DETECTION OF BRUCELLA IN *DERMACENTOR MARGINATUS* TICKS

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Abstract. Ticks are carriers of numerous pathogens, and their bacterial composition, abundance, diversity, and interaction affect both their growth and the efficiency of disease transmission. The emergence of next-generation metagenomic sequencing (NGS) technologies has expanded the possibilities for detecting and characterising microbial pathogens. Sequence data analysis can identify the presence of *Brucella* DNA in ticks and determine its genetic characteristics. In the spring of 2023, tick samples were collected in the Taskalinsky district of the West Kazakhstan Region. Sequencing of the 16S rRNA genes of bacteria in tick samples was performed using the Ion Torrent platform based on NGS technology.

In *D. marginatus*_WKR_Taskala tick samples, metagenomic analysis identified *Brucella suis bv. 3* (25%) and other *Brucella species* (75%). Analysis of the reads obtained as a result of metagenomic sequencing of the tick sample identified 3,973 reads, of which 2,966 were classified as *Brucella spp.* and 1,007 as *Brucella suis bv. 3*. The alpha diversity indices for the *D. marginatus*_WKR_Taskala sample were: Shannon = 0.797, Simpson 1−D = 0.473, Margalef = 0.241. Ticks are recognised as the main carriers of a wide range of diseases among domestic and wild animals compared to other arthropods. Despite numerous studies on the link between brucellosis transmission and ticks, the exact role of ticks in the transmission of this disease and the associated risks remain unclear.

Keywords: tick, *Dermacentor marginatus*, 16S rRNA, metagenomics, *Brucella*, alpha-diversity

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ANTIBODY RESPONSE TO *TRYPANOSOMA EVANSI* IN CAMELS OF KAZAKHSTAN

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Abstract. Surra, caused by *Trypanosoma evansi*, is a major constraint to camel health and productivity in arid and semi-arid regions. Despite Kazakhstan's large camel population, peer-reviewed data on surra epidemiology remain scarce.

A cross-sectional seroepidemiological survey was conducted in two camel-breeding regions of Kazakhstan (Mangystau and Kyzylorda) during 2024 and 2025. Serum samples (n = 2,745 in 2024; n = 2,900 in 2025) were tested for anti-*T. evansi* antibodies using the complement fixation test (CFT) and the formol gel test (FGT). Seroprevalence was expressed with 95% confidence intervals (CI), and regional/temporal differences were assessed using Pearson's chi-square test.

In Mangystau, CFT prevalence decreased from 5.0% (95% CI: 2.15–11.18) in 2024 to 0.78% (95% CI: 0.21–2.82) in 2025 (p = 0.0319), while FGT positivity declined from 65.0% to 5.88% (p < 0.001). Conversely, in Kyzylorda, CFT prevalence increased significantly from 4.0% (95% CI: 3.32–4.82) to 8.88% (95% CI: 7.87–10.01; p < 0.001), whereas FGT values rose slightly from 7.8% to 8.96% without statistical significance (p = 0.1504).

This study provides one of the first systematic multi-year assessments of *T. evansi* circulation in

camels in Kazakhstan, revealing contrasting regional dynamics, with a sharp decline in Mangystau and a significant increase in Kyzylorda. These findings underscore the heterogeneous nature of surra epidemiology and highlight the need for continued surveillance and combined diagnostic approaches to inform control strategies

Keywords: Camel (*Camelus dromedarius*), *Trypanosoma evansi*, Surra, Antibodies, Complement fixation test (CFT), Formol gel test (FGT), Seroepidemiology

Introduction

Camel husbandry plays an important role in the agricultural economy and traditional livelihoods of Kazakhstan, providing meat, milk, wool, and transport for pastoral communities. However, camel health and productivity are constrained by infectious diseases, particularly parasitic infections such as trypanosomosis. Surra, caused by *Trypanosoma evansi (T. evansi)*, is a chronic vector-borne disease affecting multiple domestic animal species, with camels serving as the principal hosts in many arid and semi-arid regions. The disease is associated with anemia, weight loss, reproductive disorders, and mortality, resulting in substantial economic losses in camel-breeding systems worldwide [1,2].

Trypanosoma evansi is transmitted mechanically by biting flies and is currently regarded as the most widely distributed pathogenic trypanosome. Its prevalence varies considerably depending on ecological conditions, host characteristics, and diagnostic methods, with serological surveys in camels frequently reporting seropositivity rates ranging from 10% to 30% [1,2,8]. In addition to its veterinary importance, sporadic cases of human infection have been reported, indicating zoonotic potential [9].

Despite the large camel population in Kazakhstan, data on the epidemiology of surra remain limited. Systematic peer-reviewed studies addressing the regional distribution and temporal dynamics of *T. evansi* infection in camels are scarce, which hampers accurate risk assessment and the development of effective surveillance and control strategies. Existing information is largely fragmentary and insufficient to characterize differences between regions with distinct ecological and management conditions.

Serological methods remain the most practical tools for large-scale surveillance of surra in camel populations, particularly in resource-limited settings. The complement fixation test and the formol gel test are widely used for epidemiological monitoring due to their technical simplicity and applicability under field conditions, although they differ in sensitivity and specificity [7,8].

The present study therefore aimed to investigate the seroprevalence of *T. evansi* antibodies in camels from different regions of Kazakhstan during 2024 and 2025, and to compare spatial and temporal patterns using complementary serological assays.

Materials and Methods

Study area and design: A cross-sectional seroepidemiological survey was conducted to investigate the prevalence of *T. evansi* infection in camels across major camel-breeding regions of Kazakhstan (Mangystau and Kyzylorda regions) during two consecutive years, 2024 and 2025. The study included administrative regions known for high camel population density, representing both desert and semi-desert ecological zones typical for camel husbandry. Sampling sites were selected in collaboration with regional veterinary services to ensure broad geographic coverage.

Sample collection: Whole blood samples were collected from camels by jugular venipuncture into plain vacutainer tubes. Animals older than one year and not subjected to anti-trypanosomal treatment in the last six months were included in the study. After collection, blood samples were allowed to clot at ambient temperature, and sera were separated by centrifugation at 1,500 × g for 10 min. The sera were transferred into cryovials and stored at –20 °C until further testing.

Serological testing: Serological detection of antibodies against *T. evansi* was performed using the complement fixation test (CFT) and the formol gel test (FGT), following protocols recommended by the World Organisation for Animal Health (WOAH) [10]. Antigen preparations and positive/negative control sera were included in each batch to ensure assay validity.

In the CFT, results were expressed according to the degree of hemolysis inhibition (0–4+ scale), with titres ≥1:5 considered positive [11].

The FGT was used as a screening tool, with visible gel formation within 30 minutes considered a positive reaction.

Statistical analysis: Seroprevalence was calculated as the proportion of antibody-positive samples among the total number tested, with 95% confidence intervals (CI) estimated using the Wilson score method. Differences in prevalence between years (2024 vs. 2025) and between regions (Mangystau and Kyzylorda) were assessed using Pearson's chi-square test (χ^2). The strength of association between categorical

variables (year, region) and seropositivity was evaluated by calculating Cramér's V coefficient. Statistical significance was defined as $p < 0.05$. Data were processed using Microsoft Excel 2021 (Microsoft Corp., Redmond, WA, USA).

Ethics statement: All sampling procedures were carried out by trained veterinarians in accordance with national animal welfare regulations. The study protocol was reviewed and approved by the Biological Ethics Committee of the Kazakh Scientific Research Veterinary Institute (Approval No. 1, dated 14 July 2023).

Results

A total of 2,745 camel serum samples were analyzed in 2024 and 2,900 in 2025 from two major camel-rearing regions of Kazakhstan (Mangystau and Kyzylorda). Serological testing using the complement fixation test (CFT) and the formol gel test (FGT) revealed clear spatial and temporal variations in *T. evansi* seroprevalence (Table 1).

Table 1 – Seroprevalence of T. evansi in camels by region and year (CFT and FGT assays, 2024–2025)

Year	Region	Number tested (N)	CFT-positive (n, %) [95% CI]	FGT-positive (n, %) [95% CI]
2024	Mangystau	100	5 (5.0%) [2.15–11.18]	65 (65.0%) [55.25–73.64]
	Kyzylorda	2,645	106 (4.0%) [3.32–4.82]	207 (7.8%) [6.86–8.91]
2025	Mangystau	255	2 (0.78%) [0.21–2.82]	15 (5.88%) [3.58–9.53]
	Kyzylorda	2,645	235 (8.88%) [7.87–10.01]	237 (8.96%) [7.92–10.13]

Note: FGT, Formol gel test; CFT, Complement fixation test.

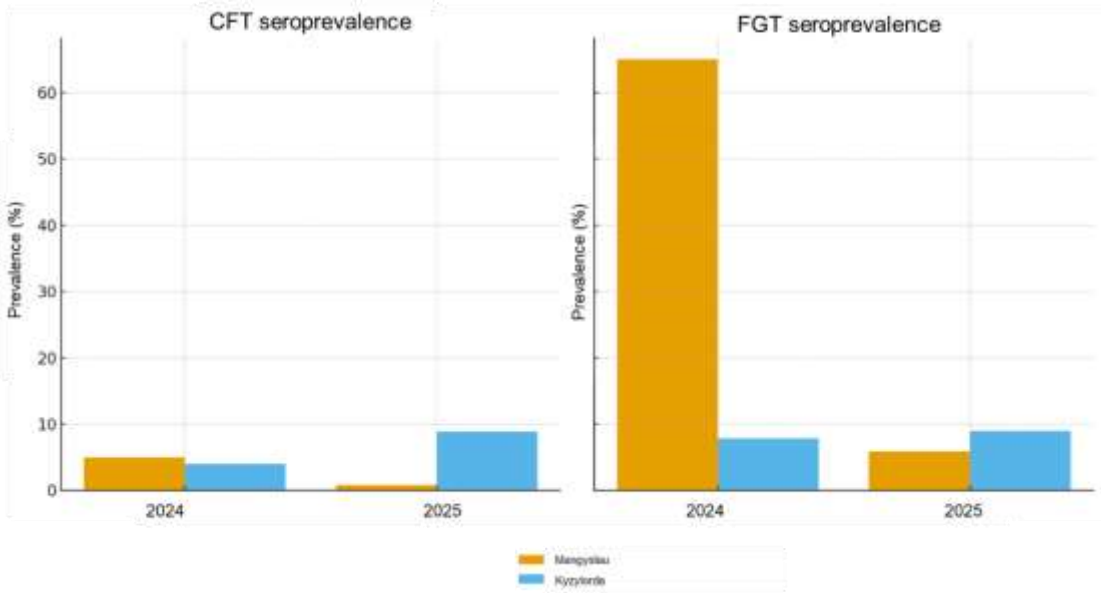

Figure 1 – Seroprevalence of Trypanosoma evansi in camels from Mangystau and Kyzylorda regions of Kazakhstan in 2024 and 2025 as determined by complement fixation test (CFT, left) and formol gel test (FGT, right)

In Mangystau Region, the CFT detected antibodies in 5.0% of camels (95% CI: 2.15–11.18) in 2024, which significantly decreased to 0.78% (95% CI: 0.21–2.82) in 2025 ($\chi^2 = 4.60$, $p = 0.0319$). Similarly, FGT results showed a dramatic decline from 65.0% (95% CI: 55.25–73.64) in 2024 to only 5.88% (95% CI: 3.58–9.53) in 2025 ($\chi^2 = 140.44$, $p < 0.001$). These results indicate a sharp reduction in *T. evansi* seropositivity within the region over one year.

In Kyzylorda Region, the CFT revealed 4.0% seropositivity (95% CI: 3.32–4.82) in 2024, which significantly increased to 8.88% (95% CI: 7.87–10.01) in 2025 ($\chi^2 = 51.36$, $p < 0.001$). In contrast, the FGT

results showed a slight increase from 7.8% (95% CI: 6.86–8.91) to 8.96% (95% CI: 7.92–10.13) across the same period; however, this difference was not statistically significant ($\chi^2 = 2.07$, $p = 0.1504$).

Overall, the findings suggest divergent regional dynamics, with a pronounced decline in both CFT and FGT seroprevalence in Mangystau and a significant increase in CFT-detected seropositivity in Kyzylorda, as shown in Figure 1.

Discussion

The present study provides new insights into the seroepidemiology of camel trypanosomosis caused by *Trypanosoma evansi (T. evansi)* in Kazakhstan and demonstrates pronounced spatial and temporal differences between the Mangystau and Kyzylorda regions during 2024–2025. The most notable finding is the sharp decline in seroprevalence in Mangystau contrasted with a significant increase in Kyzylorda, particularly when assessed by the complement fixation test (CFT).

Several factors may explain the observed decrease in seropositivity in Mangystau. This region is characterized by an arid climate, sparse vegetation, and relatively low density of hematophagous flies, which may limit the mechanical transmission of *T. evansi* [1]. In addition, local veterinary practices, including targeted treatment of clinically suspected animals, could have contributed to a reduction in infection pressure at the population level. Although detailed data on preventive and therapeutic measures were not available for the present study, similar declines in seroprevalence following control interventions have been reported in camel populations from other endemic regions [3,4].

A particularly pronounced reduction was observed in formol gel test (FGT) positivity in Mangystau. This finding requires cautious interpretation, as the FGT is known to be a highly sensitive but poorly specific assay. The FGT may detect residual antibodies persisting after previous exposure or treatment, as well as nonspecific serum protein changes [5]. Consequently, a marked decrease in FGT positivity may reflect not only a true reduction in transmission but also changes in antigenic stimulation or improvements in herd health status. In contrast, the CFT is considered more specific and is more likely to reflect sustained or recent immune responses. The parallel decline in both tests therefore supports the hypothesis of reduced circulation of *T. evansi* in this region.

In Kyzylorda, the increase in CFT seroprevalence suggests ongoing or intensified circulation of the parasite. This region hosts one of the largest camel populations in Kazakhstan and is characterized by frequent herd movements, shared grazing areas, and proximity to riverine and irrigated zones that may favor vector activity. The lack of a statistically significant increase in FGT positivity, despite a clear rise in CFT values, further highlights the differing diagnostic characteristics of the two assays and suggests that CFT may be more informative for detecting changes in active transmission dynamics.

The discrepancy between CFT and FGT results observed in this study is consistent with previous reports. The FGT tends to overestimate seroprevalence due to its susceptibility to cross reactions and persistence of antibodies following past infections or treatment. Conversely, the CFT, while less sensitive, offers higher specificity and is therefore better suited for confirmation of seropositive cases [6,7]. Differences in sample quality and handling may also contribute to variability between tests. Factors such as storage conditions, repeated freeze thaw cycles, and delays between blood collection and serum separation may disproportionately affect FGT results compared with CFT [8,9].

Another important consideration is the widespread use of trypanocidal drugs in camel herds. Chemotherapeutic treatment can reduce parasitemia and clinical signs while antibodies remain detectable for prolonged periods. This situation complicates the interpretation of serological data and may partly explain discordant test results, particularly in endemic areas where repeated exposure and treatment are common.

Taken together, these findings indicate that sharp differences between CFT and FGT results should not be interpreted as methodological inconsistencies but rather as a reflection of the distinct diagnostic properties of the tests and the complex epidemiology of surra. The combined use of both assays provides a more comprehensive picture of population exposure and immune status than either test alone.

Future studies should incorporate molecular diagnostic approaches, such as PCR based detection of *T. evansi* DNA, to confirm active infections and to distinguish between current and past exposure. In addition, entomological investigations and longitudinal sampling across different seasons would improve understanding of transmission dynamics and the role of vectors in different ecological settings. Such integrated approaches are essential for refining surveillance strategies and improving control of camel trypanosomosis in Kazakhstan.

Conclusion

This study provides the first multi-year seroepidemiological data on *Trypanosoma evansi* infection in camels in Kazakhstan. The results revealed contrasting dynamics between regions, with a marked decline in seroprevalence in Mangystau and a significant increase in Kyzylorda. These findings highlight the heterogeneous nature of surra circulation and underline the need for continuous surveillance, combined diagnostic approaches, and region-specific control strategies to protect camel health and productivity.

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Conflict of interest: The authors declare that they have no conflict of interest.

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ҚАЗАҚСТАНДАҒЫ ТҮЙЕЛЕРДЕ *TRYPANOSOMA EVANSI*-ГЕ ҚАРСЫ АНТИДЕНЕЛІК ЖАУАП

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Аннотация. *Trypanosoma evansi* қоздыратын су-ауру ауруы шөлейт және жартылай шөлейт аймақтарда түйелердің денсаулығы мен өнімділігіне елеулі зиян келтіретін негізгі факторлардың бірі болып табылады. Қазақстанда түйе санының көп болуына қарамастан, су-ауру эпидемиологиясы бойынша рецензияланатын ғылыми деректер шектеулі.

2024 – 2025 жылдары Қазақстанның түйе шаруашылығы дамыған екі өңірінде – Маңғыстау және Қызылорда облыстарында – көлденең сероэпидемиологиялық зерттеу жүргізілді. Қан сарысуының үлгілері (2024 ж. – n = 2 745; 2025 ж. – n = 2 900) *T. evansi*-ге қарсы антиденелердің болуына комплементті байланыстыру реакциясы (КБР) және формол-гель сынамасы (ФГС) әдістері арқылы зерттелді. Серопревалеттілік 95% интервалдарымен (СИ) есептелді, ал аймақтық және уақытша айырмашылықтар Пирсонның χ^2 критерийі арқылы бағаланды.

Маңғыстау облысында КБР бойынша серопревалеттілік 2024 жылы 5,0% (95% СИ: 2,15 - 11,18) көрсеткішінен 2025 жылы 0,78%-ға (95% СИ: 0,21-2,82) дейін төмендеді ($p = 0,0319$), ал ФГС бойынша оң нәтижелердің үлесі 65,0%-дан 5,88%-ға дейін азайды ($p < 0,001$). Керісінше, Қызылорда облысында КБР бойынша серопревалеттілік 4,0%-дан (95% СИ: 3,32 - 4,82) 8,88%-ға дейін (95% СИ: 7,87-10,01) сенімді түрде артты ($p < 0,001$), ал ФГС көрсеткіштері 7,8%-дан 8,96%-ға дейін шамалы өсіп, статистикалық тұрғыдан мәнді болмады ($p = 0,1504$).

Бұл зерттеу Қазақстандағы түйелер арасында *T. evansi* айналымына жүргізілген алғашқы жүйелі көпжылдық бағалаулардың бірі болып табылады және өңірлік деңгейде қарама-қайшы динамиканы – Маңғыстау облысында айқын төмендеуді және Қызылорда облысында елеулі өсуді – анықтайды. Алынған нәтижелер су-аурудың эпидемиологиясының гетерогенді сипатын көрсетіп, ауруды бақылау стратегияларын жетілдіру үшін тұрақты эпидемиологиялық мониторинг пен біріктірілген диагностикалық тәсілдерді қолдану қажеттігін негіздейді.

Түйін сөздер: түйе (*Camelus dromedarius*), *Trypanosoma evansi*, су-ауру, антиденелер, комплементті байланыстыру реакциясы (КБР), формол-гель сынамасы (ФГС), сероэпидемиология.

АНТИТЕЛЬНЫЙ ОТВЕТ НА *TRYPANOSOMA EVANSI* У ВЕРБЛЮДОВ КАЗАХСТАНА

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Аннотация. Болезнь су-ауру, вызываемая *Trypanosoma evansi*, является одним из основных ограничивающих факторов, влияющих на здоровье и продуктивность верблюдов в аридных и полуаридных регионах. Несмотря на значительную численность верблюдов в Казахстане, данные рецензируемых исследований по эпидемиологии су-ауру остаются ограниченными.

В 2024-2025 гг. было проведено поперечное сероэпидемиологическое исследование в двух верблюдоводческих регионах Казахстана – Мангистауской и Кызылординской областях. Образцы сыворотки крови (n = 2 745 в 2024 г.; n = 2 900 в 2025 г.) были исследованы на наличие антител к *T. evansi* с использованием реакции связывания комплемента (РСК) и формол-гель теста (ФГП). Серопревалентность рассчитывали с 95%-ными доверительными интервалами (ДИ), а региональные и временные различия оценивали с применением критерия χ^2 Пирсона.

В Мангистауской области распространённость по РСК снизилась с 5,0% (95% ДИ: 2,15-11,18) в 2024 г. до 0,78% (95% ДИ: 0,21-2,82) в 2025 г. ($p = 0,0319$), при этом доля положительных результатов ФГП сократилась с 65,0% до 5,88% ($p < 0,001$). Напротив, в Кызылординской области распространённость по РСК достоверно возросла с 4,0% (95% ДИ: 3,32-4,82) до 8,88% (95% ДИ: 7,87-10,01; $p < 0,001$), тогда как показатели ФГП увеличились незначительно – с 7,8% до 8,96% и не достигли статистической значимости ($p = 0,1504$).

Настоящее исследование представляет собой одну из первых систематических многолетних оценок циркуляции *T. evansi* среди верблюдов в Казахстане и выявляет контрастную региональную динамику, характеризующуюся резким снижением показателей в Мангистауской области и их значительным ростом в Кызылординской области. Полученные данные подчёркивают гетерогенный характер эпидемиологии су-ауру и указывают на необходимость продолжения эпидемиологического надзора и применения комбинированных диагностических подходов для разработки эффективных мер контроля.

Ключевые слова: верблюд (*Camelus dromedarius*), *Trypanosoma evansi*, сурра, антитела, реакция связывания комплемента (РСК), формол-гелевая проба (ФГП), сероэпидемиология.

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РАЗРАБОТКА И ИССЛЕДОВАНИЕ МАЗИ НА ОСНОВЕ ГУСТОГО ЭКСТРАКТА ЛАБАЗНИКА ОБЫКНОВЕННОГО (*FILIPENDULA VULGARIS M.)*

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Аннотация. Проведена фармацевтическая разработка мази на основе густого экстракта лабазника обыкновенного (*Filipendula vulgaris M.),* полученного методом ультразвуковой экстракции. Использование ультразвукового воздействия позволило повысить эффективность извлечения биологически активных веществ, уменьшить время экстрагирования и сохранить термолабильные компоненты растительного сырья.

Химический состав густого экстракта изучен методом газовой хромато-масс-спектрометрии (ГХ/МС). В результате анализа установлено наличие комплекса соединений фенольной природы, флавоноидов, органических кислот, эфиров и других вторичных метаболитов, обладающих выраженной антиоксидантной и противовоспалительной активностью. Полученные данные подтверждают фармакологическую значимость экстракта и целесообразность его применения в качестве действующего ингредиента при создании мягких лекарственных форм.

На основе исследованного экстракта разработано пять модельных составов мази, отличающихся соотношением компонентов основы и концентрацией активного вещества. Для каждой модели проведена оценка физико-химических, органолептических и реологических характеристик. Определены такие показатели, как консистенция, устойчивость к расслаиванию, pH, вязкость, а также равномерность распределения экстракта в мазевой массе. На основании совокупности полученных данных выделен оптимальный состав, обеспечивающий стабильность структуры и фармакотехнологические свойства, соответствующие требованиям к мягким лекарственным формам.

Проведены токсикологические исследования и фармакологические испытания разработанных образцов. Установлено отсутствие признаков острой токсичности и раздражающего действия при наружном применении. Экспериментальная оценка противовоспалительной активности выявила достоверное снижение воспалительных реакций, что свидетельствует о выраженном терапевтическом потенциале разработанной мази.

Таким образом, результаты исследования подтверждают, что густой экстракт *Filipendula vulgaris* M. является перспективным растительным источником биологически активных веществ для создания мягких лекарственных форм с противовоспалительным действием. Разработанная мазевая композиция отличается стабильностью, безопасностью и фармакологической эффективностью, что позволяет рассматривать её как основу для дальнейших доклинических и технологических исследований с целью внедрения в фармацевтическую практику.

Ключевые слова: Лабазник обыкновенный, *Filipendula vulgaris* M., ультразвуковая экстракция, густой экстракт, мазь, фармацевтическая разработка, противовоспалительная активность, токсикологическая оценка.

Введение

Современное развитие фармацевтической науки направлено на поиск и внедрение эффективных, безопасных и доступных лекарственных средств, в частности препаратов растительного происхождения. Это соответствует приоритетам Целей устойчивого развития ООН, в том числе ЦУР 3 «Хорошее здоровье и благополучие» и ЦУР 12 «Ответственное потребление и