activated. Analysis of proteomes (set of all proteins) by electrophoresis and mass spectrometry confirms that these antigens are more abundant in *M. bovis* than in *M. tuberculosis*, both inside the cells and among those secreted outside [69, 12]. Interestingly, the Ag85 protein complex (Ag85A/B/C) in both species is almost identical in structure, but the level of its production may differ depending on the infection conditions and the type of infected cells. This requires further study [70].

The secretome: a comparative analysis of pathogenic proteins. The secretome is a set of proteins that mycobacteria secrete into the external environment. It plays a key role in how the bacterium "communicates" with host cells and how it resists their defenses. Comparative analysis has shown that *M. bovis* has many more ESX-1 system proteins (including ESAT-6, CFP-10, EspA, EspC, EspD) in the secretome than *M. tuberculosis*. This helps *M. bovis* to degrade phagosome membranes, penetrate the cytoplasm, and trigger a strong inflammatory response through the cGAS-STING pathway with the production of type I interferon more actively. All this explains the peculiarities of zoonotic tuberculosis, i.e., tuberculosis transmitted from animals to humans [69]. In addition, the proteins MPB70/80 and MPB83, which strongly excite the immune system and can increase inflammation, are actively secreted by *M. bovis* and are considered good markers for the diagnosis of this species. In *M. tuberculosis*, these proteins are almost not produced due to the peculiarities of its regulatory systems [12]. *M. tuberculosis* secretome was also found to be more saturated with proteins associated with latent (hidden) state and stress, such as α-crystallin Rv2031c or Rv2623 protein. In contrast, in M. bovis, secreted proteins are directed at triggering inflammation and interacting with TLR2 receptors. Even among the common proteins, there are differences not only in quantity but also in chemical modifications, such as the composition of lipid "anchors" in lipoproteins. All this affects how the immune system recognizes and responds to bacteria [63, 64].

The significance of differences in 'omics'-profiles for pathogenesis and diagnosis. All the above shows that *M. bovis* induces a more active immune response by secreting many proteins that stimulate innate immunity. This can help the body to respond more quickly to infection, but at the same time creates a risk of chronicity, as the bacterium is able to "evade" the immune attack by rearranging the work of macrophages. *M. tuberculosis*, although close in genetics, acts differently. It prefers to trigger those genes that allow it to "hide" in the body – it activates proteins that work under conditions of stress and contribute to the transition to the latent phase. This makes human tuberculosis prone to a long, latent course [69].

Diagnostic and applied significance

How *M. bovis* produces key proteins such as MPB70, MPB83, ESAT-6 and CFP-10 plays a critical role in current diagnostic and prognostic methods for bovine tuberculosis as well as zoonotic tuberculosis in humans. Comparative studies that analyze gene activity and protein synthesis (transcriptomics and proteomics) show that the main differences between *M. bovis* and M. *tuberculosis* are not related to the genes themselves, but to how active they are. This explains the differences in bacterial behavior in different host species and influences which antigens are used in modern diagnostic tests [7, 1].

Protein expression and secretion as a basis for multicomponent diagnostics and vaccines. In *M. bovis*, the proteins MPB70, MPB80 and MPB83 are consistently produced in large quantities much higher than in *M. tuberculosis*. This is due to changes in the gene regulator RskA, which normally suppresses the activity of another gene, sigma factor K. In the case of *M. bovis*, this regulation is disrupted, and the sigma factor works without restrictions, which causes active production of these proteins [12]. These proteins are hardly produced in BCG vaccine strains, while they are very scarce in *M. tuberculosis*. Therefore, MPB70/80/83 are considered excellent markers to distinguish infected from vaccinated animals (so-called DIVA-diagnostics – Differentiating Infected from Vaccinated Animals). This is crucial for tracking the epidemic, understanding its magni-tude, and assessing how well the vaccine is working [69]. On the other hand, ESAT-6 and CFP-10 proteins, which are encoded in the

RD1 genetic region, are absent in most BCG vaccine strains but are actively produced in both *M. bovis* and *M. tuberculosis*. These proteins are the basis of modern IGRA tests (cellular immune response tests), as they induce a strong T-cell response. Current ap-proaches combine multiple antigens, such as MPB70/83 and ESAT-6/CFP-10, to improve diagnostic accuracy. These combined ELISA and IGRA tests not only allow for better detection of TB, but also for judging the stage of infection. This is possible because the immune system responds differently to these proteins at different times: first, a rapid T-cell response to ESAT-6 and CFP-10, and later the production of antibodies to MPB70 and MPB83 [71, 72].

Detection of circulating peptides and integration with host markers. Thanks to modern protein analysis methods such as LC-MS/MS and MALDI-TOF and the development of very sensitive antibodies, it is now possible to directly detect individual mycobacterial peptides (e.g. MPB70 and Ag85B) in the blood even when the number of bacteria in the body is still very low. This makes it possible to detect tuberculosis at the earliest stages, including latent and asymptomatic forms, which is beyond the scope of standard immunologic tests [72]. However, detection of such peptides alone does not always give an accurate result due to the fact that the immune system may react differently in different animals. To improve accuracy, scientists have begun to combine data on mycobacterial antigens with information on proteins and signaling molecules of the organism itself, so-called inflammatory markers, such as SAA (serum amyloid A), HP (haptoglobin), IFN-γ (interferon-gamma), IP-10, and TNF-α. This approach allows for a more comprehensive diagnostic panel that works better in real-world settings, both in laboratories and in veterinary field examinations [73]. These complex biomarkers are characterized by their ability to detect infection at stages when conventional tests (antibody or T-cell tests) have not yet been successful. Combinations such as the presence of MPB83 peptide in the blood along with high levels of IFN-γ and low levels of IL-10 are associated with a higher risk of developing active tuberculosis. This makes it possible to use such markers not only to detect the disease, but also to monitor its development, prevent it, and assess the effectiveness of treatment or vaccination [71].

Cytokine profile: role in diagnosis and prognosis. Today, scientists are actively studying how the levels of various cytokines – proteins that regulate the immune response – change in the body. Especially important are such molecules as IFN-γ, IL-22, TNF-α, IL-10, IP-10 and others. They are measured in multi-analyses used both for diagnosis of tuberculosis (e.g. IGRA tests) and to assess the risk of progression from latent to active infection. IFN-γ remains one of the main indicators of the body's early response to mycobacteria – especially to ESAT-6 and CFP-10 antigens. By monitoring IL-22 and TNF-α, it is possible to judge the current phase of the disease, the level of inflammation and the formation of granulomas (areas of inflammation characteristic of tuberculosis). By combining data on “inflammatory” cytokines (IFN-γ, TNF-α, IL-22) and “inflammation-suppressing” cytokines (IL-10, TGF-β), it is possible not only to diagnose infection, but also to divide infected individuals into groups according to their risk level – for example, those who may remain latent and those who are more likely to develop active disease. This is especially important when it comes to timely intervention, treatment or vaccination [71].

In general, these approaches, from analyzing the expression of mycobacterial proteins (MPB70/80/83, ESAT-6, CFP-10), through their direct detection in blood, to analyzing body proteins and cytokines, make it possible to move towards more accurate and “smart” diagnostics of TB, especially *M. bovis*. This helps to detect even latent cases, to apply DIVA testing (to distinguish infected from vaccinated), to monitor the effectiveness of vaccination and to predict how the infection will progress in both animals and humans.

Conclusion

This review highlights that *M. bovis* represents a unique member of the *M. tuberculosis* complex with distinct molecular features of interaction with the host immune system. This review revealed that immunoactive proteins of *M. bovis*, including components of the ESX-1 secretion system, lipoproteins, PE/PPE proteins, and MPB70/80/83 antigens, play a key role in modulating the immune response by providing intracellular persistence and evasion by phagocytic and autophagic mechanisms. Active interference in innate immunity pathways (phagocytosis, autophagy, TLR sig-naling) makes these proteins not only virulence factors but also promising targets for diagnosis, monitoring, and vaccine development. Molecular and multiomic studies support the need for a comprehensive approach to

understanding the biology of *M. bovis*, especially in the face of increasing zoonotic threat and resistance to therapy. In this context, indepth studies of immunoactive proteins offer opportunities to develop highly specific biomarkers and second-generation vaccines that can effectively control bovine TB and prevent its transmission to humans. Integration of immunoactive M. bovis proteins into multicomponent diagnostic panels and next-generation vaccine platforms rep-resents a promising strategy to enhance sensitivity, specificity and translational applicability of bovine tuberculosis control tools.

Authors' Contributions

NG: conducted a comprehensive literature search, analyzed the gathered data and drafted the manuscript. AG: analyzed the gathered data and drafted the manuscript. FZh: analyzed the gathered data and performed final revision and proofreading of the manuscript. APS and OA: designed and supervised the study, conducted a comprehensive literature search, analyzed the gathered data and drafted the manuscript. All authors have read, reviewed, and approved the final manuscript.

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Alternatives to therapeutic and preventive methods for calves in the early postnatal period

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Abstract

Background and Aim. Calves in the early postnatal period are highly susceptible to infec-tious diseases, particularly gastrointestinal disorders. These pathologies often result from poor zoo-hygienic management and inadequate feeding practices, which activate opportunistic microflora and lead to clinical symptoms. Conventional treatment strategies frequently rely on antibacterial agents, raising concerns regarding antimicrobial resistance in dairy farming. This study aimed to evaluate alternative therapeutic and preventive methods that reduce the need for antibiotics in calf manage-ment.

Materials and Methods. The study was conducted under practical farm conditions characterized by suboptimal microclimate and feeding practices that contributed to gastrointestinal and respiratory disorders in calves. A locally produced antibacterial drug, *Timutin*, was used to assess ther-apeutic efficacy. Additionally, the probiotic *Vetom 3* was administered orally to evaluate its preven-tive effect on gastrointestinal diseases.

Results. Limited use of *Timutin* demonstrated noticeable therapeutic effectiveness in calves under unfavorable housing and feeding conditions, contributing to clinical improvement in gastroin-testinal and respiratory disorders. Preventive oral administration of the probiotic *Vetom 3* showed a positive effect, supporting the formation of a stable microbiocenosis and reducing the incidence of bacterial gastrointestinal diseases accompanied by diarrheal syndrome.

Conclusion. The findings indicate that alternative therapeutic and preventive measures such as controlled use of *Timutin* and probiotic supplementation with *Vetom 3* may effectively reduce gastrointestinal morbidity in calves while limiting reliance on antibacterial drugs. These approaches represent promising strategies for preventing antibiotic resistance in dairy farming.

Keywords: calves; gastrointestinal pathologies; antibacterial drugs; probiotics; blood bio-chemistry.

Introduction

Obtaining strong and viable young animals is the foundation for developing dairy farming. The health status of newborn calves determines their subsequent growth and development. This influences their ability to adapt to adverse environmental factors and fully express their genetic potential [1, 2, 3].

However, the technological and zoo-veterinary methods used in newly established dairy farms, such as housing, calving management, and calf rearing during the milk-feeding period, as well as the untimely implementation of specific therapeutic and preventive measures, do not ensure the birth of calves with high metabolic activity and resistance. As a result of these factors, a significant number of diseases among newborn calves are reported. In the nosological structure of neonatal calf pathology, gastrointestinal and respiratory diseases are most frequently observed [4, 5, 6].

Gastrointestinal disorders accompanied by diarrhoea may occur on a large scale, resulting in high morbidity and mortality. These diseases cause significant economic losses in animal husbandry due to the costs of preventive and therapeutic measures. High disease incidence is associated with the fact that

calves are born with an underdeveloped immune system, making this age group particularly vulnerable to gastrointestinal infections and unable to resist the negative impact of conditionally pathogenic microflora ingested orally. Such microflora quickly acquires virulence within the host, leading to high mortality [7, 8].

Since 2023, a large national programme to develop dairy farms has been implemented in Kazakhstan. As a result of the active use of state support measures for dairy farming, many farmers have entered the dairy sector, importing livestock from abroad. However, farmers do not always take into account the veterinary aspects of dairy farming, which are determined by farm specialization, the concentration of large numbers of animals in limited spaces, and the cyclical nature of production technology. When diseases occur among adult animals, especially gastrointestinal and respiratory diseases in newborn calves, farmers often indiscriminately use antibiotics and chemotherapeutic agents, such as Nitox, Penestrel, and Tylosin, which are widely available and sold without prescription. In such cases, antibiotics are used to combat the negative consequences of poor man-agement conditions [9, 10, 11].

The widespread use of antibacterial drugs for these purposes, along with their positive therapeutic effects, produces adverse side effects. Prolonged, unsystematic use of antibacterial agents reduces their effectiveness and leads to the emergence of resistant microorganism strains.

Such a disease control protocol in dairy farming is unacceptable. In many countries with ad-vanced dairy industries, researchers have emphasised the risk of antibiotic resistance associated with such practices [12, 13, 14, 15]. Hence, there is an urgent need to intensify practical measures and scientific research on preventive strategies for animal health protection and public health standards in Kazakhstan. The aim of our research was to study alternative methods of therapy and disease prevention in calves during the early postnatal period, while minimising the use of antibiotics and chemotherapeutic agents [16-20].

Materials and Methods

To expand their dairy farming potential, in 2023, the Arystanov farm constructed a modern cowshed for 400 head of cattle and associated facilities, including a calf barn, a milking unit, and a maternity ward. In the second half of July 2024, the first batch of Holstein heifers arrived from Germany. The supplier, having artificially inseminated the heifers, planned mass calving for Decem-ber 2024 and January 2025. However, the contractors failed to construct the necessary farm facili-ties on time, including the calf barn, maternity ward, and veterinary station.

Consequently, after calving, the cows and newborn calves were kept in group pens in an adapted old cowshed. Monitoring of these housing conditions revealed several shortcomings: low temperatures, lack of sunlight, and twice-daily feeding with colostrum and, subsequently, milk, of-ten not from individual nipple drinkers. Due to these factors, 15 calves showed clinical signs of gas-trointestinal and respiratory diseases. Thermometry revealed elevated body temperature in most an-imals, reaching 40 °C.

The conditions for the Simmental calves at the Tolengut farm were somewhat better organ-ised. Here, the calves were kept in draft-free individual pens at an acceptable temperature (8-10 °C). Nevertheless, due to violations of sanitary and hygienic standards, such as improper cleaning and disinfection of feeding equipment (nipple drinkers) and infrequent cleaning of pens, some calves developed diarrhoea. Clinical signs of gastrointestinal lesions, including diarrhoea and elevated body temperature, were observed in four calves. For treatment, they received a five-day course of intramuscular injections of the drug *Timutin* (a relatively new drug produced in Kazakhstan from the group of diterpene antibiotics. It is included in the State Register of Veterinary Drugs and Feed Additives of the Republic of Kazakhstan. Produced by Epsilon LLP in Uralsk, West Kazakhstan region.) and the probiotic *Vetom 3*, administered with milk. The probiotic *Vetom 3* was produced by Research Center, LLC, Koltsovo, Novosibirsk Region (batch no. 070524, production date May 2024, shelf life: 4 years).

During the research period, a comparative analysis of the biochemical parameters of blood serum was performed for clinically healthy calves and those with gastrointestinal disorders at 1.5-2 months of age. Blood samples for biochemical studies were collected from the jugular vein using vacuum systems with clot activators. Laboratory analyses were performed on an automatic biochemical analyser. Biometric processing of the obtained results was conducted using Student's t-test for determining the significance of differences. Given the insufficiently favourable housing and feeding conditions,

to prevent complications associated with gastrointestinal and respiratory diseases, the relatively new domestically produced antibacterial drug *Timutin* was used at 1 ml per 12.5 kg of live weight for therapeutic purposes. The treatment course lasted 4 to 5 days, depending on clinical symptoms, with 1 injection administered per day. The drug *Timutin* is a relatively new preparation containing the active substance tiamulin, a semisynthetic derivative of the diterpene antibiotic pleuromutilin, produced by *Pleurotus mutulis*. According to GOST 12.1.007-76, *Timutin* is classified as a moderately hazardous substance (hazard class 3).

For the remaining 57 calves, the probiotic *Vetom 3* was administered orally with milk at 50 mg per 1 kg of live weight for prophylactic purposes. The veterinary specialists were advised to follow a prophylactic protocol with Vetom 3 through enteral administration three times daily for two weeks.

Results and Discussion

The application of the new domestic preparation, *Timutin*, and the probiotic *Vetom 3* demonstrated positive outcomes. After *Timutin* administration, clinical improvement was observed within three days, and diarrhoea symptoms ceased by day four or five. Table 1 presents the results of treatment and prevention of diseases in calves.

Table 1 – Results of Therapeutic and Preventive Drug Use at Arystanov and Tolengut Farms

№	Farm name	Number of calves (under 2 months)	Number of sick calves	Treatment course with Timutin (days)	Number of dead calves	Recovery rate (%)	Probiotic prevention (Vetom 3)	Disease recurrence
1	Arystanov	74	17	4–5	2	88	74	No recurrence
2	Tolengut	68	4	5	–	100	68	No recurrence

However, stopping all use of antibacterial agents remains difficult at present. Their rational use is still justified for sick calves, especially during the first 10 days of life, as this period does not involve forced slaughter and poses no risk of antibiotic residues or antibiotic resistance that could affect public health. Furthermore, the reasonable use of antibacterial agents in our study is justified by the fact that the heifers were not immunised during pregnancy, meaning no antibodies were produced against gastrointestinal diseases for subsequent transfer via colostrum.

Gastrointestinal diseases in calves are characterised by changes in blood biochemical parameters compared with healthy animals. The comparative characteristics of blood serum indicators are presented in Table 2.

Table 2 – Biochemical indicators of calf blood serum

Blood serum indicators	Clinically healthy calves	Sick calves
Total protein, g/L	66.4±8.53	54.3±14.8
Calcium, mmol/L	4.9±0.08	3.8±0.24
Phosphorus, mmol/L	1.9±1.48	1.3±0.25
Iron, μmol/L	32.1±11.6	20.12±12.3
Urea, mmol/L	2.2±0.8	2.4±0.94

Blood serves as a test system that reflects metabolic disturbances and the effects of poor veterinary and sanitary conditions, as well as improper feeding. These factors influence the gastrointestinal tract function in calves, as reflected in their biochemical blood parameters.

Among biochemical indicators, total protein concentration is one of the objective criteria that characterise the level of metabolism and the functional state of the animal. When protein metabolism is impaired, the immune system cannot provide effective protection against potential pathogens. In sick calves, the total protein concentration decreased by 18.2%. The calcium and phosphorus levels in sick calves were also lower than in healthy ones, may be associated with dehydration and insufficient

mineral intake through feeding. Iron plays an important physiological role in metabolism, as it is part of haemoglobin, myoglobin, and enzymes involved in biological oxidation and haematopoiesis. In clinically healthy calves, iron levels were 37.4% higher than in diseased animals. A characteristic feature of calf blood and urea chemistry is the nitrogen content. The urea concentration in the blood of calves with gastrointestinal disease increased slightly (2.4±0.94 mmol/L vs. 2.2±0.8 mmol/L), which may reflect the activation of protective mechanisms to maintain homeostasis during illness.

As noted earlier, the main factors contributing to gastrointestinal diseases in calves were violations of zoo-veterinary housing standards, feeding regulations, and non-compliance with veterinary and sanitary requirements. To reduce the use of antibiotics and limit the spread of antibiotic resistance, we applied the probiotic *Vetom 3* as an alternative to prevent diarrhoea and dysbiosis in calves. The preparation was administered orally, both individually and in groups, mixed with milk as a freshly prepared solution at a dose of 1.5 g per head twice daily. The probiotic was given to 57 calves aged up to 1.5 months for 12 days. During and after the administration of the probiotic *Vetom 3*, no cases of diarrhoea were observed in calves, which can be explained by its antagonistic action against pathogenic microflora and its ability to neutralise accumulated toxic products.

Conclusion

The results of this study indicate that the occurrence of gastrointestinal diseases in calves during the early postnatal period, as well as the prevalence, severity, and outcome, depend on the overall body condition of the animal, its natural resistance, and the conditions under which the calf is kept after birth and during subsequent rearing. The feeding, housing, and management deficiencies identified at Arystanov and Tolengut farms contribute to reduced body resistance and typically lead to more severe disease.

At present, a complete rejection of antibacterial agents is difficult. It is acceptable to use such drugs for diseased calves, especially during the first ten days of life, because forced slaughter is not practised in this period, meaning that the development of antibiotic resistance and the potential impact on public health are minimal. Furthermore, the rational use of a relatively new, domestically produced antibacterial drug is justified, as the heifers were not immunised during pregnancy with vaccines that promote immunity; thus, no antibodies against gastrointestinal diseases were produced and transferred through colostrum. The probiotic Vetom 3 demonstrated sufficient effectiveness in the prophylaxis of gastrointestinal diseases, as it contributes to the formation of a healthy microbiocenosis, helping calves avoid bacterial infections associated with diarrheal syndromes.

Authors' Contributions

GA and AZh: Conceptualized and designed the study, conducted a comprehensive literature search, analyzed the gathered data and drafted the manuscript. IZh and AJ: Conducted the final revision and proofreading of the manuscript. All authors have read, reviewed, and approved the fi-nal manuscript.

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Letter to the Editor

Dear Editor,

We thank our colleagues for their interest in our work and for their remarks concerning the detection of *Anoplocephala perfoliata* and *Cylicocyclus insigne* in tugai deer, as reported in our article published in *Herald of Science of S. Seifullin Kazakh Agrotechnical Research University: Veterinary Sciences,* 2025, No. 3(011), pp. 36–42. https://doi.org/10.51452/kazatuvc.2025.3(011).1986).

The arguments presented regarding the strict host specificity of these parasites in perissodactyls are scientifically well grounded and merit careful consideration.

We acknowledge that accidental sample contamination during field collection, transportation, or laboratory processing cannot be entirely excluded and is, in all likelihood, the most plausible explanation for these isolated findings. As is well known, such technical factors may occasionally result in erroneous identifications in parasitological studies.

We appreciate our colleagues for their constructive comments, which allowed us to clarify specific aspects of our results and highlighted the importance of stringent control measures to prevent potential cross-contamination in parasitological research. We intend to place increased emphasis on these aspects in our future work.

Sincerely,
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Using indirect hemagglutination assay for the diagnosis of cattle brucellosis

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