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DETERMINATION OF TRICHOPHYTOSIS OF CATTLE BY POLYMERASE CHAIN REACTION METHOD

ANNOTATION

In recent years, the increasing veterinary and biomedical importance of dermatomycosis infection [1, 2, 3], in particular fungi of the genus Trichophyton [4, 5, 6], has been noted everywhere. This is due to the increased incidence of diseases caused by them, as well as their atypical forms, which are difficult to differentiate with mycoses of other etiologies and diseases of non-fungal origin [7, 8]. **At the same time, the importance of laboratory diagnostic methods increases significantly [9]; however, in practice, their actual informativeness is relatively low [10] due to the slow growth of dermatomycosis pathogens on nutrient media and the frequent formation of atypical forms of these fungi as a result of previous (usually local) antifungal therapy [11, 12].**

In this regard, the need for new, more effective ways of detecting dermatomycetes in biological substrates and methods of their identification is of particular importance. In this context, molecular genetic diagnostic technologies based on polymerase chain reaction seem to be the most promising, as it has been shown that they combine both high specificity and high sensitivity and can be used for reliable species-specific detection of some dermatomycosis pathogens [13, 14, 15].

Key words: *Trichophyton verrucosum, polymerase chain reaction, sensitivity, specificity, diagnostic efficiency.*

Introduction Dermatomycoses (dermatophytosis) - superficial diseases of the skin and its appendages, caused by microscopic fungi - dermatomycetes (dermatophytes), today remain an urgent problem of veterinary medicine and medicine in general [16].

Dermatomycosis is spread by contact (direct and indirect contact). The disease is transmitted by direct contact with the source of mycotic infection (sick person, sick animal or carrier) or by contact with environmental objects contaminated with dermatomycetes [16].

Currently, there is continued interest in dermatomycoses caused by zoophilic fungi of the genus Trichophyton.

On the territory of the Republic of Kazakhstan, where there are livestock farms it is always possible to meet infection of animals with trichophytosis, mainly up to 35-40 percent among young farm animals. Therefore, routine vaccination of animals against fungal infection is necessary [17].

One of the most difficult questions in the epizootology (epidemiology) of zooanthroponotic dermatomycoses is to determine the source of infection [18].

In the etiologic structure of trichophytosis, the pathogen Tr.verrucosum was more often present [19, 20, 21, 22, 23, 24, 25]. The source of infection for trichophytosis caused by Tr.verrucosum was cattle in most cases [23, 24, 25].

To date, a classification of mycoses that fully meets the needs of the practicing physician has not yet been proposed. Among mycoses there are acute and chronic (by course), superficial and deep (by depth of skin and mucous membrane lesions), localized and disseminated forms [26, 27, 28, 29, 30].

As already noted, zooanthroponous dermatomycoses continue to be relevant at present [31, 32].

Trichophytia is a fungal disease of the skin and coat caused by various species of fungi of the genus Trichophyton [16].

In recent years, many researchers have noted changes in the clinical picture of zooanthroponous dermatomycoses, the appearance of their erased and atypical forms. Low-symptomatic, erased, sluggish forms of microsporia of smooth skin are usually mistaken for manifestations of seborrheic dermatitis, seborrhea, streptoderma, chronic trichophytia [33, 34].

In the context of the above, it seems promising to use methods for the diagnostic detection of the causative agent of trichophytia using molecular genetic methods, in particular polymerase chain reaction, characterized by exceptionally high sensitivity and specificity [36]. At the same time, time costs can be significantly reduced and it becomes possible to express detection of the pathogen in various clinical samples.

Currently, the possibility of using polymerase chain reaction (PCR) in the diagnosis of mycoses is being widely and intensively studied. The diagnostic sensitivity, specificity and speed of the reaction often exceed those of microscopic and cultural methods of examining pathological material [37].

The PCR method has a number of advantages over other methods of laboratory diagnostics.

1. Direct determination of the presence of pathogens. The identification of a specific DNA site of the pathogen by PCR gives a direct indication of the presence of the pathogen in the clinical material [37].

2. The high specificity of the PCR method is due to the fact that a unique DNA fragment characteristic only of this pathogen is revealed in the studied material [37].

3. The high sensitivity of the PCR method makes it possible to detect even minimal amounts of nucleic acids of microorganisms in clinical material with a negative response from bacteriological and microscopic methods [37].

4. The universality of the procedure for detecting various pathogens. The PCR method is based on the identification of DNA or RNA fragments that are specific to a particular microorganism. The universality of the genetic code of all nucleic acids allows the use of standardized methods of laboratory research, which makes it possible to diagnose several pathogens in one bioassay. Hair, skin scrapings, blood, serum, etc. can be used as the test material [37].

5. High speed of obtaining the analysis result. PCR analysis does not require isolation and cultivation of a culture of the pathogen, which take a long time. The standardized method of biomaterial processing and detection of reaction products, automation of the amplification process make it possible to conduct a complete analysis in 4-5 hours. In this way, it compares favorably with other laboratory methods that give delayed results.

6. The possibility of diagnosing not only acute, but also latent infections. Since the PCR method avoids the difficulties associated with growing microorganisms in the laboratory, this makes it especially effective in identifying difficult-to-cultivate, uncultivated, demanding a complex nutrient medium and persistent forms of microorganisms, which are often encountered in latent and chronic infections [37].

7. The possibility of differential species diagnostics [37].

Since the late 90s, researchers all over the world have been actively searching for methods of molecular genetic diagnosis of dermatomycosis [38, 39]. Already in 2000, it was reported that the results of PCR and the culture method coincided when using primers based on 18S rRNA and ITS for the detection of a number of pathogens of human and animal dermatomycosis [40].

In recent years, many foreign researchers have reported on the high sensitivity and specificity of the PCR method in relation to some representatives of Trichophyton and Microsporum species [41]. At the same time, research remains relevant in the direction of creating test systems for the detection of pathogens of both zoonotic and anthroponotic dermatomycoses [42, 43]. An important aspect is that the PCR method, having a higher sensitivity and specificity, will be the fastest for accurate diagnosis of dermatomycosis [44].

However, despite the possible prospects of this method, the high cost of research using test systems created abroad excludes its widespread use in dermatomycology [45]. Therefore, it seems advisable to create domestic developments in this direction.

In addition, the widespread introduction of molecular genetic diagnostic methods into the practice of healthcare and veterinary services is limited by the high cost of test systems created abroad. Russian researchers have developed methods for detecting anthropophilic dermatomycetes. The creation of domestic test systems for the detection of pathogens of zooanthroponous dermatomycosis, in particular trichophytia, seems promising enough.

In connection with the above, the purpose of this study was to study the morphophysiological and molecular features of pathogenic fungi Trichophyton verrucosum and to evaluate the informativeness of the PCR method in identifying Tr. verrucosum using objective criteria of sensitivity, specificity, diagnostic efficacy, prognostic value of the test and the values of the likelihood index in comparison with traditional methods of laboratory diagnosis of microsporia and trichophytosis.

Materials and methods of research. The material for the study was 141 samples of clinical material taken from calves with dermatomycosis from farms in Almaty, Turkestan and Kyzylorda regions. The collection of clinical material was carried out for the period from 2018-2024. The material was obtained from 98 animals with dermatomycosis.

After preliminary stratification, the body of research material was distributed into two samples – the main one and the comparison group.

43 animals with skin diseases of non-fungal origin were selected to form a control group (comparison).

Wool, skin scrapings, scales, and crusts were used as clinical material.

The collection of pathological material was carried out from the periphery of lesions of cattle that were not subjected to drug treatment. Crusts with remnants of wool, as well as a certain amount of wool, were pulled out with tweezers from infected areas (if possible less contaminated), placed in test tubes with cotton plugs or paper bags. The samples were provided with a label indicating the region, district, farm, age and degree of damage to the animal, as well as the date of taking the material for research in the department.

The material for the study was collected in sterile Petri dishes and delivered to the laboratory within 0.5-1 hour from the time of its receipt, where it was subjected to pathogen detection by PCR.

At the same time, the pathological material was studied in native preparations. A 20% KOH solution was used to clear the preparations, i.e. to dissolve the horny masses.

For microscopic examination of the hair, a small drop of 20% KOH solution was applied to a slide and the affected wool was placed into it with a dissecting needle. The drop with the hair was slightly heated over the flame of an alcohol lamp until vapors appeared above the surface of the liquid or the rim of crystals fell out along the edge of the alkali drop. After covering with a cover glass, the excess alkali was removed with filter paper. To avoid crushing the hair, the cover glass was not pressed down with a dissecting needle.

Epidermis scales were also placed on a slide in a drop of 20% KOH solution and heated by adding alkali during evaporation. The cooled unpainted preparation was covered with a cover glass, the cover glass was pressed down with a dissecting needle.

The preparations were examined first under a small (× 10) magnification, and then under a large (× 40) magnification microscope.

After preliminary examination of biosubstrates under a microscope, clinical material (wool, skin scrapings, scales, crusts) was seeded on nutrient media.

As the main medium for the isolation of pathogens of microsporia and trichophytia, medium No. 2 GRM (Saburo) (FBG SSC PMB, Russia), selective medium 188 for the isolation of dermatophytes (HiMedia, India), as well as susloagar were used. A special medium M1026 with rice extract (HiMedia, India) was used to form characteristic morphological features and stimulate conidium formation. The crops were incubated for 2-3 weeks at a temperature of 25-30 ° C.

The sown cups were scanned on the second day after sowing in order to detect the onset of growth of pathogens and differentiate them from environmental saprophytes that could contaminate crops.

Total DNA from clinical samples was isolated by nucleosorption (Boom, 1990). During the work, 300 µl of a lysing solution (5M guanidine thiocyanate, 1% TritonX100) was added to disposable test tubes. Next, the required amount of material was added to the tubes with the lysing solution. The samples were thoroughly mixed on a vortex (BioSan, Latvia) and heated for 5 minutes at a temperature of 65 °C. They were centrifuged on a MiniSpin microcentrifuge (Eppendorf Manufacturing Corporation, Germany) for 5 minutes at 10000 g and a filler liquid was used to isolate DNA, transferring it to a new test tube. 25 µl of resuspended sorbent (silica gel) was added to each tube with a separate tip and mixed on a vortex. Then the tubes were placed in a tripod for 2 minutes, mixed again and left in the tripod for 5 minutes. Next, the sorbent was deposited in test tubes by centrifugation for 30 seconds at 2000 di, the filler liquid was removed using an OM-1 vacuum suction device (Utes LLC, Russia) and a separate tip for each sample. 300 ml of washing solution (5M guanidine thiocyanate) was added to the samples and mixed on a vortex until the sorbent was completely resuspended. The sorbent was precipitated by centrifugation for 30 seconds at 2000 g. The filler fluid was removed using a vacuum suction device. Then 950 ml of washing solution (70% ethyl alcohol) was added to the samples, stirred on a vortex until the sorbent was completely resuspended and centrifuged for 30 seconds at 2000 g. The filler liquid was removed, the tubes were placed in a thermostat (BioSan, Latvia) for 5-10 minutes to dry the sorbent at a temperature of 65 ° C. At the same time, the caps of the test tubes were left open. Next, 50 microliters of TE buffer for DNA elution were added to the tubes, mixed and placed in a thermostat at 65 ° C for 5 minutes, periodically shaking on a vortex. After that, the tubes were centrifuged for 1 min at 10000 g. As a result, a filler liquid containing purified DNA was obtained, ready for PCR.

The PCR method was used for selective in vitro amplification of certain DNA sites. The reaction mixture with a volume of 30 µl contained 1 µl of total DNA, 3 µl of a 10-fold buffer for Taq polymerase supplied in a kit with the enzyme used, 3 µl of dNTP, 1 µl of each primer and 1 µl of Taq polymerase (SibEnzyme, Russia). The DNA content in the samples ranged from 2 to 37 ng/µl. In order to avoid evaporation of the liquid, 50 µl of mineral oil was layered on the surface of each reaction mixture. PCR was performed in a Tertsik amplifier (DNA Technology, Russia). At the initial stage, DNA denaturation was performed at 94 ° C for 2 minutes, followed by 25-30 amplification cycles, each of which included a DNA detanuration stage for 20 seconds at 94 ° C, a primer annealing stage lasting 20 seconds at a temperature from 58 ° C to 64 ° C (depending on the length and the nucleotide sequences of the primers used), and the elongation stage for 10-20 seconds at a temperature of 72 ° C, optimal for the operation of Taq polymerase. The final stage of amplification, namely, the "elongation" of the amplicons was carried out at 72 ° C for 1 minute. In some cases, a "hot start" was used to optimize PCR and reduce the number of non-specific amplification products. According to this procedure, Taq polymerase, diluted in 10 µl of the corresponding 1-fold buffer, was introduced into the reaction mixture only after heating the latter to 94 ° C, which excluded the possibility of non-specific annealing of primers on nucleotide sequences with low homology. The amplification results were analyzed after electrophoretic separation of the products.

To prepare the agarose gel, a calculated amount of agarose powder (1.5 g) was added to a measured volume of an electrophoretic buffer (2 ml of 50x TAE buffer and 100 ml of purified water). The mixture was heated in a microwave oven until the gel was completely melted (2-2.5 min). The solution was cooled to 50 ° C, and a cleanly washed glass plate was placed in the filling chamber. The edges of the chamber were treated with cooling agarose to avoid further leakage of agarose from the chamber. A plastic comb was mounted on one of the edges of the camera so that its teeth formed holes in the gel for DNA samples. A warm agarose solution with a thickness of no more than 5-6mm was carefully poured into the mold. 1 liter of 1-fold TAE buffer was prepared. After complete hardening of the agarose (15-20 min) carefully took out the comb, trying not to damage the formed pockets. A plate with gel from the chamber was placed in an electrophoretic chamber. A buffer was poured into the chamber so that it covered the agarose from above with a thin layer of 2-3 mm.

10 µl were taken from vials with amplification products, 3 µl of bromophenol blue dye with xylenzanol and glycerin were added. They were mixed with a pipette. The dye samples were slowly applied to the gel wells under the buffer layer. The terminals of the device were connected to the power source so that (–) was at the start, and (+) was at the finish. The power supply was turned on and the voltage was set to 120 V, DNA separation was performed for 30-40 minutes. A plate with gel was taken out and placed in a cuvette for staining. A weak solution of ethidium bromide was poured into the cuvette. They were stained for 10-15 minutes. The dye was poured into a flask. The gel was washed with

running water. It was placed on the transilluminator glass. Next, the gel was photographed on the GelCameraSystem photosystem (UVP, Inc., USA).

Data on dermatomycetes detected in clinical material using microscopic and cultural methods, as well as by PCR, were entered into an electronic database in the form of an object-feature table. These data were subjected to step-by-step mathematical and statistical processing.

To conduct a comparative analysis of the informativeness of the Tr. verrucosum detection methods used, indicators of specificity, sensitivity, diagnostic effectiveness, prognostic value and likelihood ratio were calculated [36, 46, 47].

The clinical material, first of all, was examined by regulated methods of laboratory diagnostics. As a result of cultural and morphological studies, 141 samples of dermatomycetes were detected in 98 (69.5%) cases, and 43 (30.5%) samples, pathogens of dermatomycosis were not detected.

Results. One of the unsolved problems of molecular diagnostic detection of mycotic pathogens is the low efficiency of high-quality DNA separation methods due to the presence of chitin in the cell walls of micromycetes.

Considering that the effectiveness of PCR is largely determined by the quality and quantity of the isolated DNA, he gave a comparative assessment of the effectiveness of several approaches to separating it from samples in order to determine the optimal method for working with clinical samples in which the composition of fungi varies widely and which usually contain mysterious amplification inhibitors.

The following methods were used for this purpose:

- salt extraction;
- phenol-chloroform extraction;
- nucleosorption.

To increase the depth of solubility (lysis), in addition to the extract of the clinical sample, 15 μl of chitinase (Sigma-ALDRICH, Germany) was injected, despite comparable methods, at the stage of homogenization in the tissue.

As a result of the conducted scientific research, it was found that when using the phenol-chloroform extraction method, the smallest amount without DNA chitinase was released from clinical samples, when using this method, DNA release increased slightly with the addition of chitinase. The DNA salt extraction method was more effective compared to Phenol-chloroform, and, in addition, DNA yield also increased when using chitinase. The largest amount of DNA of the studied pathogenic dermatomycetes was obtained by pretreatment of samples with chitinase by nucleosorption (Fig.1).

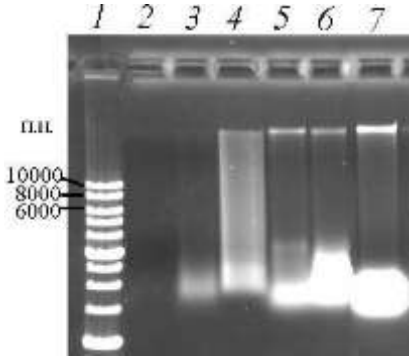

Figure 1 – Electrophoregma of the results of DNA isolation from clinical material by various methods:

1 - control (marker 1Kb, SibEnzyme, Russia);
2 - phenol-chloroform extraction method;
3 - the method of extraction with phenol-chloroform with the addition of chitinase;
4 - salt extraction method;
5- salt extraction method with the addition of chitinase;
6 - nucleosorption method;
7 - the method of nucleosorption with the addition of chitinase.

Thus, it was decided to continue using this method in DNA division.

The DNA concentration was then measured by various methods. As a result of the analysis, it was shown that the minimum DNA concentration is typical for samples isolated by phenol-chloroform extraction.

However, when using chitinase, the DNA concentration doubled, but remained low.

The highest concentration of DNA is typical for DNA samples isolated by nucleosorption, while the addition of chitinase also contributed to an increase in the concentration of DNA in the sample (Fig.2).

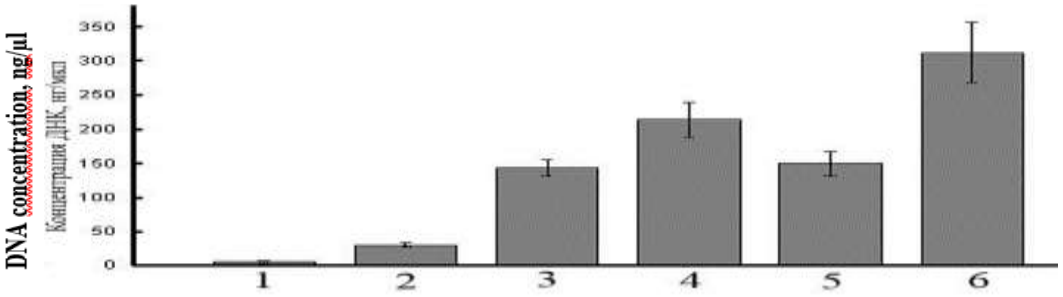

Figure - 2 DNA concentration in various separation methods:
1 - phenol-chloroform extraction method;
2 - the method of extraction with phenol-chloroform with the addition of chitinase;
3 - salt extraction method;
4 - salt extraction method with the addition of chitinase;
5 - the method of nucleosorption;
6 - the method of nucleosorption with the addition of chitinase.

The complete genome of the affected fungus Tr. verrucosum has not been sequenced, and it is almost impossible to select a unique site for PCR discrimination for each of them. Therefore, for PCR analysis of these microorganisms, it is necessary to use conservative DNA sites with changing sites.It is known that all eukaryotes 18S, 5s, 5.8 S and 28S genes encoding rRNA are the most conservative.In addition, the internal parts of the 18s, 5s, 5.8 S and 28S rrnc genes are so conservative that they do not allow us to determine which type they belong to, and the primers selected for these genes hybridize with DNA, which leads to false positive results. In this regard, the choice of primers for variable regions of these conservative genes, which are internally transcribed spacers ITS1 and ITS2, which flank 5.8 rrnk genes on both sides of these genes, is being decided (Fig.3).

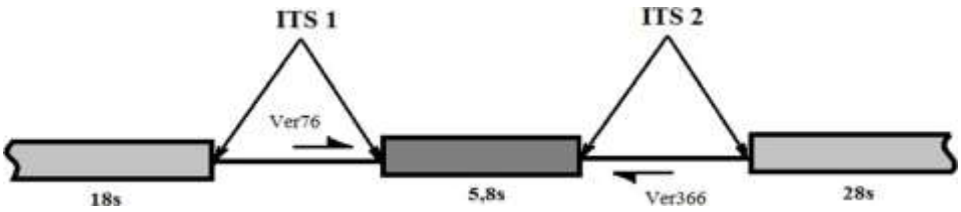

Figure 3 – Is a physical map of the DNA region, including internal transcribed spacers (ITS1, ITS2) and the 5.8 S rRNA gene primers for distinguishing Tr. verrucosum DNA (shown by arrow).

Next, GenBank searched for the nucleotide sequence of the 5.8 rRNA genes and their related its1 and ITS2.As a result of the work carried out, the nucleotide sequences of 5.8 rRNA genes with flanking ITS1 and ITS2 regions of all the studied dermatomycetes were identified.

The nucleotide sequence of Tr. verrucosum was registered in GenBank under the number Z98003, respectively.

In order to select specific primers for PCR, the alignment of the corresponding dermatomycetes using the lasergene program to determine the sequence of the 5,8 rRNA nucleotide genes and adjacent ITS2.

PCR and differentiation of Tr. verrucosum primers Ver76F/Ver366R were selected to determine the species, and the theoretically calculated amplicon size was 231 bp. The firing temperature of the primer should have been about 57 °C. The location of the annealing sites of a special primer is shown in Figure 4.

As a result of the work carried out, 1 pair of primers was selected. Theoretical studies using the primerselect program have shown that these primers do not form dimers and studs, the optimal annealing temperature exceeds 55 ° C, and the melting temperature difference did not exceed 2 ° C.

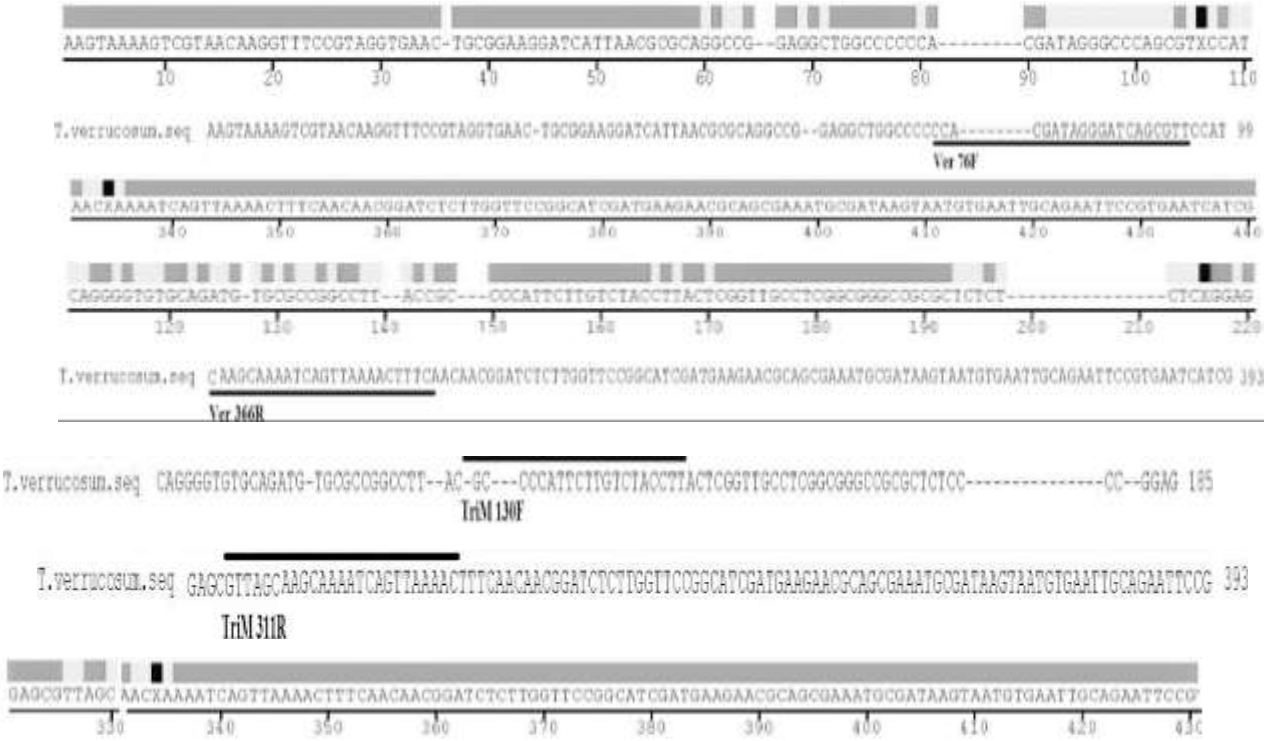

Figure 4 – M 5,8 rRNAs and adjacent ITS1 and ITS2 Tr. verrucosum, results of sequence alignment of nucleotide genes and the location of annealing sites of selected specific primers.

All this allows you to be sure that the selected primers will work in the clinical material.

All pairs of primers were synthesized and used for further experiments. The results of the work on the selection of optimal oligonucleotide primers are shown in Table 1.

The dark gray color corresponds to a complete coincidence of the nucleotide sequence, and the light gray color corresponds to medium-variable areas.

Table 1 – Theoretically calculated primer and PCR parameters selected during the work performed

The name of the primer	The sequence of nucleotides of the primer	Annealing temperature	Expected product size (Ph.D.)
Ver76F/Ver366R (TverFor/TverRev)	5'-ccacgatagggatcagcgtt-3' 5'-gaaagttttaactgattttgcttg-3'	57°C	231

From the bacteriological laboratory of the State Medical Institution "Republican Skin and Venereological Dispensary No. 1" in Ufa, a constructed primer from the museum culture Tr. verrucosum was used.

This is Tr.this allowed us to make an assumption about the possibility of using a primer developed for verrucosum.

PCR differentiation - the Ver76F/Ver366R primer was used to determine the type of Tr. verrucosum.

Next, PCR is performed using Ver76F/Ver366R primers. The type of Tr. verrucosum was determined (Fig.5).

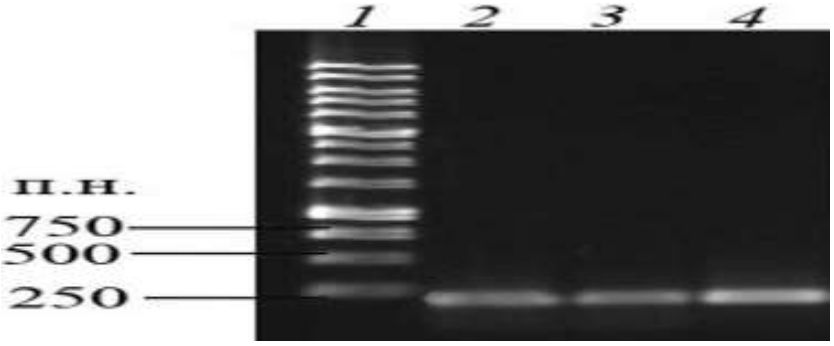

Figure 5 – Ver76F / Ver366R. 1 – when using primers. Electrophoregma of the PCR results of the ITS1 and ITS2 sites adjacent to the 5.8 S rRNA gene from the Tr. verrucosum museum culture is a molecular weight marker (1Kb marker, SibEnzyme, Russia), 2-4 are specific amplicons with a size of 231 bp.

As a result, special PCR products were obtained and sequenced. It has been shown that the sequenced site corresponds to the sequence registered in GenBank, and, consequently, the actual DNA Tr.verrucosum sites are amplified.

Work was also carried out to optimize the PCR conditions. The most optimal conditions for Ver76F/Ver366R primers were: initial denaturation 94 °C-2 min, 30 cycles – denaturation 94 ° C-20 c, annealing of primers 61 ° C-20 c, elongation 72 °c – 20 c, terminal elongation - 1 min.

After testing the selected primers, the task is to evaluate their specificity. For this purpose, PCR was established both with the DNA of the studied species and with other DNA that theoretically occur in clinical material.

In turn, Tr. when determining the specificity of the Ver76F/Ver366R primers selected for the detection of verrucosum, a positive PCR result only on Tr. verrucosum occurred with DNA (Fig.6).

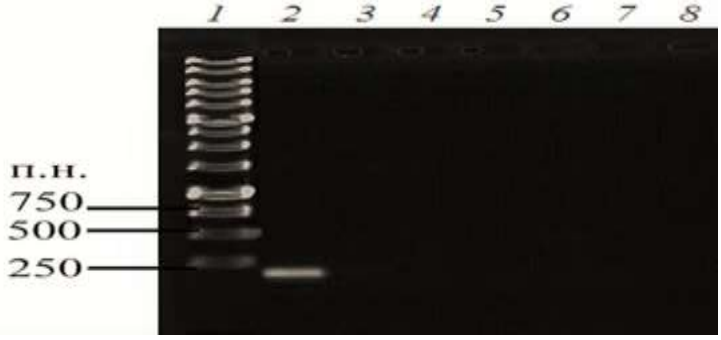

Figure 6 – PCR isolation - determination of the specificity of Ver76F/Ver366R primers selected for Tr. verrucosum:

1 - molecular weight marker (marker 1kb, SibEnzyme, Russia);

2 - positive control (Tr. verrucosum DNA).

After determining the optimal PCR conditions and confirming the specificity of the selected primers, work was carried out to determine the sensitivity of the developed test systems. For this purpose, the control DNA of Tr. verrucosum (10-1, 10-2, 10-3, 10-4 and 10-5) was prepared for 10-fold serial cultivation, each of which was studied by PCR. The concentration in the initial samples for Tr.verrucosum DNA was 433 ng/µl.

At the same time, positive PCR results were obtained by 10-1, 10-2, 10-3, 10-4 exploration even when using Ver76F/Ver366R primers. Thus, the lowest detectable DNA concentration is Tr. verrucosum - 43.3 pcg/µl.

The results obtained indicate the high sensitivity of the developed test systems.

To assess the performance of primers in the clinical material, 427 clinical samples were used, including in 234 cases by microscopic and/or growth methods. the presence of Tr. verrucosum has been established.

This growth method of Tr. verrucosum was detected in sample 189 (80.8%), Tr by microscopy. verrucosum received positive results in 204 (87.2%) cases. Tr in the study of clinical samples by DNA

PCR. It was found in verrucosum-229 (97.9%). The positive results of PCR indicate the high sensitivity and specificity of the PCR method compared with microscopic and cultural methods.

Microscopy of clinical samples showed that when smooth skin was affected, the scales contained filaments of poorly dyed thin mycelium of various lengths, 2-4 microns in diameter, straight, sometimes branching and septic. Quite often, mycelium of an uncharacteristic shape was present in the preparations, especially in samples from long-term sick animals. Polymorphic spores of irregular shape, located freely or in the form of chains, were often found. This indicated the fungal nature of the lesions, but morphological identification of pathogenic fungi in such samples proved difficult.

When examining the affected hair, attention was paid to the peculiarities of the location of the spores (inside or outside the hair) and their size.

During trichophytosis, the "enveloping" of the root part of the wool with spores from the outside and their arrangement in the form of parallel chains were noted. The diameter of the spores varied. In general, the above morphological data in the study of clinical samples, despite their number and attempts at morphometry, did not allow us to establish reliable criteria for differentiating dermatomycetes directly in clinical samples.

In the course of the conducted studies, 79 strains of dermatomycetes were obtained. When determining the species, relatively characteristic features were established, which allowed them to be attributed to the species Tr. verrucosum. The identification of dermatomycetes was quite a difficult task, this is due to the difficulties of cultivating this species. However, the formation of characteristic morphological features could be achieved by changing the cultivation conditions, namely by increasing the temperature to 37 ° C and adding thiamine to the medium.

The colonies are slow-growing, in the form of a disk with a towering leathery wrinkled center of grayish-yellow color and a powdery whitish peripheral zone. Microscopy revealed a lot of branching mycelium, micro- and macroconidia were very rare. A characteristic feature of the pathogen was the formation of terminal and intercalary chlamydospores in the form of rosaries along the mycelium.

Thus, using regulated methods, the presence of dermatomycetes was established in 98 clinical samples. However, light microscopy allowed to obtain a positive result only in 86 (87.8%) cases, and culture on nutrient media – in 79 (80.6%) cases. Despite the fact that microscopy of clinical material, as a method of preliminary diagnosis, did not allow the identification of dermatomycetes to the species, in 19 (19.4%) cases of absence of growth of the pathogen in culture, a positive result of direct microscopy was considered the only confirmation of mycotic infection. It should be noted that the diagnosis was confirmed by two regulated methods at once in only 67 (68.4%) cases, which is due to the insufficient specificity and sensitivity of these methods.

Species-specific PCR detection of Tr. verrucosum DNA in clinical material was carried out in accordance with the method developed by us. As a result, specific PCR products were obtained in 96 (98%) cases.

To assess the informative value of the PCR method for the detection of T.verrucosum in clinical material in comparison with traditional methods of laboratory diagnosis of dermatomycosis, the criteria of sensitivity, specificity, diagnostic effectiveness, prognostic value of the test and the likelihood ratio were used.

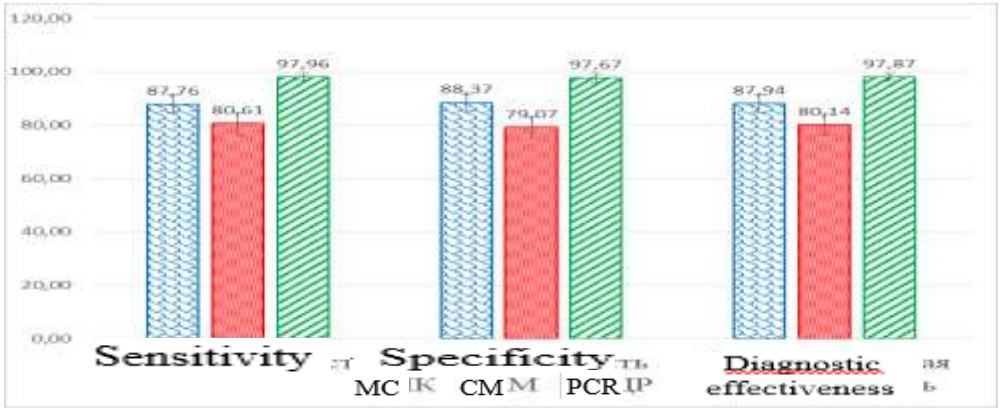

Figure 7 – Comparative assessment of sensitivity, specificity and diagnostic effectiveness of microscopy of pathological material (MC), culture method (CM) and PCR in the diagnosis of trichophytia.

The results of the studies revealed higher values of indicators for PCR. Sensitivity was 97.96% (96.53-99.39, p<0.05), specificity was 97.67% (96.15-99.19, p<0.05), and diagnostic efficacy was 97.87% (96.41-99.33, p<0.05) (Figure 7). At the same time, the probability of the presence of the disease with a positive result of PCR for trichophytia due to Tr. verrucosum, estimated through the prognostic value of a positive result (PC+) was also the highest (98.9%), as well as the value of the likelihood ratio (OP+=32.3), determining the presence of a true disease, with the lowest probability of obtaining a negative result (OP–=0.03) (Table 2).

Table 2 – Indicators of prognostic value (PC+; PC–) and likelihood ratio (OP+; OP–) of microscopy (MC), culture method (CM) and PCR in the diagnosis of trichophytia caused by Tr. verrucosum

Method	Predictive value PV(%)		Iikelihood relations IR(шанс)	
	PV+	PV–	IR+	IR–
MC	94,50549	76	7,3	0,2
CM	89,77273	64,15094	3,8	0,3
PCR	98,96907	95,45455	32,3	0,03

The data obtained indicate that the PCR method makes it possible to detect and identify Tr. verrucosum in various clinical samples and has significantly higher diagnostic efficiency, sensitivity and specificity in the laboratory diagnosis of zooanthroponous dermatomycoses, compared with microscopic and cultural methods.

Conclusion. The incidence of zooanthroponous dermatomycosis is characterized by the occurrence of periodic epizootological (epidemiological) outbreaks, both in various regions of the Republic of Kazakhstan and abroad. The instability of the epizootological (epidemiological) situation in the country is evidenced by the continued decrease in the incidence of these dermatomycoses in some territories and their growth in others. In addition, an increase in the number of atypical and erased forms currently significantly complicate the clinical diagnosis of these diseases.

It should be noted that the problem of rapid, highly specific and sensitive detection of pathogens of microsporia and trichophytia in clinical material has not been solved so far, even despite a number of currently used laboratory diagnostic methods, since their informativeness, for a number of reasons, leaves much to be desired.

Therefore, in order to form objective ideas about the extent of the spread of trichophytia, the most promising is the introduction of molecular genetic methods capable of providing highly sensitive and specific direct detection of the genetic material of the pathogen. However, PCR and its modifications for the detection of pathogens of zooanthorponous dermatomycosis are practically not used in our country and are not regulated in any way. These problems could be solved with the introduction into laboratory practice of the PCR method for detecting Tr. verrucosum in clinical material.

In the course of the work, the morphophysiological features of Tr. verrucosum cultures detected in clinical samples with mycotic lesions of the skin and coat were studied. It was shown that dermatomycetes had a relatively characteristic morphology for them, on the basis of which their differentiation was possible only in animals that had become ill relatively recently and had not received specific antimycotic drugs. Mycological examination of samples from long-term sick animals mainly revealed atypical cultures.

A comparative analysis of the information content of laboratory methods (PCR, microscopic and cultural) in the diagnosis of zooanthroponous dermatomycosis showed that PCR was characterized by the highest diagnostic efficiency (97.87% (96.41-99.33, p<0.05)) in the diagnosis of trichophytia caused by Tr. verrucosum, and provided statistically significantly higher sensitivity indicators (97.96% (96.53-99.39, p<0.05), specificity (97.67% (96.15-99.19, p<0.05)) and the prognostic value of a positive result (PC + =98.9%) compared with the results of traditional (regulated) research methods. At the same time, a high value of the "likelihood ratio" indicator increased the probability of an accurate diagnosis in PCR studies by 32.3 times, whereas microscopic and cultural studies increased the chances of an accurate diagnosis by only 7.3 and 3.8 times, respectively. It should be noted that in cases where Tr. verrucosum acted as the causative agent of the disease, the indicators of the informative value of the cultural method (sensitivity – 80.61% (76.62-84.6, p<0.05), specificity – 79.07% (74.96-83.18, p<0.05), diagnostic effectiveness – 80.14% (76.11-84.17, p<0.05) were significantly lower than the corresponding values obtained by microscopic examination (sensitivity – 87.76% (84.45-91.07, p<0.05), specificity – 88.37%

(85.13-91.61, p<0.05), diagnostic efficacy – 87.94% (84.86-91.23,p<0.05). This is due to the fact that Tr. verrucosum is difficult to cultivate. This fact makes the results obtained in the PCR study even more valuable, especially considering that cultural research is still considered the main one in the laboratory diagnosis of trichophytia.

The data obtained indicate a significantly higher diagnostic efficiency, sensitivity and specificity of the PCR method for detecting Tr. verrucosum in clinical material compared with microscopic and cultural methods.

These substantiate the need for widespread introduction of molecular genetic studies into the laboratory diagnosis of mycoses, as well as the need to optimize the laboratory component of the diagnostic subsystem not only for epizootological (epidemiological), but also for veterinary supervision and control of trichophytia. This is due to the well-known fact that animals are the main sources of infection for humans. In this regard, the adequate implementation of both types of surveillance (epidemiological and veterinary) requires, in each specific case of trichophytia, PCR detection of the pathogen, for the objective identification of epizootological (epidemiological) links between sources of infection, factors and routes of infection, identification of contaminated objects. At the same time, time costs can be significantly reduced and it becomes possible to express detection of the pathogen in various clinical samples.

As a result of our research work, we received the Patent of the Republic of Kazakhstan for the «Method of specific detection of Trichophyton verrucosum in cattle» No. 8224 dated 06/39/2023.

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Sustainable development of the agro-industrial complex and safety of agricultural products.

Ensuring veterinary safety.

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ТҮЙІН

Соңғы жылдары дерматомикозды инфекцияның ветеринарлық және биомедициналық маңыздылығының жоғарылауы байқалады [1, 2, 3], атап айтқанда Trichophyton тұқымдас саңырауқұлақтар [4, 5, 6]. Бұл аурудың көбеюіне, сондай-ақ олардың атипті түрлеріне байланысты[7, 8], оларды басқа этиологияның микоздарымен және саңырауқұлақ емес аурулармен ажырату қиын[9,10,11, 12].

Осыған байланысты биологиялық субстраттардағы дерматомицеттерді анықтаудың жаңа, тиімдірек әдістеріне және оларды анықтау әдістеріне қажеттілік ерекше маңызға ие. Сондықтан полимеразды тізбекті реакцияға негізделген молекулалық-генетикалық диагностика технологиялары ең перспективалы болып табылады, өйткені олар жоғары ерекшелікті де, жоғары сезімталдықты да біріктіретіні көрсетілген және кейбір дерматомикоз қоздырғыштарын сенімді түрге тән анықтау үшін пайдаланылуы мүмкін[13, 14, 15].

РЕЗЮМЕ

В последние годы повсеместно отмечается возрастание ветеринарной и биомедицинской значимости дерматомикозной инфекции[1, 2, 3], в частности грибов рода Trichophyton[4, 5, 6]. Это связано с увеличением заболеваемости, а также их атипичными формами[7, 8], которые трудно дифференцировать с микозами другой этиологии и заболеваниями негрибкового происхождения*[9,10,11, 12]*.

В связи с этим особое значение приобретает потребность в новых, более эффективных способах обнаружения дерматомицетов в биологических субстратах и методах их идентификации. Поэтому наиболее перспективными представляются технологии молекулярно-генетической диагностики, основанные на полимеразной цепной реакции, поскольку показано, что они сочетают в себе как высокую специфичность, так и высокую чувствительность и могут быть использованы для надежного видоспецифичного выявления некоторых возбудителей дерматомикозов[13, 14, 15].