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# TESTING METHODS FOR TUBERCULOSIS

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**ANNOTATION:**

**Identifying the causative agent of tuberculosis, sending pathological materials to laboratories, conducting laboratory tests, ensuring food safety through biosynthesis and improving disease prevention measures.**

**Keywords: tuberculosis, abscess, sick animals, risk level, epizootic processes, biochemical properties, biological preparations.**

**RELEVANCE OF THE TOPIC:**

It is important to better provide the country's population with high-quality and affordable livestock products, to increase the production of livestock products, and to improve their fodder and sanitary qualities. Despite the fact that they caused great economic damage to livestock farms, the development of measures to prevent the disease remains relevant today.

This, in turn, requires the development and implementation of effective methods of disease prevention.

**MATERIALS AND RESEARCH METHODS:**

When diagnosing tuberculosis in the laboratory, microscopic, bacteriological and biological methods are used to excrete from the nose, sputum, tracheal mucus, feces, urine. Microscopic staining is carried out according to the Sil-Nilsin method. The processed material is grown on selective nutrient media: egg starch, potato-glycerin broth, foreign microorganisms that inhibit growth.

Tuberculosis is a chronic infectious disease of humans, mammals and poultry, characterized by the formation of specific nodules (tubercles) in the affected organs and tissues.

Microbacteria are common in nature, among pathogenic and saprophytic species. Saprophytes live in soil, water bodies, manure, milk and grass. The causative agents are mainly mycobacterium tuberculosis, which cause diseases in humans and animals. Mycobacteria include leprosy in humans and paratuberculosis in large animals.

The causative agents of tuberculosis belong to the Actinomycetales family, the Mycobacteriaceae family, the Mycobactyerium genus. All mycobacteria sometimes migrate to humans or other species and cause disease.

The species M. tuberculosis, M.bovis, M. avium play an important role in the pathology of farm animals and humans. Tuberculosis is predominantly latent. The patient is examined for an allergic reaction using tuberculin in order to detect the animal in time.

The causative agent is usually a thin, straight or slightly curved rod with twisted ends. They are fickle. It varies depending on the type of bacteria, environment and growing conditions. Compared to M. tuberculosis,

M.bovis is shorter and thicker and mycobacteria are longer than those grown in animal tissues and nutrient media.

Mycobacterium tuberculosis is presented in filtered form, L-shaped mycobacteria. They can be seen in phase contrast microscopy as small grains or spherical bodies of various sizes.

Due to the hydrophobic nature of the shell, a modified Gram-Mux method is used for Gram staining of mycobacteria. The greases are heated with methyl violet carbol until steam is generated. Drain the paint and fill with Lugol's solution. Then it is decolorized, respectively, with a mixture of 5% nitric acid, 3% hydrochloric acid, acetone and alcohol. Finally, it is additionally dyed with saffron or diluted fuchsin. Under the microscope, blue mycobacteria are visible on a red background. Immunofluorescence is currently used. The causative agent of tuberculosis is highly aerobic. Optimum growth temperature: M. tuberculosis -37-38$^0$C, M. bovis-38-39$^0$C, M.avium -39-41$^0$C. pH environment 6.8-7.4. Microbes do not grow in normal nutrient media. For its cultivation, special glycerin, electroprotective and protein-free (synthetic) nutrient media are used.

Tuberculosis bacilli are more resistant to physical and chemical influences. In crops, they usually die after 8-10 months. The bacteria live 7-140 months in dried sputum, 2-6 months in decomposed organs, 7-10 months in manure, 2 months in water, more than 2 years in soil and die after 30 minutes when milk is heated to 85°C and 3- Boil for 5 minutes. Under the influence of disinfectants 5% phenol, 20% fresh slaked lime, 3-5% lysol, 3% formaldehyde, the tubercle bacillus dies after 12-24 hours. The manure is subjected to biothermal disinfection. Immunity is the nostril cells in tuberculosis.

Diagnostics includes serological and allergic tests. Bacterial diagnosis is very important. The final diagnosis is made on the basis of positive results of pathological or bacteriological examination on the farm. Determining the type of mycobacteria is necessary to find the source of the infection.
Bacteriological examinations include microscopy, culture and biological methods.

**Serological diagnosis:**

During implantation, we studied PR, AG, DPR, ABR, HR, hemolysis reactions. PR and AG didn't work in animals. Only in chickens the drip AG method gave reliable results.

**Allergic diagnostics:**

Altuberculin and dehydrated tuberculin PPD (purified protein derivative) are currently used. They are produced in biofactories. For diagnostic purposes, tuberculin is injected subcutaneously, subcutaneously, or instilled into the conjunctiva.

Cattle, sheep, goats and chickens are injected with tuberculin subcutaneously. It is directed to the neck of cattle, to the chest in calves, to the base of the tail in goats, to the inner thighs of sheep and to the base of the outer surface of the ears of pigs. Goes to the chickens in earrings. The dose is 0.2 ml for animals and 0.1 ml for poultry.

It is counted after 72 hours in cattle, after 48 hours in goats, sheep and pigs, and after 30-36 hours in poultry. In cattle, the result is positive if the skin layer is 3 mm or more thicker than normal skin.

**Pathological material:**

From sick animals, nasal discharge, sputum, tracheal mucus, feces and urine are taken. From the dead - removed parts of the affected organs, bronchial, pharyngeal, umbilical, lymph nodes.
The body of a dead bird will be sent. Upon receipt of the pathological material, it is necessary to follow the rules of asepsis,

personal prophylaxis, and technical safety. The pathological material is sent to the laboratory immediately upon receipt. If this is not possible, 30-40% of glycerin can be preserved or frozen.

**Microscopy:**

The causative agent belongs to a group of bacteria that are resistant to the action of acid-alcohol-alkalis. Its shell contains steoric acids and waxy substances. These substances do not transfer water, dyes, acids and alkalis into the cell. This is why the dye is difficult for tuberculosis bacteria to take. Specially colored by the Sil-Nielsen method.

1. Place a special filter paper on the fixed grease and fill it with TB carbolic fuchsia. An alcohol lamp is heated on a flame until steam appears and kept on the bridge for 5-7 minutes.
2. Remove the filter paper and apply 3-5% sulfuric acid solution on it for 5-7 seconds.
3. Rinse thoroughly with water.
4. Additional Leffler staining with methylene blue for 4-5 minutes.
5. Rinse off grease with water and dry with filter paper.

Under the microscope, moisture-resistant bacteria are red, and unstable bacteria are blue. Pour TB carbolic fuchsia into the grease and heat for 1-2 minutes until steam appears or boils. It is washed with water, decolorized with 5% sulfuric acid, then the grease is washed first with alcohol, then with water and stained with methylene blue.

Gram-stained grease shows gram-positive rod-shaped bacteria with a length of 1.5-5-6 microns and a diameter of 0.3-0.6 microns. M. tuberculosis is thin, slightly curved, M. bovis is short, thick, M. avium is a relatively small, polymorphic rod. One ball in grease ball. It is inactive, does not form spores and capsules. The long, ipsimon-shaped form is also found in fat prepared from the culture.

**Bacteriology:**

First, the pathological material is processed by one of the methods of Gon and Alikayev.

Ghosn's method: thoroughly crush the material in a sterile mortar and mix with an aqueous solution of 10-12% sulfuric acid in a ratio of 1: 4. The resulting suspension is centrifuged for 10-15 minutes at a speed of 3000 rpm. Exposure (acidic effect) should not exceed 20-30 minutes. The sediment is used for the preparation of ointments and for cultivation in food. For biosyns, the sediment is washed 1-2 times with sterile saline.

Alikayev's method: it is often used when the material is fresh and less contaminated. The material is ground in a sterile mortar with a volume of 0.5 cm3 and filled with an aqueous solution of 10-6% sulfuric acid. It will take 10-20 minutes. The exposure time and preservation of the acid depends on the degree of contamination of the material. After 10-20 minutes, the acid is drained, replaced with saline and left for 8 minutes. Then the saline is poured and ground thoroughly in a mortar. A suspension is prepared in physiological solution; 5-6 test tubes are inoculated into the nutrient medium.

The processed material is grown in media that inhibit the growth of foreign microorganisms - eggs, starch, potatoes - glycerin - broth, often using Lowenstein-Jensen, Gelberg media, as well as GPB and GPA glycerol.

The causative agent of tuberculosis is aerobic, slow-growing. With prolonged cultivation in glycerin broth, tuberculin toxin accumulates at 8 weeks. This substance does not affect a healthy body, but only poisons animals with tuberculosis. Used to diagnose tuberculosis. In a liquid medium, the pathogen grows and forms a shell after 10-30 days.

M. tuberculosis forms a thick membrane, M. bovis forms the reticular

membrane of the tumor, and M.avium forms a thin, thin, whitish membrane on days 7-10 with a strong wrinkle on the 21st, first dry, then the mucous membrane. In dense nutrient media, barely noticeable microcolonies are formed, and then they grow. Small or large, glossy or opaque, smooth or wide 1-2 colonies appear on the surface of the nutrient medium, or the colonies coalesce, forming a single white layer on the surface. The duration of the bacteriological study is 2 months. Results are recorded every 4-5 days.

**Biosinov:**

1 ml of the suspension is injected under the skin of the guinea pig, 2 ml - into the vein of the rabbit's ear, 1-2 ml - into the vein under the wing of the chicken. The observation period is 3 months. Animals taken for biosin are pre-tested for tuberculosis with an allergy to tuberculin. For biosynthesis, only those that gave a negative result are used. The dead animal is dissected, the characteristic tubercles are used to prepare ointments and introduced into the culture medium.

**IDENTIFICATION OF PATHOGENS:**

M.bovis culture causes generalized tuberculosis in guinea pigs and rabbits.

M. tuberculosis spreads in guinea pigs and localizes in the lungs of rabbits.

M. avium rabbits are killed by a septic process. This sometimes creates a local process. In guinea pigs, only a local process causes abscesses at the sowing site.

Three tests are carried out to differentiate low-virulent cultures from acid-fast saprophytes.

1. Catalase activity is determined by measuring gas bubble formation in mm with a 50% perhydrol solution. This property is higher than that of saprophytes.
2. Formamidase activity. The blue ring appears in culture solution treated with formamide solution and several chemicals. This is observed only in saprophytes.
3. Susceptibility to drugs is studied on a nutrient medium with the addition of tiberculostat drugs (streptomycin, ftivazide, PASK).

**CONCLUSION:**

1. Diagnosis of tuberculosis is based on clinical signs, pathological changes, epizootological data and laboratory results.
2. We protect animal and human health through early detection and development of disease control measures.
3. We will ensure economic efficiency through timely detection of tuberculosis, its correct diagnosis, isolation of animals, isolated storage, high-quality disinfection, disinsection and deratization of the farm.

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