MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE

STATE BIOTECHNOLOGICAL UNIVERSITY

Faculty of Veterinary Medicine

PHARMACOLOGY AND PARASOTOLOGY DEPARTMENT

WORKBOOK

for laboratory classes of educational discipline «Veterinary Parasitology»

for student ___ group _____ year

second master's level in speciality 211 – Veterinary medicine

Part I

_____

(Surname and Name)

Lecturer: PhD. _____

Surname

_____

Name, patronymic

Kharkiv – 2022

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Faculty of Veterinary Medicine SBTU
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Authors: Nikiforova O.V., Mazanny O.V., Fedorova O.V.

Reviewer: associate professor of department of epizootology and veterinary management, Candidate of Veterinary Sciences Savenko M.M.

Workbook for laboratory classes of educational discipline «Veterinary Parasitology» for students of IV-V years of second master's level in speciality 211 – Veterinary medicine. / Yu.O. Prykhodko, O.V. Nikiforova, O.V. Mazanny, O.V. Fedorova, P.V.Lulin − Kh., 2022. − Part I. − 63 p.

Basic foundation of general Parasitology, Veterinary protozoology and Trematodology have been stated. The data on the morphology and biology of agents of invasive disease of ruminants, horses, pigs, carnivorous, rabbits, birds and bees have mentioned.

For training at higher educational institutions III-IV accreditation level on the specialty 211 – «Veterinary medicine".

Second edition.

Translated and layout created by O.V. Nikiforova, cand. of vet. sci., associate professor of Pharmacology and Parasitology department of SBTU

CONTENTS

RECOMMENDED BOOKS

1. Timothy M. Goater, Cameron P. Goater, Gerald W. Esch. Parasitism. The diversity and ecology of animal parasites. Second edition, Cambridge, University Press, 2001, 2014, 524 p.
2. Gregory v. Lamann. Veterinary parasitology. Nova biomedical Press, Inc. New York, 2010, 323 p.
3. G.M.Urquhart, J.Armour, J.L.Duncan at all. Veterinary parasitology. The faculty of veterinary medicine, the University of Glasgow, Scotland, 2nd edition 1996, 307 p.
4. Dwight D. Bowman Charles M. Hendrix David S. Lindsay Stephen C. Barr. Feline Clinical Parasitology. Iowa State University Press. 2002. 469 c.

LABORATORY CLASS № (SELF-STUDY) **«____» _____ 20__ p.**

TOPIC: Introductory lesson

(History of the department. Methodology of studying discipline, safety measures and personal hygiene in the conditions of the department and at the enterprise. Special literature. Preparation and performance of course project.)

Class location – classroom, museum of the department

Purpose of the lesson: Definition of discipline of parasitology as a science, purpose and tasks and structural-logical scheme. The role of discipline in the formation of doctor of veterinary medicine at the current stage of development of agro-industrial complex, environmental protection and health of the population, ensuring stable epizootic welfare of farm animals in accordance with the Law of Ukraine «On Veterinary Medicine» and other legal acts of Ukraine. Place of veterinary science and practice in the prevention of animals invasive diseases.

History of the department. Methodology of studying discipline, safety measures and personal hygiene in the conditions of the department and at the enterprise. Special literature. Preparation and performance of course project.

Task: Prepare for the lesson by yourself – by recommended books.

Auditory work.

Task performance:

SHORT HISTORY OF THE PARASITOLOGY DEPARTMENT

The department of parasitology of Kharkiv Veterinary Institute was created in October, 1932 on the basis of parasitology office. Professor *S. V. Ivanitskiy* was appointed to the position of the head of the department.

A. F. Nosik was appointed to the position of the head of parasitology department of Kharkiv Veterinary Institute in July, 1941. In 1944 Kharkiv Veterinary Institute returned from the evacuation (Frunze (presently Bishkek, Kirghizia)). Doctor of veterinary science, professor *M. O. Palimpsestov* was elected the head of parasitology department in the same year.

In 1961 Kharkiv Veterinary Institute moved to Malaya Danilivka. The parasitology department got a separate, new, educational building with classrooms for practical classes, a laboratory, clinic and boxes for stationary maintenance and treatment of sick animals and a spacious manege. From that time the duties of the head fulfilled a lecturer *N. T. Litvishko*. Professor, doctor of veterinary science *V. K. Chernucha* was the 3rd head of the department of parasitology and clinical diagnostics from 1973 to 2000.

Professor *V. K. Chernucha* together with the staff of the department (Byrka V.I., Ponomarenko V. Ya., Ponomarenko A. M.) published the first in Ukraine textbook in Ukrainian language for higher educational institutions «Parasitology and invasive diseases of farm animals» in 1996.

From 2000 the parasitology department was headed by doctor of veterinary science, the well-known arachno-enthomologist *I. A. Mashkey*.

Candidate of veterinary science, a lecturer *Byrka V.I.* was elected to the position of the head of parasitology department in 2001.

Doctor of veterinary science, professor, Corresponding member of National Academy of Agricultural Sciences of Ukraine *Prihodko Yu.O*. was appointed to the position of the head of the department in 2003 by the order of Rector.

Candidates of veterinary sciences work in the department: associate professors P.V. Lyulin, O.V. Fedorova, O.V. Mazanny, O.V. Nikiforova.

METHODOLOGY OF DISCIPLINE «VETERINARY PARASITOLOGY»

Preparing for each class, the student should know:

1. Definition of each disease.
2. Place of infectious agents in the animal classification.
3. Morphological and biological peculiarities of pathogens and characteristics of their eggs or larvae. To know how they are different from other parasites.
4. Complex life diagnostics (features of epizootology, pathogenesis, clinical signs, special (laboratory) diagnostics), differential diagnosis.
5. Post-mortem diagnostics of invasions, taking into account the site and type of zooparasites, the intensity of invasion and the nature of the pathoanatomical changes.
6. Measures to control invasions: a). medicines and schemes of their use; b). features and main ways of prevention.

REQUIREMENTS OF THE PARASITOLOGY DEPARTMENT TO STUDENTS

1. Have and wear clean overalls – white gown and hat.
2. Have with you a workbook for laboratory classes on parasitology with completed homework.
3. Have the material provided for the home preparation (see Methodology of discipline).
4. The missed classes will be worked out during next week after the student has started classes – on the day of the duty of the teachers assigned to this group.
5. In time, pass the modules according to the work plan and resit the module during the next week if you receive negative assessment.

The duty of students is a systematic and deep mastery of knowledge, practical skills, professional skills, improving the general cultural level. (Article 52 of the Law of Ukraine "On Education" dated 23 May 1991. № 1060-XI)

SAFETY MEASURES IN CLASSES AT PARASITOLOGY DEPARTMENT

1. Elementary medical ethics or a culture of behavior and work in the workplace.
2. Appear to the classes in clean technical clothing (white gown, hat), because the Department of Parasitology is the department of a group of infectious diseases, including: zooanthroponosis (toxoplasmosis, trichomonosis, echinococcosis, fasciollosis, toxocarosis, trichinellosis, etc.), accidental scattering of the invasive onset is possible when receiving sick animals or demonstrating material.
3. You should wash your hands after class, whether or not there has been contact with a sick animal, test material (it is advisable to limit food intake in the department).
4. Gently treat virulent and poisonous substances, including acids and alkalis.
5. To follow the fire safety rules, since there may be flammable substances such as alcohols, ether, benzene, xylene, etc. in the classroom.
6. To follow the rules of electrical safety, carefully handle electrical appliances.
7. Before the treatment (cure) of animals, especially small one's (dogs and cats) – listen to workplace safety training.
8. Delivering parasitological material to the department's laboratory, it must be preserved (alcohols, Barbogallo liquid, 10% formaldehyde (organs)) and carefully packed, preventing scattering of invasive onset in the environment.

Parasitology (from Greek *parasitos* – parasite, *logos* – doctrine) is a complex biological science that studies the systematics, morphology, cycle of development of various parasitic organisms and the diseases that they cause.

THE COURSE OF VETERINARY PARASITOLOGY CONSISTS OF THE FOLLOWING SECTIONS:

- General parasitology;
- Protozoology – the science of the parasitic protozoa and the diseases they cause;
- Helminthology – the science of parasitic worms and the diseases they cause;
- Arachnoentomology – the science of parasitic mites and ticks and insects, which are ectoparasites of animals and humans.

Webster's Third New International Dictionary of the English Language defines '**parasite**' as follows: (Greek ***para*** – near, ***sitos*** – nutrition) an organism living in or on another living organism obtaining from it part or all of its organic nutrient, and commonly exhibiting some degree of adaptive structural modification – such an organism that causes some degree of real damage to its host.

BIOLOGICAL BASES OF PARASITOLOGY

- **Ecology –** the relationships between the air, land, water, animals, plants etc., usually of a particular area, or the scientific study of this.

- **Species** – a set of animals in which the members have similar characteristics to each other and can breed with each other. Largest group of organisms in which any two individuals of the appropriate sexes or mating types can produce fertile offspring, typically by sexual reproduction.
- **Population** – all the animals of a particular type or group who live in one country, area or place.
- **Biocenosis** – (biotic community, biological community, ecological community, life assemblage) describes the interecting organisms living together in a habitat (biotope).
- **Biotope** is an area of uniform environmental conditions providing a living place for a specific assemblage of plants and animals. *Biotope* is almost synonymous with the term *habitat*.

TYPES OF INTERRELATIONS OF ORGANISMS IN NATURE

Parasitism is one of at least four complex symbiotic relationships.

- **Symbiosis**, a term coined by de Bary in 1879, literally means 'living together of differently named organisms.' It describes the relationship in which a symbiont lives in, or on, another living host.
- Symbiotic interactions, or symbioses, include a tremendous variety of intimate partnerships in nature. In the broadest sense, there is no implication with respect to the length or outcome of the association, nor does it imply physiological dependence or benefit or harm to the symbionts involved in the partnership. Given such a broad definition of symbiosis, a functional separation can be made in relation to the feeding biology of one or both of the symbiotic partners, as well as the degree of host exploitation. Thus, categories of symbiosis relate to trophic relationships, and if and how energy is transferred between the partners. Such categories are best viewed as a continuum with overlapping boundaries.
- If there is no trophic interaction involved in the symbiotic interaction, then the relationship is called **phoresy**. In this case, the symbiont (=phoront) merely travels with its host; there is no metabolic commitment by either partner. Protists or fungal microbes that are mechanically carried by insects are examples of phoretic associations.
- Phoresy grades into **commensalism**, a symbiotic interaction that implies a trophic relationship between the partners. Commensalism means 'eating at the same table.' Here the benefit gained is unidirectional. The smaller commensal partner typically benefits via food transfer and increased dispersal opportunities, while the host is neither harmed nor benefited. When sharks feed on large prey, they scatter fragments of food that are made available to remoras. Yet, some remoras also feed on ectoparasites of their shark hosts, implying an indirect metabolic linkage.
- Commensalism therefore grades into **mutualism** in many cases. Many mites are commensals, hitching a ride and sharing food with hosts as diverse as insects and molluscs to birds and mammals.
- When there is a direct transfer of energy between the partners, the interaction may be either **mutualistic or exploitative**. Obligate mutualists are metabolically dependent on one another. A classic example of an obligatory mutualism is the mutualistic

relationship between ruminant mammals and the ciliated protists and microbes in their stomach is similar. The biochemical complexity of these, and many other mutualistic associations found throughout nature, is the product of a long coevolutionary history between the partners. Such coevolved mutualisms are regarded as being creative forces in the adaptive radiations of many taxa.

- If more than one organism is attacked, but typically is not killed, then the aggressor is called a **micropredator**. Hematophagous organisms such as mosquitoes, and some leeches and biting flies, for example, are considered micropredators, taking frequent blood meals from several hosts. Some micropredators are often considered as ectoparasites, e.g., leeches.
- If more than one organism (considered as prey) is attacked and always killed, then the aggressor is considered a **predator**.
- If only one specific host is attacked and is almost always killed, then the aggressor is usually referred to as a **parasitoid**, most of which are wasps and flies
- If only one host is attacked, but typically is not killed outright, the aggressor is a **parasite**.

Parasites can have parasites too! The parasites living in/on other parasites are called **hyperparasites**. Parasite biodiversity will increase exponentially when we fully understand how common hyperparasitism is in nature. The sea louse, Lepeophtheirus salmonis, for example, is a common skin ectoparasite of salmonid species.

Parasitism's place' within the context of symbiotic relationships. This is one way of looking at parasitism and it is based, initially, on trophic relationships, followed by 'harm,' and finally, quantity of hosts involved. The final criterion, number of hosts attacked, is meaningful only if restricted to a single life history stage. For example, adult parasitoids may parasitize many host individuals but their larvae live in, and consume, only a single individual. Likewise, a typical helminth parasite may have both intermediate and definitive hosts, but each life-cycle stage will infect only a single host individual. These categories are arbitrary and, often, there is considerable overlap between many of the relationships.

LEVELS OF PARASITISM

Depending on the localization, the parasite method has distinguished by the following parasite levels.

1. Genetic level of parasitism – host contact occurs at the level of the cell genome. These are viruses – obligate parasites of cells.

2. Cellular level of parasitism. This is when prokaryotic and karyotic organisms have exposed to the animal as intracellular parasites that have reduced in the cytoplasm. Viruses, mycoplasmas, chlamydia, eimeria, toxoplasma, pyroplasmids, sarcocysts and other pathogens are intracellular parasites.

3. The tissue level of parasite is the larvae of insect helminths and other parasites (ascarid, larvae, trichinella, gadfly).

4. The organ-cavity level of parasitism has found in most sexually mature nematodes of the gastrointestinal and respiratory tracts (ascarid, oxyuriate, trichocephalus, etc.).

There are also mixed levels: cell-tissue-cavity, tissue-cavity while larval and imaginal stages are parasiting and also while localizing parasites from different groups (protozoa, helminths, insects, etc.) at the same time.

CLASSIFICATION OF ZOOPARASITES AND THEIR HOSTS

- **Temporary parasites** are those that live, reproduce in the environment, and use animals only for feeding (mosquitoes, clegs, fly flies, Ixodidae ticks, etc.).
- **Stationary parasites** live and reproduce inside or outside the nourishment organism for a long time. These parasites have also divided into two groups: **permanent** and **periodic**.
- **Stationary-permanent** parasites are those that from birth to death are inside or outside the body, where all stages of their life occur. These are lice, bovicola, scabies, trichinella.
- **Stationary-periodic** parasites invade hosts only for a long time being in the larvae or pubescent stages.
- **Endoparasites** include those that are confined within the host's body. They include the protists, microsporidian and myxozoans as well as the 'worm' parasites such as flukes, tapeworms, acanthocephalus and nematodes. A variety of holdfast adaptations often serve to anchor these endoparasites to specific sites within their specific hosts. The holdfasts of elasmobranch cestodes, for example, are often exquisitely adapted to match the microstructure of the intestines of their specific elasmobranch hosts.
- Parasites found on the surface of the host's body have called **ectoparasites**. Most parasitic arthropods and monogeneans are ectoparasitic.
- **Larval (parasitic) parasites** are when animal and human diseases are caused by the larvae of parasites – helminths (cysticercus, coenur, echinococcus, trichinella), insects (ovad or gadfly).
- **Imaginal parasites** cause infestation in the mature stage (imaginal cestodes).
- The organism in, or on, which a parasite reaches sexual maturity, is the **definitive host**. Many parasites have a simple, direct life cycle, requiring only one host for transmission to occur. All monogeneans, and many nematode and arthropod parasites, have **direct life cycles**.
- Many animal parasites, however, have obligate **intermediate hosts** in which the parasites undergo developmental and morphological changes.
  - **Intermediate hosts** may be the prey of the predatory definitive host in the life cycles of parasites. Thus, parasites with intermediate hosts in their complex life cycles are often transmitted trophically to definitive hosts via food web interactions.
  - Life cycles in which more than one host are required are referred to as **indirect life cycles**. Many parasites have remarkably complex life cycles with several hosts and both free-living and obligate parasitic larval stages.

Some protists and filarial nematodes have **vectors** as hosts. Vectors are **micropredators** that transmit infective stages from one host to another.

- A vector may be an intermediate or a definitive host, depending on whether the sexual phase of the parasite's life cycle occurs in it or not. For example, the insect vectors for species of Plasmodium, the causative agents of malaria, are certain species of female mosquitoes that actively inoculate infective stages of the parasite into the next host during their blood meals. Sexual reproduction

occurs within the stomach of the mosquito; consequently, mosquitoes are the definitive hosts for the parasite.

- A number of parasites may use hosts in which there is no development and that are not always obligatory for the completion of a parasite's life cycle. These are called **paratenic or transport hosts**. Such hosts are most frequently used to bridge an ecological, or trophic, gap. For example, adults of the fluke, *Halipegus occidualis*, live under the tongue of green frogs. Snails in the genus *Helisoma* are obligate first intermediate hosts, whereas aquatic micro-crustaceans such as ostracods are obligate second intermediate hosts. But green frogs do not normally consume these small crustaceans.
- A number of animals are normal hosts for parasites that may also infect humans. These are called **reservoir hosts**. These non-human hosts act as reservoirs of infection for certain parasites.

Diseases of animals that are transmissible to humans are called **zoonotic diseases, or zoonoses**. Thus, giardiosis, trichinellosis, and schistosomosis are examples of zoonoses. Similarly, rats are important reservoir hosts for the nematode, Trichinella spiralis.

Ecologically, reservoir hosts are similar to paratenic hosts since they may greatly increase transmission rates, and also help prevent local extinction of the parasite. The potential for controlling zoonotic diseases in humans is greatly complicated by the presence of these reservoir hosts.

Furthermore, reservoir hosts greatly complicate the zoonotic parasite's epidemiology. Epidemiology is the study of all the many complex, inter-related ecological factors responsible for the transmission and distribution of a human disease.

A related term is **epizootiology**. Epizootiology usually refers to the factors involved in the transmission and distribution of non-human parasites, often in reference to epizootics.

Types of pathogenic effects of zooparasites

- Mechanical
- Trophic
- Toxic
- Allergic
- Inoculatory

The influence of the parasitic organism depends on:

- the pathogenicity,
- the virulence,
- the specificity of parasites,
- the reactivity of the organism,
- the natural and acquired resistance of the host

PRINCIPLE OF ANTI-PARASITIC MEASURES

An ideal anthelmintic should possess the following properties:

(1) **It should be efficient against all parasitic stages of a particular species.** It is also generally desirable that the spectrum of activity should include members of different genera, for example in dealing with the equine slrongyles and *Parascaris equorum.* However in some circumstances, separate drugs have to be used at different times of year to control infections with unrelated helminths; the trichostrongyles responsible for ovine parasitic gastroenteritis and the liver fluke *Fasciola hepatica* are one such example.

(2) It is important that any anthelmintic **should be non-toxic to the host,** or at least have a wide safety margin. This is especially important

in the treatment of groups of animals such as a flock of sheep, where individual body weights cannot easily be obtained, rather than in the dosing of individual companion animals such as cats or dogs.

(3) In general, an anthelmintic **should be rapidly cleared and excreted by the host,** otherwise long withdrawal periods would be necessary in meat and milk producing animals. However, in certain circumstances and in certain classes of animals, drug persistence is used to prophylactic advantage, for example the use of closantel to control *Haemonchus* in sheep.

(4) Anthelmintics **should be easily administered,** otherwise they will not be readily accepted by owners; different formulations are available for different domestic animal species. Oral and injectable products are widely used in ruminants, and pour-on preparations are available for cattle. Anthelmintic boluses arc also available for cattle and sheep. Palatable in-feed and paste formulations are convenient for use in horses, while anthelmintics are usually available as tablets for dogs and cats.

(5) **The cost of an anthelmintic should be reasonable.** This is of special importance in pigs and poultry where profit margins may be narrow.

USE OF ANTHELMINTICS

Anthelmintics are generally used in two ways, namely, therapeutically, to treat existing infections or clinical outbreaks, or prophylactically, in which the timing of treatment is based on a knowledge of the epidemiology. Clearly prophylactic use is preferable where administration of a drug at selected intervals or continuously over a period can prevent the occurrence of disease.

THERAPEUTIC USAGE

When used therapeutically, the following factors should be considered.

- First, if the drug is not active against all stages it must be effective against the pathogenic stage of the parasite.
- Secondly, use of the anthelmintic should, by successfully removing parasites, result in cessation of clinical signs of infection such as diarrhoea and respiratory distress; in other words, there should be a marked clinical improvement and rapid recovery after treatment.

PROPHYLACTIC USAGE

Several points should be considered where anthelmintics are used prophylactically.

- First, the cost of prophylactic treatment should be justifiable economically, by increased production in food animals, or by preventing the occurrence of clinical or subclinical disease in, for example, horses with strongylosis or dogs with heartworm disease.
- Secondly, the cost-benefit of anthelmintic prophylaxis should stand comparison with the control which can be achieved by other methods such as pasture management or, in the case of dictyocaulosis, by vaccination.
- Thirdly, it is desirable that the use of anthelmintics should not interfere with the development of an acquired immunity, since there are reports of outbreaks of disease in older stock which have been overprotected by control measures during their earlier years.
- Finally prolonged prophylactic use of one drug should be avoided as this may encourage the development of anthelmintic resistance.

«____» _____ 20 p. Signatures: Student_____ Lecturer _____

LABORATORY CLASS № 1 **«____» _____ 20 p.**

TOPIC: Diagnostics and differential diagnosis of babesiidoses of ruminants, horses, dogs and cats

Class location – classroom, laboratory, museum of the department

Purpose of the lesson: To know the definition, content and history of development of veterinary protozoology. To learn the principles of taxonomy of protozoa pathogens. To study morphological and biological features of pathogens of babesiidoses: cattle *(Babesia bigemina (Piroplasma bigeminum), B. bovis (B. colchica, B. argentina))*, small cattle *(B. ovis, B. motasi (Piroplasma ovis))*, horses *(B. caballi (Piroplasma caballi), B. equi (Nuttalia equi))*, pigs *(B. trautmanni, B. perroncitoi)*, carnivorous *B. (Piroplasma) canis, B. gibsoni, B. vogeli, B. (Nuttallia) felis, Theileriidoses of* cattle *(Theileria annulata, Th. parva, Th. sergenti, Th. mutans, Th. ovis, Th. hirci)*. To master methods of life-time and post-mortem diagnostics and the principles and specific pathogenic therapy. To know the prevention of protozooses of animals.

Task: To study the morphological features of pathogens using macro- and micropreparations. To master features of diagnostics and differential diagnosis of these diseases. To study the samples of medicines, their use for therapeutic and preventive purposes. To master practically basic methods of laboratory diagnostics of a group of protozoa diseases of animals.

Independently prepare for classes using recommended books (1−4), lecture material and electronic files from the discipline «Veterinary Parasitology» at the «Distance learning portal (MOODLE) of SBTU».

Auditory work. To study and make a drawing of the basic diagnostic features of the agents of pyroplasmidoses of animals using the museum material (macropreparations), temporary and permanent micropreparations. Conduct hematological examination of blood samples from animals. Get acquainted with the arsenal of medicines recommended for control this group of diseases.

Task performence:

1. The place of pathogens of pyroplasmidoses of animals in the world animals system (classification):

Kingdom _____ Subkingdom _____

Phylum _____ Class _____ Order _____

Family _____ Genus _____ Genus _____

Family _____ Genus _____ Genus _____

Definition: _____

_____

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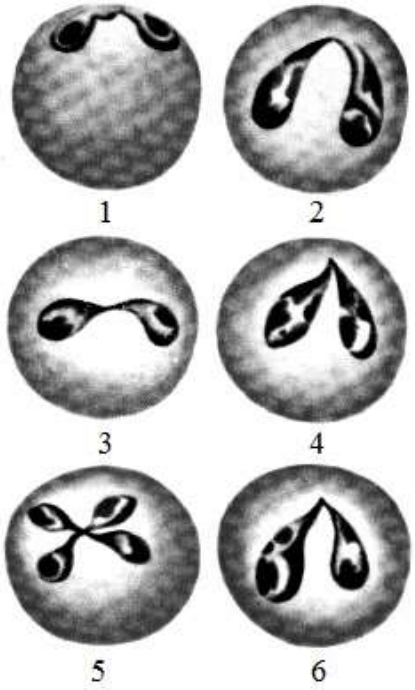
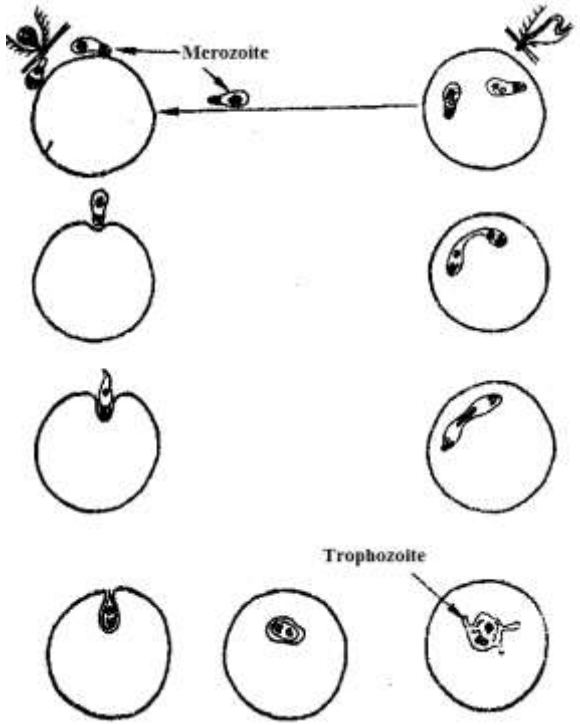
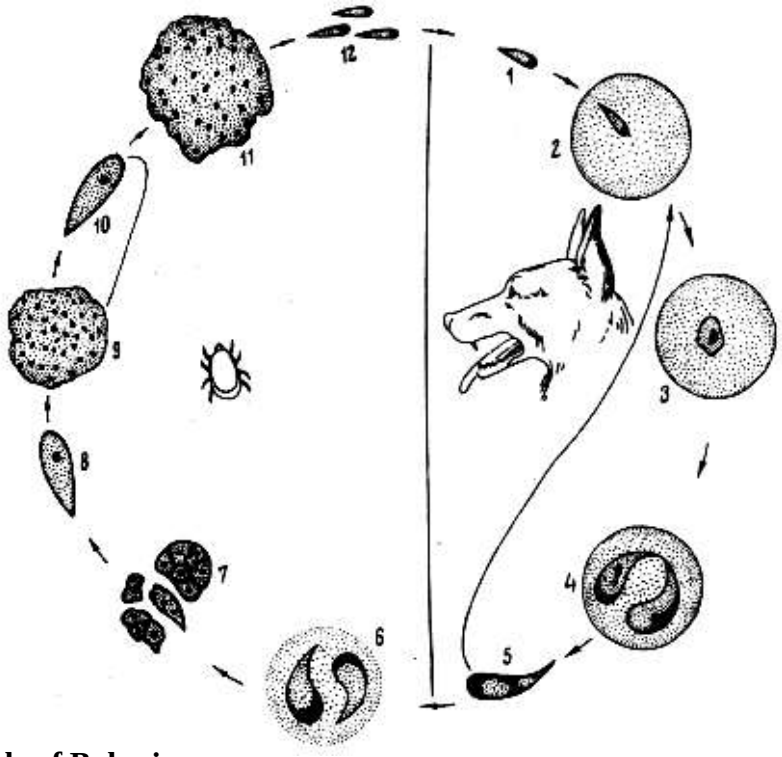
2. Morphological and biological characteristics of pathogens of babesiidoses:



Typical forms of babesia in erythrocytes:
1,3 – *B. bovis*; 2 – *B. bigemina*; 4 – *B. caballi*;
5 – *B. equi*; 6 – *B. canis*.



Penetration of Babesia bovis into erythrocytes and development in the vertebrate organism



Life cycle of Babesia
1, 12 - merozoites; 2-4 - development of merozoite in erythrocytes;
5 - exit of the pathogen from erythrocyte; 6 - re-entry into red blood cells;
7 - development of the parasite in the gut of the tick; 8 - stage (ookinetes) in ticks;
9-11 - merogony in the gut of the tick.

Short characteristic of pyroplasm parasitic in animals

Species of pathogen	Size, μm		The characteristic shape of		Rates of erythrocytes ,%	Susceptible animals	Vectors
	in erythrocytes	in RES cells	in erythrocytes	in RES cells			
Babesia bigemina	2,2 – 6	–	Pyriform	–	10–15	Cattle	**Ixodidae ticks *Boophillus calcaratus, Rhipicephalus bursa, Haemaphysalis punctata***
Babesia bovis	1,5 – 2,4	–	– " –	–	40–70	– " –	***Ixodes ricinus***
Babesia caballi	6	–	– " –	–	6–10	Horses	***Hyalomma plumbeum, Dermacentor marginatus, D. pictus, D. silvarum***
Babesia equi	1 – 4	–	Cruciform	–	30–60	– " –	***Hyalomma scupense, H. plumbeum, Rhipicephalus bursa, Rh. turanicus, Dermacentor marginatus***
Babesia motasi	2 – 3,8	–	Pyriform	–	45	Sheep, goat	***Rhipicephalus bursa***
Babesia ovis	0,5 – 2,5	–	– " –		1–5	– " –	**– " –**
Babesia canis	2 – 7	–	– " –		5–10	Dogs	***Dermacentor pictus, Rhipicephalus sanguineus***
Theileria annulata	0,5 – 2,9	8–20	Round, rod-shaped	Pomegranate bodies	80–95	Cattle	***Hyalomma detritum, H. plumbeum, H. scupense, H. anatolicum***
Theileria ovis	– " –	– " –	– " –	– " –	90–95	Sheep, goat	***Rhipicephalus bursa*****, кліщі роду *Hyalomma***

3. Sources and ways of invasion of animals by piroplasmidoses: _____

_____

_____

4. Features of life-time and post-mortem diagnostics and differential diagnosis of piroplasmidoses of animals:

Clinical signs _____

_____

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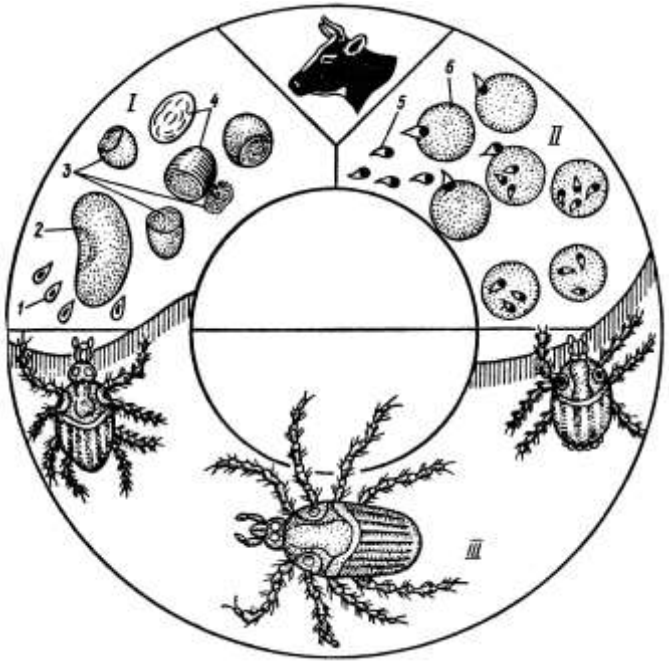
Morphological and biological characteristics of pathogens *Theileria*:



Life cycle of *Theileria annulata*:
I. 1 – sporozoites; 2 – lymph node; 3-4 – lymphocytes with micro- and macromerontes;
II. 5 – micromerozoites; 6 – penetration micromerozoites into red blood cells;
III. – the formation of sporozoites in the tick

Pathoanatomical changes _____
_____
_____
_____
_____
_____
_____

Special laboratory diagnostics _____
_____
_____
_____
_____
_____
_____

5. Measures of control and ways of prevention of piroplasmidoses of animals. Therapeutic drugs.

Treatment _____
_____
_____

Prevention _____
_____
_____
_____

Material and technical supply. Microscopes, magnifying glass, temporary and permanent micro preparations. Intermediate hosts. Tables, schemes. Invasive animals. Samples of drugs.

«____» _____ 20 p. Signatures: Student_____ Lecturer _____

LABORATORY CLASS № 2 **«____» _____ 20 p.**

TOPIC: Testing equipment of laboratory diagnostics of animals' blood parasite protozooses

Class location - classroom, laboratory, museum of the department

Purpose of the lesson: To know testing equipment of laboratory diagnostics of animals' blood parasite of protozooses.

Task: To workout the methodology and to know the features of special diagnostics of blood and parasitic diseases in animals.

Independently prepare for classes using recommended books (1−4), lecture material and electronic files from the discipline «Veterinary Parasitology» at the «Distance learning portal (MOODLE) of SBTU»

Auditory work. To conduct a clinical and parasitological examination of a sick animal or a freshly obtained blood sample from it and make a diagnosis.

Task performance:

Microscopic examination is the main way to diagnose blood parasite diseases in animals. The detection of the pathogen in sick animals depends mainly on the technique of material selection, on the method of preparation and research. Most often prepare a thin smear or crushed drop, because the quality of blood smear is of great importance in the diagnosis of blood and parasitic diseases.

1). Preparation of slides

To obtain a high-quality thin smear, first of all, clean degreased slides are needed. To this end, they are boiled, if they new, for up to 5 minutes in soapy water, and those that have been in use for 1 hour in a 5-10 % solution of baking soda. Then they are thoroughly washed in clean water and wiped dry with a towel. To store ready-made glasses in a jar with a ground stopper in alcohol or a mixture of alcohol with ether (1:1). Before using the glass, remove it, wipe it with a clean towel and wrap in clean paper, preferably paraffin or parchment. Control quality of slides clearence has made with a drop of water: on clear slide, the water drop will spread, on greasy – retains its shape.

2). Blood tests

a). Preparation of a thin smear.

The blood in domestic animals is taken from the peripheral vessels of the ear or from the tip of the tail, in poultry – from the crest or earring, and more often from the drop of blood by puncture the infraorbital vein. To do this, the animal securely fixate, prepare a skin area for blood sampling – cut hair, pluck feathers, release skin from dirt, first with a cotton swab and then with alcohol or ether. The superficial vein is punctured with a sterile needle from a syringe, using the first drops of blood, which have the highest number of parasites. The slide is held with the thumb and forefinger of the left hand and gently touch it with the protruding drop of blood (the size of millet grain) at a distance of 3-5 mm from the edge of the slide. Then, with the thumb and forefinger of the right hand, a polish slide or roof is taken and a narrow edge of it at an angle of 45º to the plane of the slide touches the drop of blood. Due to the capillarity of blood, it is evenly distributed over the edge of the polish glass. (To practice without a drop of blood on a dry slide!). After that, the polish glass is quickly but

evenly moved over the slide. The resulting smear is air-dried. 3-4 thin smears are prepared from each animal and after drying with a simple pencil or the needle tip from the syringe indicate the species of animal on each smear, its number and the date it was taken. When native smears prepared, they should be protected from flies.

In the cold season, smears should be protected from the action of moisture, which may lead to hemolysis of red blood cells. In this regard, the slides are first heated on the lid of a sterilizer or other hot water dish; the finished smear is also dried on a warm surface, after which it is wrapped in clean paper. Hemolysis can also be caused if the prepared smears are placed on the palm and clamped in the hand.

The smears should be thin, with smooth lateral edges and a torn end (beard). The largest number of erythrocyte parasites are accumulated on the sides and at the end of the smear.

б). Preparation a thick drop.

A drop of blood from a cut on the ear or from the jugular vein of animals is applied to a degreased slide and the second slide is circularly placed with a thick layer in the diameter of a 10-dime coin. It is then air-dried, preferably under a glass cap or thermostat.

в). Lymph node puncture examination.

This technique is used in the early diagnosis of cattle Theileriosis. To obtain punctate use superficial lymph nodes. To do this, the operating field is excised and disinfected. Thereafter, a sterile needle inserted into the syringe is inserted into the depth of the fixed gland. The plunger of the syringe is slowly withdrawn and sucked lymph into the needle. Ordinary thin smears are prepared from the obtained punctate, dried, fixed, stained and examined under a microscope.

It is better to keep the smears fixed, because after about 1 month, the non-fixed smears become unfit for study.

3). Fixation of smears.

The dried smears are fixed in methyl alcohol (5 minutes) or ethyl alcohols (90-95º) (10-20 minutes), or in a mixture of alcohol-ether (1:1) for 10-15 minutes, in denaturate – 30 min, in acetone – 5 min. The fixer is poured into a glass cuvette, the smears are immersed into fix solutions.

4). Stainting of thin smears

The advantage is that the components of the parasite (cytoplasm, nucleus, flagella) perceive the dye differently, that is, stained in various colour.

EXAMINATION OF BLOOD AND LYMPH

Thin blood smears stained with Romanovsky dyes, such as Giemsa or Lcishman, and examined under an oil immersion lens are commonly used for the detection of trypanosomes, babesial and theilerial piroplasms and rickettsial infections such as anaplasmosis, ehrlichiosis and eperythrozoonosis. On other occasions, needle biopsies of enlarged lymph nodes may be similarly stained for the detection of trypanosomes (especially *Trypanosoma brucei* or *T. vivax)* or theilerial schizonts.

In trypanosomosis, the parasitaemia may be light and the chance of a positive diagnosis is increased if a thick blood smear, dehaemoglobinized by immersing the slide in water before eosin staining, is used. For this a drop of fresh blood, with no added

anticoagulant, is gently stirred on a slide to cover an area of about 10 mm diameter and allowed to dry. Subsequently it may be stained by Field's technique as follows.

Field's stain Preparation of solutions

Solution A	***Methylene blue***	***D.4g***
Azure I	***0.25 g***	
Solution B	***250 ml***	
Solution B	***Na,HP0412H20***	***25.2g***
KH2PO4	***12.5 g***	
Distilled water	***1000 ml***	
Solution C	***Eosin***	***0.5 g***
Solution B	***250 ml***	

These solutions do not keep and should be freshly prepared each day.

(1)	Dip slide in solution A	
(2)	Rinse in solution B	1 **to 3 seconds**
(3)	Dip slide in C	2 **to 3 seconds**
(4)	Rinse in tap water	1 **to 3 seconds**
(5)	Stand upright to drain and dry	2 **to 3 seconds**

This technique is commonly used in large-scale survey work in the field.

A particularly efficient diagnostic technique for trypanosomosis, described earlier in the text, is the examination, under darkground illumination, of the expressed buffy coat of a microhaematocrit tube for the detection of motile trypanosomes.

The inoculation of mice with fresh blood from suspected cases of *Trypanosoma congolense* or *T. brucei* infection is another common technique practised in the field. Three days later the tail blood of such mice should be examined and subsequently daily thereafter for about three to four weeks to establish if trypanosomes are present.

The detection of specific antibody in a specialist laboratory may also be useful in the diagnosis of several protozoal diseases such as theileriosis, trypanosomosis, including *T. cruzi* infection, babesiosis, cryptosporidiosis and rickettsial infections such as anaplasmosis and ehrlichiosis. However a positive result does not necessarily imply the presence of a still active infection, but simply that the animal has at some time been exposed to the pathogen. An exception to this interpretation is the diagnosis of suspected toxoplasmosis in sheep, where rising antibody levels over a period of several weeks are reasonable evidence of recent and active infection.

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Material and technical supply. Microscopes, magnifying glass, temporary and permanent micropreparations. Tables, diagrams. Animals invaded.

«____» _____ 20 p. Signatures: Student_____ Lecturer _____

LABORATORY CLASS № 3 «____» _____ 20 p.

TOPIC: Diagnostics and differential diagnosis of coccidiidoses (eimeriosis) of poultry, rabbits, ruminants and fish

Class location– classroom, laboratory, museum of the department

Purpose of the lesson: To study the morphological and biological characteristics of pathogens of coccidiidoses of cattle *(Eimeria bovis, E. zuernii, E. ellipsoidalis, E. cylindrica, E. auburnensis, E. bukidnonensis, E. alabamensis, E. canadensis, E. subsperica)*, small cattle *(E. arloingi, E. ninaekohljakimovae, E. intricata, E. faurei, E. arloingi)*, pigs *(E. suis, E. debliecki, E. scabra, E. perminuta, E. spinosa, E. guevarai, E. polita, E. neodebliecki, E. porci, E. residualis, E. betica)*, carnivorous *(E. vulpis, E. adleri, E. bacanensis, E. imantanica)*, rabbits *(E. stiedae, E. perforans, E. magna, E. media, E. irresidua)*, poultry *(E. tenella, E. necatrix, E. maxima, E. acervulina)*. To master methods of life-time and post-mortem diagnosis of coccidia.

Task: To study the morphological features of pathogens using macro- and micropreparations. To master features of diagnostics and differential diagnosis of these diseases. To study the samples of medicines, their use for therapeutic and preventive purposes. To master practically basic methods of laboratory diagnostics of a group of protozoa diseases of animals.

Independently prepare for classes using recommended books (1−4), lecture material and electronic files from the discipline «Veterinary Parasitology» at the «Distance learning portal (MOODLE) of SBTU».

Auditory work. To study and make a drawing of the basic diagnostic features of the agents of eimerias of animals using the museum material (macropreparations), temporary and permanent micropreparations. Carry out a coproscopic examination of freshly obtained faeces samples from rabbits, pigs, cattle and poultry, examine them for oocysts. Get acquainted with the arsenal of medicines recommended for control this group of diseases.

Task performance:

1. The place of pathogens of animals in the world animals system (classification):

Kingdom _____ Order _____

Subkingdom _____ Family _____

Phylum _____ Subfamily _____

Class _____ Genus _____

Definition: _____

_____

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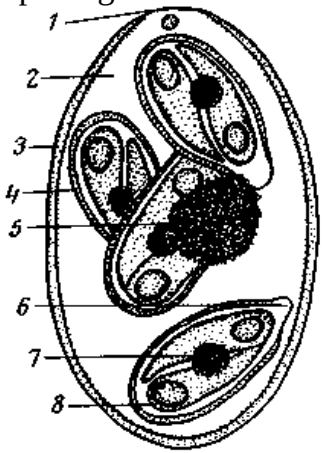
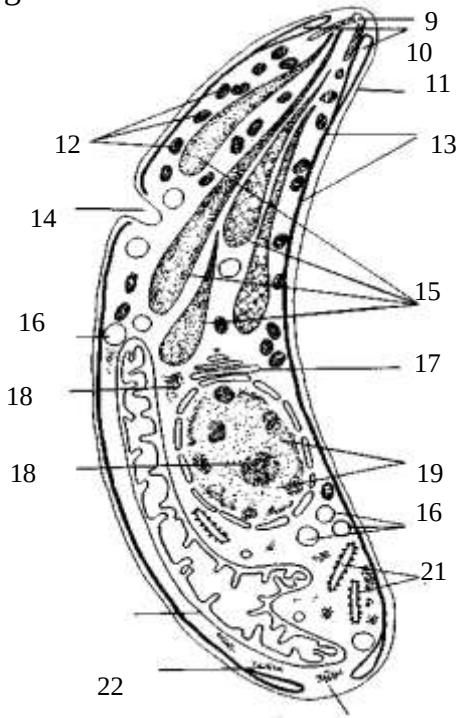
2. Morphological characteristics of pathogens of coccidiidoses:



Sporulated oocyst of Eimeria subfamily *Eimeriinae*



Sporozoite of coccidia

1 – _____

2 – _____

3 – _____

4 – _____

5 – _____

6 – _____

7 – _____

8 – _____

9 – _____

10 – _____

11 – _____

12 – _____

13 – _____

14 – _____

15 – _____

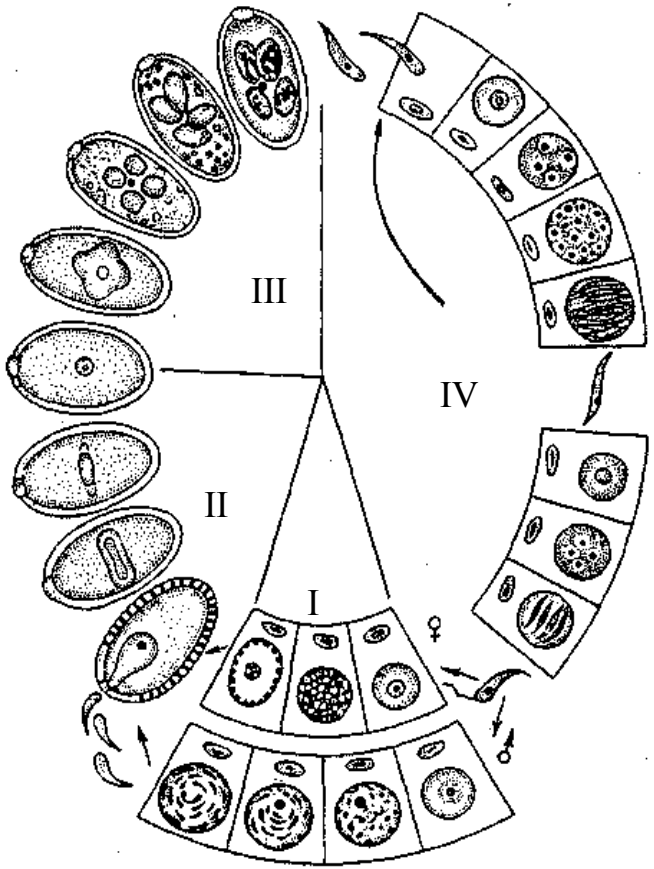
16 – _____

17 – _____

18 – _____

19 – _____

20 – _____



Life cycle of Eimeria:

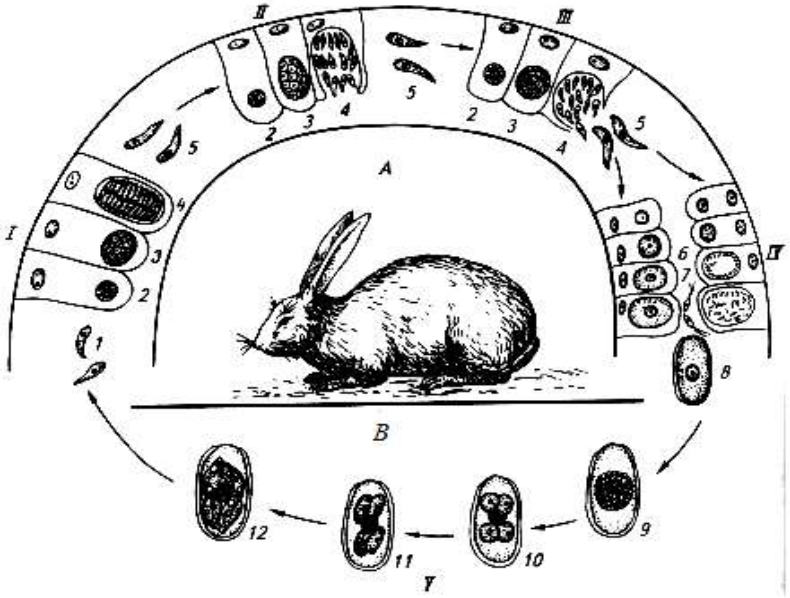
I – gametogony; II - fertilization;

III – sporogony; IV - merogony.

21 – _____

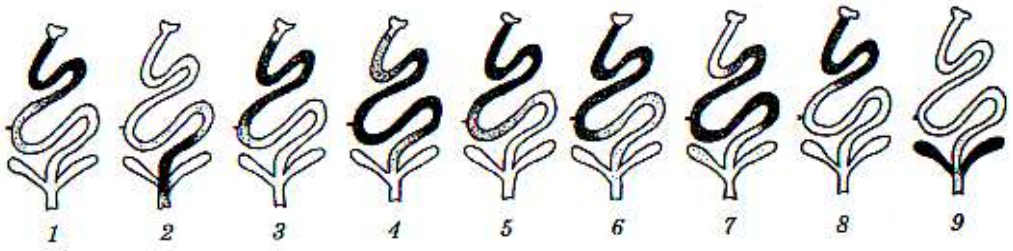
22 – _____

23 – _____



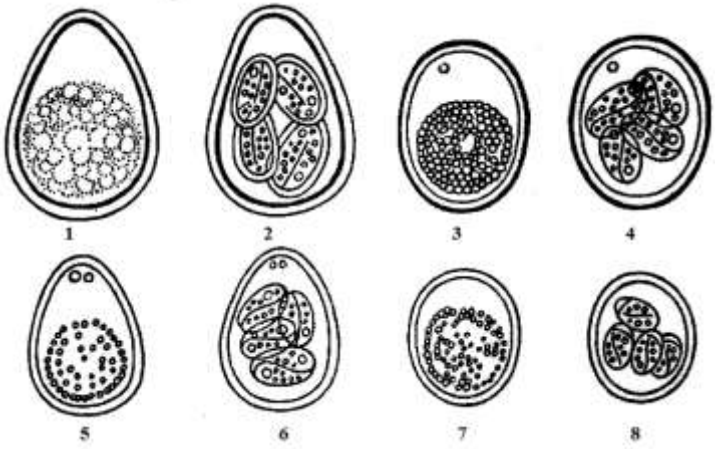
Life cycle of Eimeria

А – in rabbit's intestinal; Б – in the environment; I, II, III – merogony; IV – gametogony; V – sporogony; 1 – sporozoites; 2 – 4 – development of schizonts; 5 – merozoites; 6 – development of macrogametes; 7 – microgametes; 8 – zygote; 9 – unsporulated oocyst; 10 – 12 – sporogony.

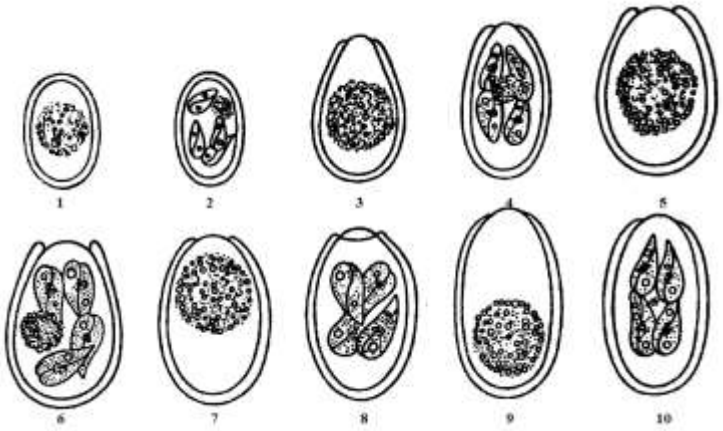


Site of Eimeria in hen's intestinal

1 – *Eimeria acervulina*; 2 – *E. brunetti*; 3 – *E. hagani*; 4 – *E. maxima*; 5 – *E. mivati*; 6 – *E. mitis*; 7 – *E. necatrix*; 8 – *E. praecox*; 9 – *E. tenella*.



OOocysts of Eimeria of hen: 1, 2 – *Eimeria maxima;* 3,4 – *E. tenella*; 5, 6 – *E. acervulina*; 7, 8 – *E. necatrix*.



Oocysts of Eimeria of rabbit:

1, 2 – *Eimeria perforans*;
3, 4 – *E. media*; 5, 6 – *E. magna*;
7, 8 – *E. irresidua;* 9, 10 – *E. stiedea.*

3. Sources and ways of invasion of animals by coccidiidoses: _____

_____

_____

4. Features of life-time and post-mortem diagnostics and differential diagnosis of coccidiidoses of animals and poultry:

Clinical signs _____

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_____

_____

_____

Pathoanatomical changes _____

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Special laboratory diagnostics _____

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5. Measures of control and ways of prevention. Therapeutic drugs.

Treatment _____

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Prevention _____

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Material and technical supply. Microscopes, magnifying glass, permanent macro preparations, temporary and permanent micro preparations. Tables, schemes. Invasive animals. Carry out a coproscopic examination of freshly obtained faeces samples from rabbit, pigs, cattle and poultry, examine them for oocystis. Samples of drugs.

«____» _____ 20 p. Signatures: Student_____ Lecturer _____

LABORATORY CLASS № 4 «____» _____ 20 p.

TOPIC: Diagnostics and differential diagnosis of animals' isosporinoses – toxoplasmosis, sarcocystosis, cystoisosporosis

Class location– classroom, coproscopic lab, museum of the department.

Purpose of the lesson: To study the morphological and biological characteristics of pathogens of toxoplasmosis *(Toxoplasma gondii),* and sarcocystosis *(Sarcocystis bovihominis, S. bovicanis, S. bovifelis, S. ovicanis, S. ovifelis, S. ovihominis, S. suicanis, S. suifelis, S. hominis)*, cystoisosporosis of carnivorous *(Cystoisospora canis, C. ohioensis, C. felis, C. rivolta, C. canivelocis, C. vulpina, C. buriatica, C. pavlodarica, C. neorivoeta, C. burrowsi)*, determine their place in the animal world.

To master methods of life-time diagnosis of toxoplasmidoses, features of their differential diagnosis. To study the peculiarities of life-time and post-mortem diagnosis of carnivorous cystoisosporosis and their differential diagnosis. To get acquainted with medical preparations and with the peculiarities of their use in different types of animals.

Task: To study the morphological features of pathogens of this subfamily using macro- and micropreparations, to know the peculiarities of their development. To master features of diagnostics and differential diagnosis of these diseases. To study the samples of medicines, their use for therapeutic and preventive purposes. To master practically basic methods of laboratory diagnostics of a group of protozoa diseases of animals.

Independently prepare for classes using recommended books (1−4), lecture material and electronic files from the discipline «Veterinary Parasitology» at the «Distance learning portal (MOODLE) of SBTU».

Auditory work. To study and make a drawing of the basic diagnostic features of the agents of toxoplasmidoses of animals using the museum material (macropreparations), temporary and permanent micropreparations. Carry out clinical and parasitological examination of animals on toxoplasmosis, isosporosis, cystoisosporosis, make a diagnostics and differential diagnosis. Get acquainted with the arsenal of medicines recommended for control this group of diseases.

Task performance:

1. The place of pathogens of these diseases in the world animals system (classification):

Kingdom _____	Subkingdom _____	Phylum _____
Class _____	Order _____	Family _____
	Subfamily _____	
Genus _____	Genus _____	Genus _____

Definition: _____

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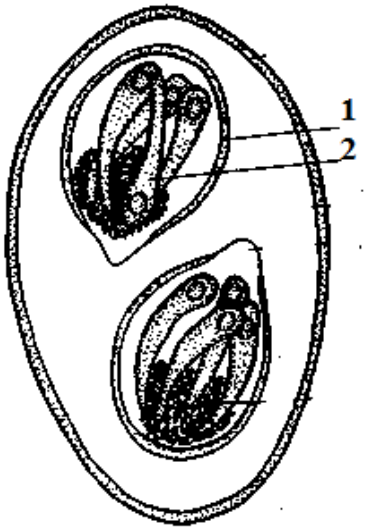
2. Morphological characteristics of pathogens of toxoplasmosis:

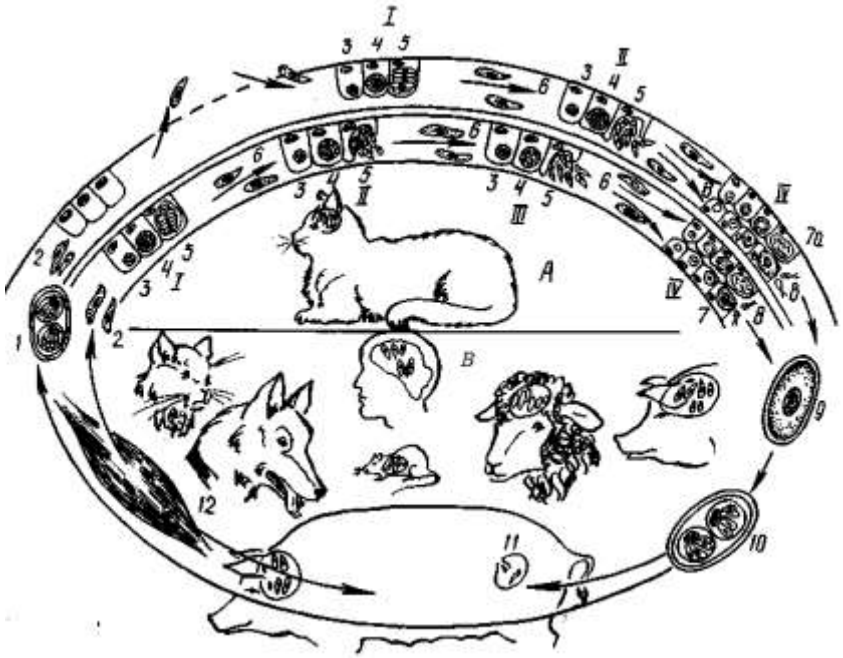


Scheme of structure of sporulated oocyst of Subfamily Isosporinae

1 – _____

2 – _____

Life cycle of toxoplasma:



A – <u>in definitive host</u>

B – <u>in intermediate hosts</u>

I, II, III – _____

IV – _____

1 – _____

2 – _____

3, 4, 5, 6 – _____

7 – _____

7 a – _____

8 – _____

9 – _____

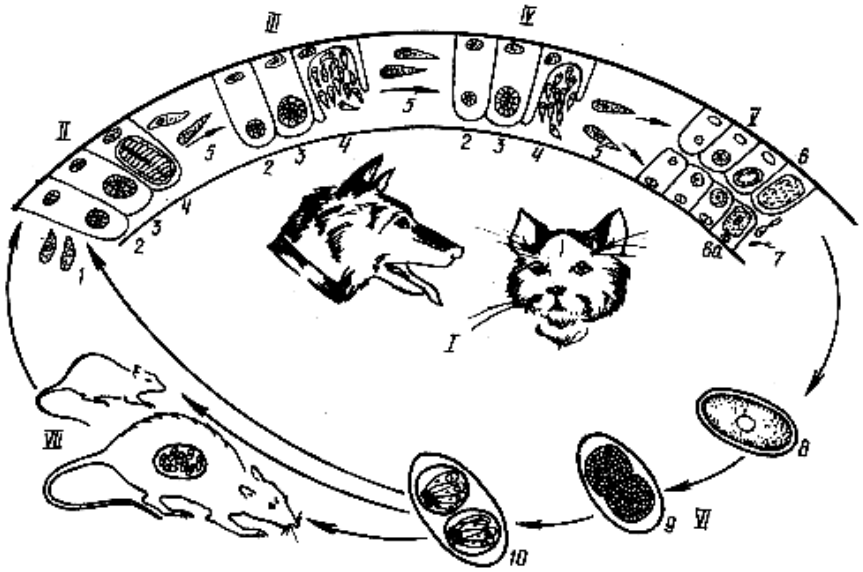
10 – _____

11 – _____

12 – _____

3. Morphological characteristics of pathogens of *Sarcocystis* and *Cystoisospora*:

Life cycle of *Cystoisospora* in carnivorous:



Life cycle of of pathogens of *Sarcocystis*

I – definitive hosts; II, III, IV – 3 generations of merogony in the intestine of carnivorous; V – gametogony; VI - development of oocysts in the environment; 1 – sporozoites; 2, 3, 4 – the stages of the merogony; 5 – merozoites; 6 – forming microgametes; 7 – the formation of macrogametes; 8 - unsporulated oocyst (immature); 9 – oocyst formation; 10 – sporulated oocyst

4. Ways to invasive of animals for toxoplasmosis, cystisosporosis and sarcocystosis:

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5. Features of life-time and post-mortem diagnostics, differential diagnosis of toxoplasmosis animals, sarcocystosis and cystoisosporosis in carnivorous:

Clinical signs _____

_____

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_____

Pathoanatomical changes _____

Special laboratory diagnostics _____

6. Measures of control and ways of prevention of toxoplasmosis, sarcocystosis and cystoisosporosiss. Therapeutic drugs.

Treatment _____

Prevention _____

Material and technical supply. Microscopes, magnifying glass, permanent macro preparations, temporary and permanent micro preparations. Intermediate hosts. Tables, schemes. Invasive animals. Freshly obtained faeces from them, all that is necessary for carrying out a coproscopic study by the method of Fuleborn. Samples of drugs.

«____» _____ 20 p. Signatures: Student_____ Lecturer _____

LABORATORY CLASS № 5 **«____» _____ 20 p.**

TOPIC: Testing equipment of laboratory diagnostics of animals' coccidioses

Class location– classroom, laboratory, museum of the department

Purpose of the lesson: To master the technique of laboratory diagnosis of eimerioses in animals.

Task: To workout the methodology and to know the features of special diagnostics of coccidiidoses of animals.

Independently prepare for classes using recommended books (1−4), lecture material and electronic files from the discipline «Veterinary Parasitology» at the «Distance learning portal (MOODLE) of SBTU».

Auditory work. To conduct a clinical and parasitological examination of a sick animal or a freshly obtained faeces from them and to diagnose.

Task performance:

COLLECTION OF FAECES

Faecal samples should preferably be collected from the rectum and examined fresh. If it is difficult to take rectal samples, then fresh faeces can be collected from the field or floor. A plastic glove is suitable for collection, the glove being turned inside out to act as the receptacle. For small pets a thermometer or glass rod may be used. Ideally, about 5 g of faeces should be collected, since this amount is required for some of the concentration methods of examination. Since eggs embryonate rapidly the faeces should be stored in the refrigerator unless examination is carried out within a day. For samples sent through the post the addition of twice the faecal volume of 10 % formalin to the faeces will minimize development and hatching.

METHODS OF EXAMINATION OF FAECES

Several methods are available for preparing faeces for microscopic examination to detect the presence of eggs or larvae. However, whatever method of preparation is used, the slides should first be examined under low power since most eggs can be detected at this magnification. If necessary, higher magnification can then be employed for measurement of the eggs or more detailed morphological differentiation. An eyepiece micrometer is very useful for sizing populations of eggs or larvae.

Direct smear method.

A few drops of water plus an equivalent amount of faeces are mixed on a microscope slide. Tilting the slide then allows the lighter eggs to flow away from the heavier debris, a cover slip is placed on the fluid and the preparation is then examined microscopically. It is possible to detect most eggs or larvae by this method, but due to the small amount of faeces used it may only detect relatively heavy infections.

Flotation methods.

The basis of any flotation method is that when worm eggs are suspended in a liquid with a specific gravity higher than that of the eggs, the latter will float up to the surface. Nematode and cestode eggs float in a liquid with a specific gravity of between 1.10 and 1.20;

tremalode eggs, which are much heavier, require a specific gravity of 1.30-1.35.

The flotation solutions used for nematode and cestode ova are mainly based on sodium chloride or sometimes magnesium sulphate. A saturated solution of these is prepared and stored for a few days and the specific gravity checked prior to usage. In some laboratories a sugar solution of density 1.2 is preferred.

Whatever solutions are employed the specific gravity should be checked regularly and examination of the solution containing the eggs or larvae made rapidly, otherwise distortion may take place.

Direct flotation

A small amount of fresh faeces, say 2.0 g, is added to 10 ml of the flotation solution and following thorough mixing the suspension is poured into a test tube and more flotation solution added to fill the tube to the top. A cover glass is then placed on top of the surface of the liquid and the tube and coverslip left standing for 10 to 15 minutes. The cover slip is then removed vertically and placed on a slide and examined under the microscope. If a centrifuge is available the flotation of the eggs in the flotation solution may be accelerated by centrifugation.

McMaster method

This quantitative technique is used where it is desirable to count the number of eggs or larvae per gramme of faeces. The method is as follows:

(1) Weigh 3.0 g of faeces or, if faeces are diarrhoeic, 3 teaspoonfuls.

(2) Break up thoroughly in 42 ml of water in a plastic container. This can be done using a homogenizer if available or in a stoppered bottle containing glass beads.

Pour through a fine mesh sieve (aperture 205/tm, or 100 to 1 inch).

(3) Collect filtrate, agitate, and till a 15 ml test tube.

(4) Centrifuge at 2000 rpm for 2 minutes.

(5) Pour off supernatant, agitate sediment and fill tube to previous level with flotation solution.

(6) Invert tube six times and remove fluid with pipette to fill both chambers of McMaster slide. Leave no fluid in the pipette or else pipette rapidly, since the eggs will rise quickly in the flotation fluid.

(7) Examine one chamber and multiply number of eggs or larvae under one etched area by 100, or two chambers and multiply by 50, to arrive all the number of eggs per gram of faeces (epg):

If 3 g of faeces are dissolved in 42ml
Total volume is 45ml
Therefore lg 15 ml

The volume under etched area is 0.15 ml. Therefore the number of eggs is multiplied by 100.

If two chambers are examined, multiply by 50.

An abbreviated version of this technique is to homogenize the 3 g of faeces in 42 ml of salt solution, sieve, and pipette the filtrate directly into the McMaster slide. Although a faster process the slide contents are more difficult to 'read' because of their dark colour.

It is impossible to calculate from the epg the actual worm population of the host, since many factors influence egg production of worms and the number of eggs also varies with the species. Nevertheless, egg counts in excess of 1000 are generally considered indicative of heavy infections and those over 500 of moderate infection. However, a low epg is not necessarily indicative of very low infections, since patency may just be newly established; alternatively, the epg may be affected by developing immunity. The eggs of some species, such as certain ascarids, *Sirongyioides*, *Oxyuris, Trichuris* and *Capillaria,* can be easily recognized morphologically. However, with the exception of *Nematodirus* spp., the common trichostrongyle eggs require measurement for differentiation.

While this technique will detect the eggs and larvae of most nematodes, cestodes, and coccidia, it will not demonstrate trematode eggs which have a higher specific density. For these, a flotation fluid of higher specific gravity such as a saturated solution of zinc sulphate lias to be used or a sedimentation method employed as described below.

Material and technical supply. Microscopes, magnifying glass, permanent macro preparations, temporary and permanent micro preparations. Tables, schemes. Invasive animals, Freshly obtained faeces from them, all that is necessary for carrying out a coproscopic study by the method of Fuleborn.

«____» _____ 20 p. Signatures: Student_____ Lecturer _____

LABORATORY CLASS № 6 «____» _____ 20 p.

TOPIC: Diagnostics and differential diagnosis of animals' zoomastygophoroses – trichomonosis of cattle and histomonosis of poultry and trypanosomosis of solipeds

Class location– classroom, laboratory, museum of the department

Purpose of the lesson: To study the morphological and biological characteristics of pathogens of trichomonosis of cattle *(Trichomonas foetus)*, poultry *(T. gallinae, T. gallinarum, T. cberi, T. anseri, T. anatis)*, histomonosis of poultry *(Histomonas meleagridis)*, trypanosomosis of solipeds *(Trypanosoma eguiperdum, T. evansi)*. Their place in classification of zooparasites. To master methods of life-time and post-mortem diagnosis and condact their differential diagnosis. To get acquainted with medical preparations and with the peculiarities of their use in different types of animals.

Task: To study the morphological features of pathogens of this family using macro- and micropreparations, to know the peculiarities of their development. To master features of diagnostics and differential diagnosis of these diseases. To study the samples of medicines, their use for therapeutic and preventive purposes. To master practically basic methods of laboratory diagnostics of a group of protozoal diseases of animals.

Independently prepare for classes using recommended books (1−4), lecture material and electronic files from the discipline «Veterinary Parasitology» at the «Distance learning portal (MOODLE) of SBTU».

Auditory work. To study and make a drawing or mark in pictures the basic diagnostic features of these diseases and the pathogen agents using the museum material (macropreparations), temporary and permanent micropreparations. Carry out clinical and parasitological examination of animals, make a diagnostics and differential diagnosis, appoint treatment. Get acquainted with the arsenal of medicines recommended for control this group of diseases.

Task performance:

1. The place of pathogens of these diseases in the world animals system (classification):

Kingdom _____ Phylum _____

Subkingdom _____ Class _____

Order _____ Order _____

Family _____ Family _____ Family _____

Genus _____ Genus _____ Genus _____

2. Sources and ways of invasion of animals by pathogens of this group of protozooses:

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_____

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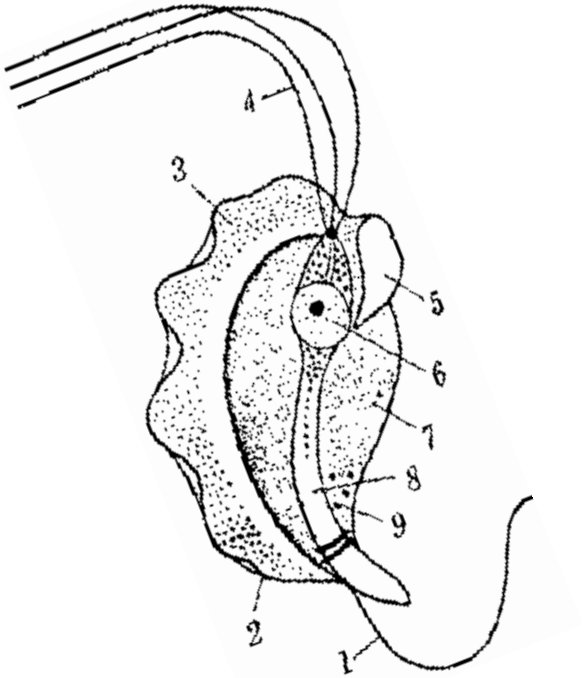
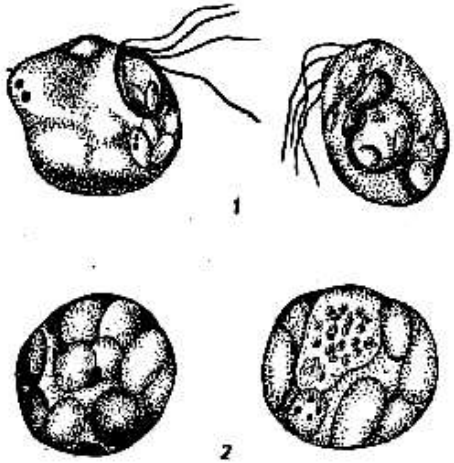
_____

_____

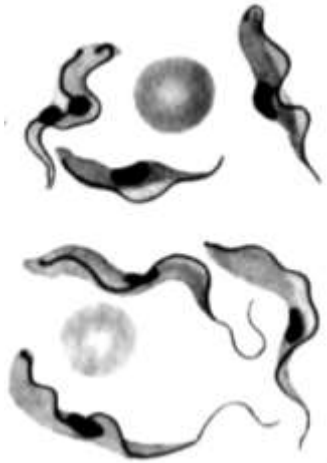
3. Morphological characteristics of pathogens:



Causative agent of trichomonosis



Causative agent of histomonosis
1 – flagella shape; 2 – unflagella shape



Causative agent of trypanosomosis of animals

1 – _____

2 – _____

3 – _____

4 – _____

5 – _____

6 – _____

7 – _____

8 – _____

9 – _____

Definition: _____

_____

_____

_____

_____

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_____

_____

4. Features of life-time and post-mortem diagnostics, differential diagnosis:

Clinical signs _____

Pathoanatomical changes _____

Special laboratory diagnostics _____

5. Measures of control and ways of prevention of this group of protozoa diseases. Therapeutic drugs.

Treatment _____

Prevention _____

Material and technical supply. Microscopes, permanent macro preparations, temporary and permanent micro preparations. Tables, schemes. Sick animals or feces from them. Samples of drugs.

«____» _____ 20 p. Signatures: Student_____ Lecturer _____

LABORATORY CLASS № 7 «____» _____ 20 p.

TOPIC: Diagnostics and differential diagnosis of pigs' balantidiosis, ruminants' anaplasmosis, poultry's borreliosis
Content module I. «Veterinary protozoology and protozooses of animals»

Class location– classroom, laboratory, museum of the department

Purpose of the lesson: To study the morphological and biological characteristics of pathogens of balantidiosis of pigs *(Balantidium suis)*, anaplasmosis of ruminant *(Anaplasma marginale, A. centrale, A. ovis)* and borreliosis of poultry *(Borrelia anserinum, B. (Treponema) hyodisenteria)*, determine their place in the current classification. To draw or indicate diagnostic features. To master methods of life-time, post-mortem and differential diagnosis. To get acquainted with modern medicines and features of their use in carrying out therapeutic and prophylactic treatments in animals.

Task: To study the morphological features of pathogens of this family using macro- and micropreparations, to know the peculiarities of their development. To master features of diagnostics and differential diagnosis of these diseases. To study the samples of medicines, their use for therapeutic and preventive purposes. To master practically basic methods of laboratory diagnostics of a group of protozoal diseases of animals.

Independently prepare for classes using recommended books (1−4), lecture material and electronic files from the discipline «Veterinary Parasitology» at the «Distance learning portal (MOODLE) of SBTU».

Auditory work. To study and make a drawing of the basic diagnostic features of the agents of this group of animals disease using the museum material (macropreparations), temporary and permanent micropreparations. Carry out clinical and parasitological examination of animals, to make diagnosis, prescribe treatment. Get acquainted with the arsenal of medicines recommended for control this group of diseases.

Task performance:

1. The place of pathogens of these diseases in the world animals system (classification):

Kingdom _____	Kingdom _____	
Phylum _____	Phylum _____	
Class _____		
Order _____	Order _____	Order _____
Family _____	Family _____	Family _____
Genus _____	Genus _____	Genus _____

Definition:_____
_____
_____
_____

2. Sources and ways of invasion of animals by pathogens of this group of protozooses _____

_____

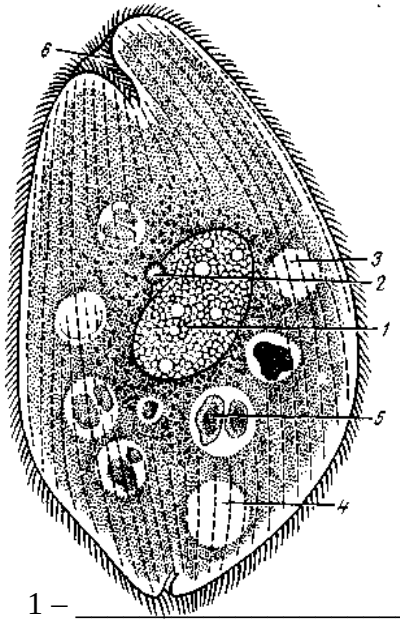
_____

3. Morphological characteristics of pathogens of this group of parasites:

To sketch

Causative agent of anaplasmosis in erythrocytes of ruminant

Causative agent of borreliosis in blood of poultry



1 – _____

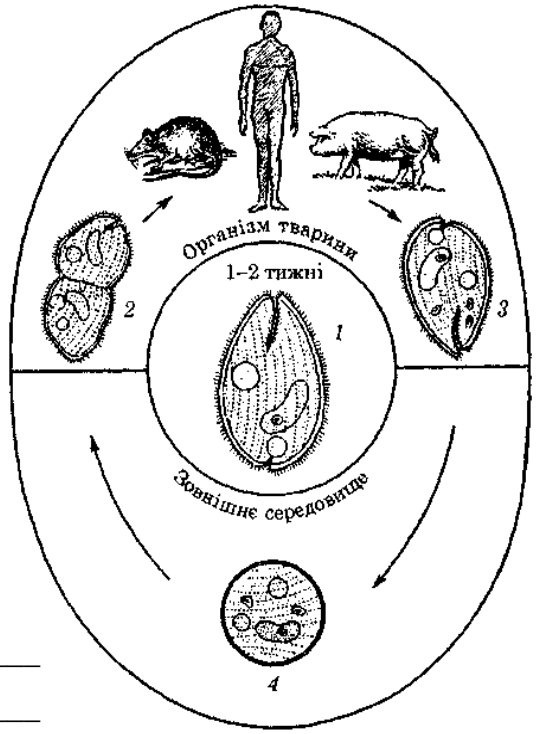
2 – _____

3 – _____

4 – _____

5 – _____

6 – _____



Life cycle of Balantidia:

1, 3 – vegetative form;

2 – cell's fission; 4 – cyst form.

4. Features of life-time and post-mortem diagnostics, differential diagnosis of this group of parasites:

Clinical signs _____

_____

_____

_____

Pathoanatomical changes _____

Special laboratory diagnostics _____

5. Measures of control and ways of prevention of this group of protozoa diseases. Therapeutic drugs.

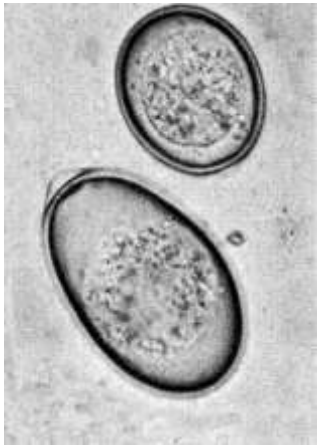
Treatment _____

Prevention _____

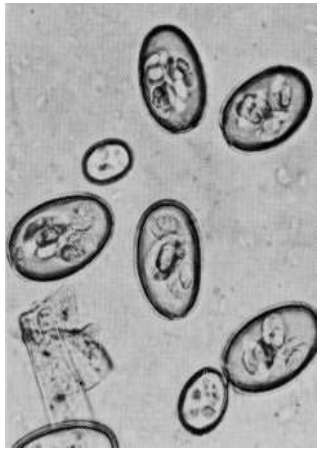
Material and technical supply. Microscopes, permanent macro preparations from museim, temporary and permanent micro preparations. Tables, schemes. Sick animals or feces from them. Samples of drugs.

«____» _____ 20 p. Signatures: Student_____ Lecturer _____

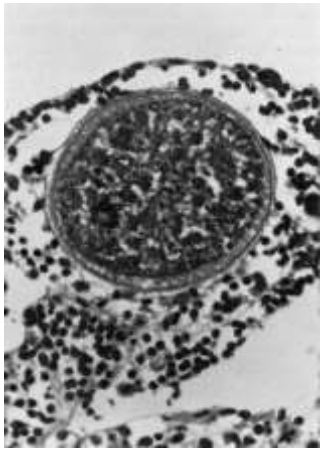
PICTURES FOR SECTION «VETERINARY PROTOZOOLOGY OF ANIMALS»



1. Oocysts of *Eimeria bovis.*

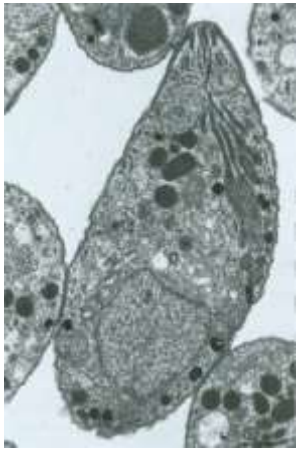


2. Oocysts of Eimeria in rabbits' faeces.

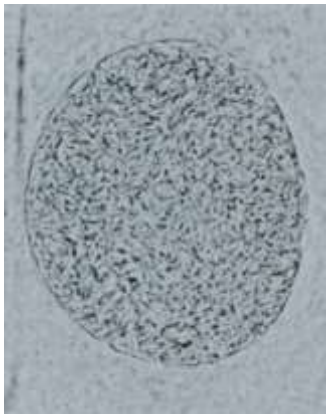


3. Schizont of *Eimeria bovis*.

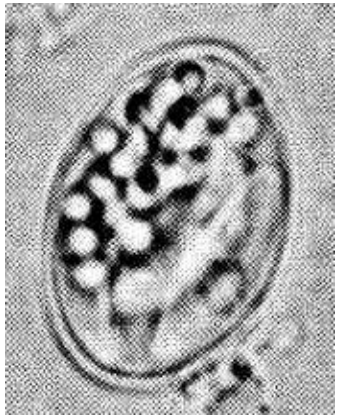
4. Oocysts of *Toxoplasma gondii* in cat's feaces.



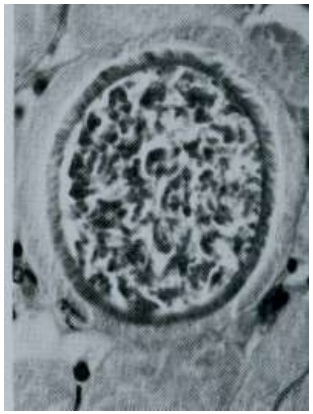
5. Endozoites of *T. gondii.*



6. Cystozoit of *T. gondii* in mouse's brain

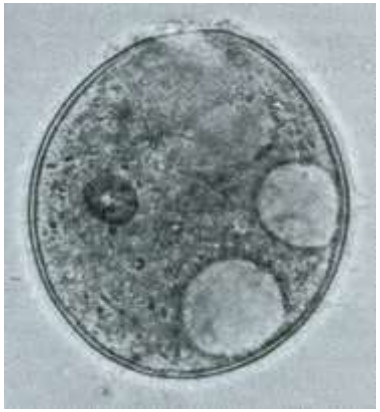


7. Oocyst of *Sarcocystis. suicanis*



8. Cysts of *S. bovifelis.*

9. Cysts of *S. bovicanis*



10. Trophozoite of *Balantidium coli*

LABORATORY CLASS № 8 **«____» _____ 20 p.**

TOPIC: Characteristics of the class Trematoda. Diagnostics and differential diagnosis of fasciolosis and paramphistomidoses in ruminants

Class location– classroom, laboratory, museum of the department

Purpose of the lesson: To study the morphological and biological characteristics of pathogens of representetives of class *Trematoda* and family *Fasciolidae (Fasciola hepatica, F. gigantica)* and *Paramphistomidae* (*Paramphistomum cervi (*syn. *Liorchis scotiae), P. ichikawai).* Their place in classification of parasitic worms. To master methods of life-time and post-mortem diagnosis and differential diagnosis. To get acquainted with anthelmintic preparations and with the peculiarities of their use in different types of animals. To study the eggs and larval stages of these parasites and draw them.

Task: To study the morphological features of pathogens of this family using macro- and micropreparations, to know the peculiarities of their development. To master features of diagnostics and differential diagnosis of these diseases. To study the samples of anthelmintic preparations, their use for therapeutic and preventive purposes. To master practically basic methods of laboratory diagnostics of a group of trematodous diseases of animals.

Independently prepare for classes using recommended books (1−4), lecture material and electronic files from the discipline «Veterinary Parasitology» at the «Distance learning portal (MOODLE) of SBTU».

Auditory work. To study and make a drawing or mark in pictures the basic diagnostic features of pathogents of these diseases using the museum material (macropreparations), temporary and permanent micropreparations. Carry out clinical and parasitological examination of animals, make a diagnostics and differential diagnosis, to appoint treatment. Get acquainted with the arsenal of medicines recommended for control this group of diseases.

Task performance:

Helminthology – _____

_____

_____

Veterinary helminthology – _____

_____

_____

Types of pathogenic effects of zooparasites for the body of definitive host: _____

_____

_____

_____

Diagnostics of helminthoses.

Helminthoscopy – _____

_____

_____

Helminthoovoscopy – _____

_____

_____

Helmintholarvoscopy – _____

_____

_____

Immunological diagnostics – _____

_____

_____

Post-mortem diagnostics – _____

_____

_____

_____

General characteristic of Class Trematoda _____

_____

_____

_____

_____

_____

1. The place of pathogens of these diseases in the world animals system (classification):

Phylum _____ Suborder _____ Suborder _____

Class _____ Family _____ Family _____

Order _____ Genus _____ Genus _____

Definition of fasciolosis: _____

_____

_____

_____

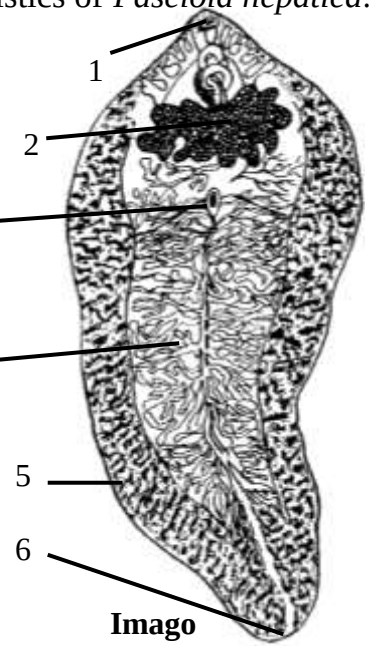
_____

_____

2. Morphological characteristics of *Fasciola hepatica*:

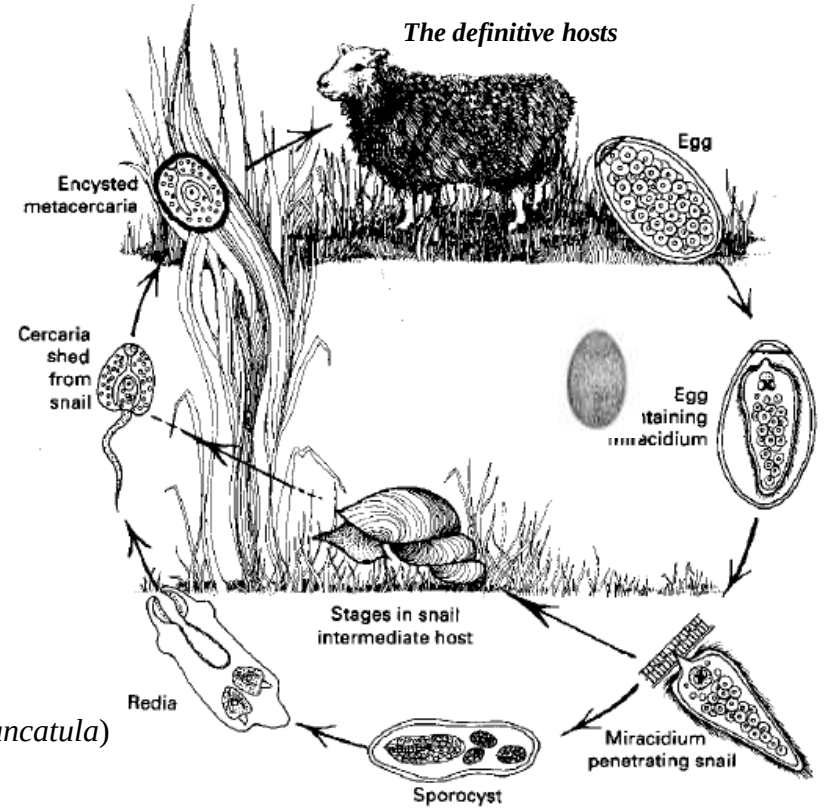
To sketch

Egg of *Fasciola hepatica*



Imago

Pond snails (*Limnaea truncatula*)



Life cycle of *Fasciola hepatica*

(https://lupinepublishers.com/dairy-veterinary-science-journal/fulltext/an-introductory-to-fasciolosis.ID.000139.php)

1 – _____

2 – _____

3 – _____

4 – _____

5 – _____

6 – _____

Definition of paramphistomidoses: _____

_____

_____

_____

_____

_____

Morphological characteristics of *Paramphistomum cervi*

The definitive hosts

To sketch

Egg of *Paramphistomum cervi*

1 – _____

2 – _____

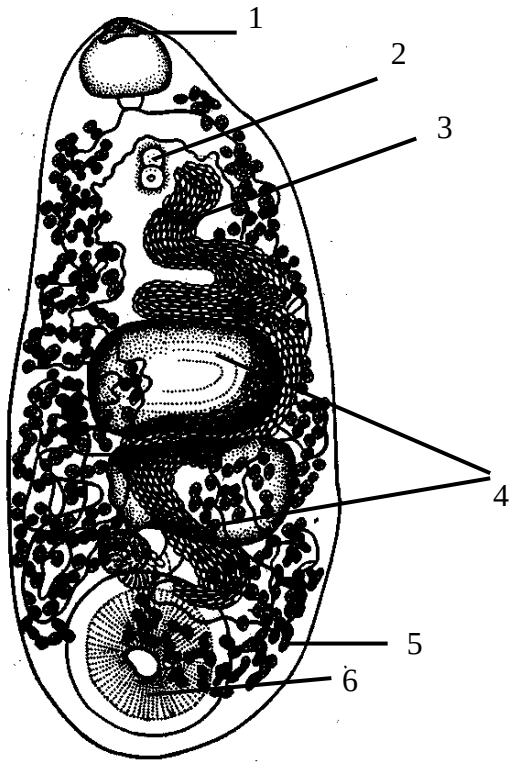
3 – _____

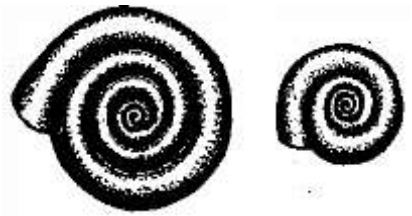
4 – _____

5 – _____

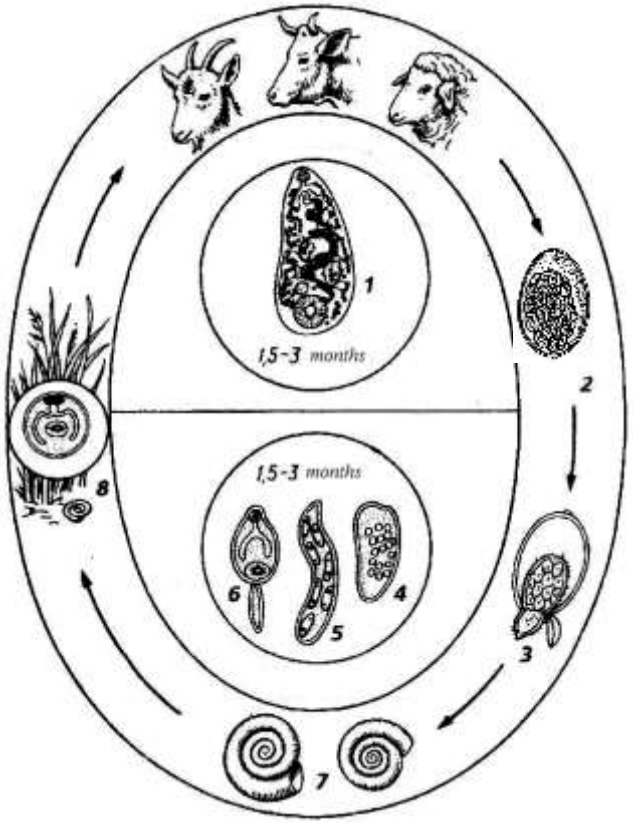
6 – _____

Imago





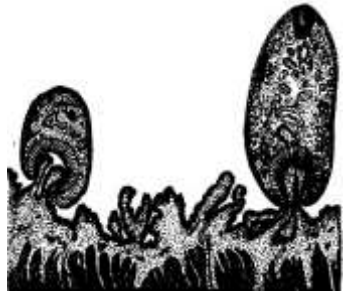
Orb snails (*Planorbis planorbis*)



Intermediate hosts

Life cycle of *Paramphistomum cervi*:

1 – marita; 2 – egg; 3 – the output of miracidium from the eggs; 4 – sporocyst; 5 – redia; 6 – cercaria; 7 – orb snails; 8 – encysted metacercaria.



***Paramphistomum cervi* in the rumen**

4. Sources and ways of invasion ruminant by *Paramphistomum cervi* and *Fasciola hepatica*: _____

_____

_____

_____

5. Features of life-time and post-mortem diagnostics, differential diagnosis of fasciolosis and paramphistomidoses in ruminant:

Clinical signs _____

_____

_____

_____

_____

Pathoanatomical changes _____

_____

_____

_____

_____

Special laboratory diagnostics _____

_____

_____

6. Measures of control and ways of prevention of fasciolosis and paramphistomidoses in ruminant. Therapeutic drugs.

Treatment _____

_____

_____

_____

Prevention _____

_____

_____

_____

_____

Material and technical supply. Microscopes, permanent macro preparations, temporary and permanent micro preparations. Tables, schemes, photoes. Freshly obtained faeces from ruminant animals, all that is necessary for carrying out a coproscopic study by the sedimentation method. Samples of drugs.

«____» _____ 20 p. Signatures: Student_____ Lecturer _____

LABORATORY CLASS № 9 **«____» _____ 20 p.**

TOPIC: Diagnostics and differential diagnosis of dicrocoeliosis, eurytremosis and hasstilesiosis in ruminants

Class location– classroom, laboratory, museum of the department

Purpose of the lesson: To study the morphological and biological characteristics of pathogens of representetives of family *Dicrocoeliidae (Dicrocoelium lanceatum, Eurytrema pancreaticum)* and family *Brachylamidae* (*Hasslilesia ovis*). Their place in classification of parasitic worms. To master methods of life-time and post-mortem diagnosis and differential diagnosis. To get acquainted with anthelmintic preparations and with the peculiarities of their use in different types of animals. To study the eggs and larval stages of these parasites and draw them.

Task: To study the morphological features of pathogens of this family using macro- and micropreparations, to know the peculiarities of their development. To master features of diagnostics and differential diagnosis of these diseases. To study the samples of anthelmintic preparations, their use for therapeutic and preventive purposes. To master practically basic methods of laboratory diagnostics of a group of trematodous diseases of animals.

Independently prepare for classes using recommended books (1−4), lecture material and electronic files from the discipline «Veterinary Parasitology» at the «Distance learning portal (MOODLE) of SBTU».

Auditory work. To study and make a drawing or mark in pictures the basic diagnostic features of pathogents of these diseases using the museum material (macropreparations), temporary and permanent micropreparations. Carry out clinical and parasitological examination of animals, make a diagnostics and differential diagnosis, appoint treatment. Get acquainted with the arsenal of medicines recommended for control this group of diseases.

Task performance:

1. The place of pathogens of these diseases in the world animals system (classification):

Phylum _____	Class _____	Suborder _____
	Family _____	Family _____
Genus _____	Genus _____	Genus _____

Definition of dicrocoeliosis: _____
_____
_____
_____
_____

Definition eurytremosis: _____
_____
_____
_____

Definition hasstilesiosis: _____

_____

_____

_____

2. Sources and ways of invasion ruminant by such tremadotes _____

_____

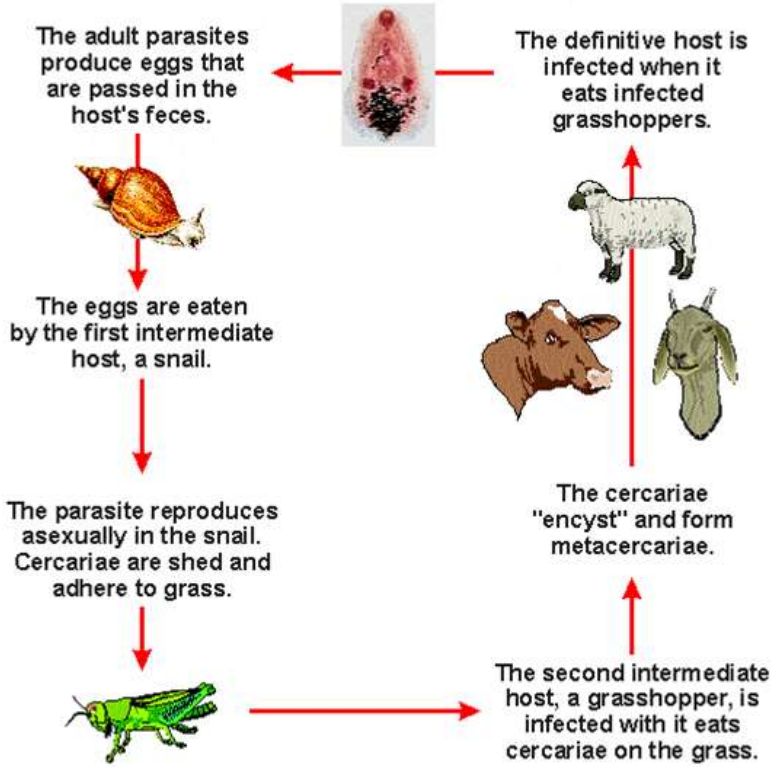
_____

_____

3. Morphological characteristics of such group of trematodes and their life cycles

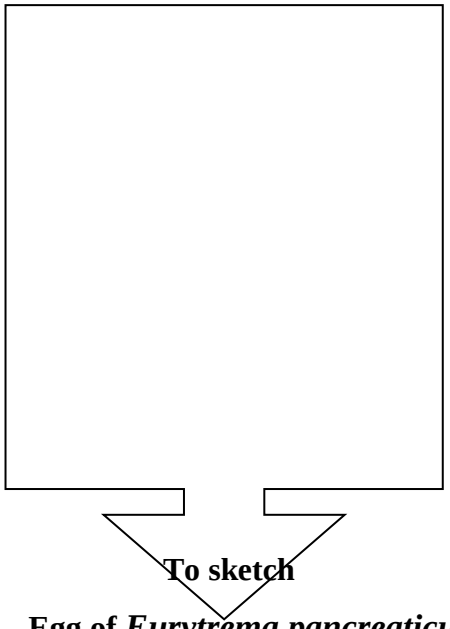
Morphological characteristics of *Eurytrema pancreaticum*

Eurytrema pancreaticum



Life cycle of *Eurytrema pancreaticum*

(https://www.animalhealthoasis.com/veterinary-services/parasite-treatment/)



To sketch

Egg of *Eurytrema pancreaticum*

Morphological characteristics of *Dicrocoelium lanceatum*

To sketch

Egg of *Dicrocoelium lanceatum*

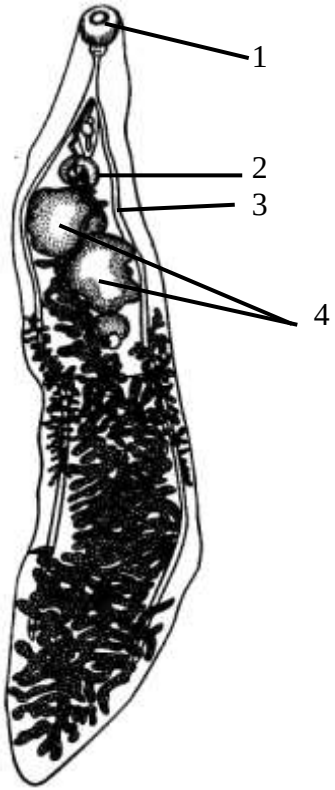
1 – _____

2 – _____

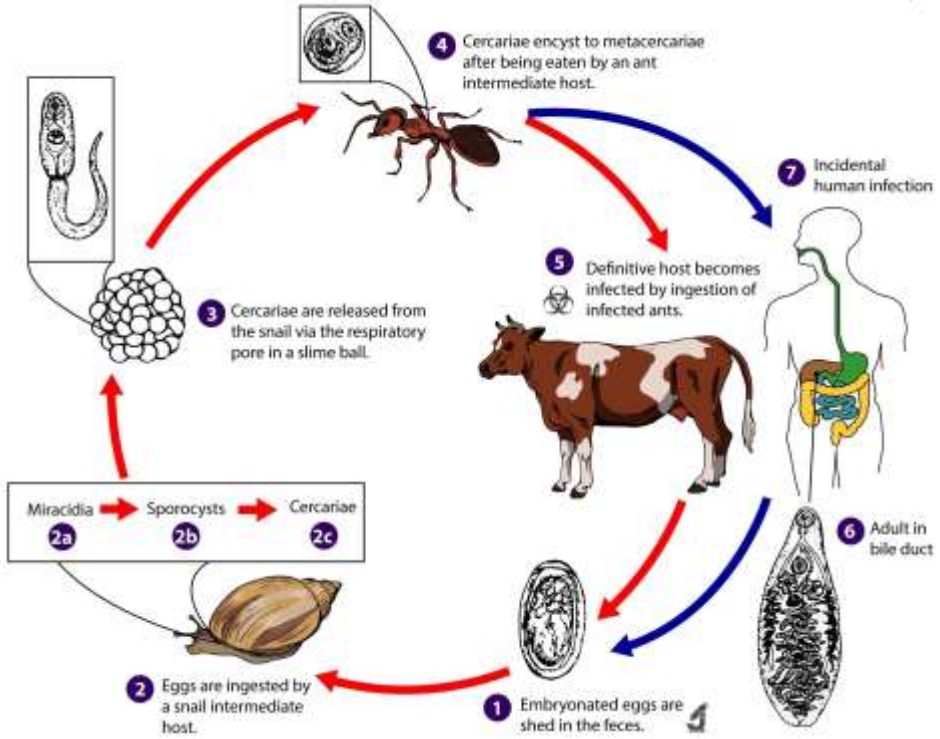
3 – _____

4 – _____

Intermediate host – land snails of genus *Helicella* **and** *Chondrula*



Marita of *Dicrocoelium lanceatum*



Life cycle of *Dicrocoelium lanceatum*

(https://www.cdc.gov/dpdx/dicrocoeliasis/index.html)

Second intermediate host – ants of genus *Formica*

4. Features of life-time and post-mortem diagnostics, differential diagnosis of this group of trematodoses:

Clinical signs _____

_____

_____

_____

Pathoanatomical changes _____

Special laboratory diagnostics _____

5. Measures of control and ways of prevention of such group of trematodoses in ruminant. Therapeutic drugs.

Treatment _____

Prevention _____

Material and technical supply. Microscopes, permanent macro preparations, temporary and permanent micro preparations. Tables, schemes, photoes. Freshly obtained faeces from ruminunts, all that is necessary for carrying out a coproscopic study by the sedimentation method. Samples of drugs.

«____» _____ 20 p. Signatures: Student_____ Lecturer _____

LABORATORY CLASS № 10 «____» _____ 20 p.

TOPIC: Diagnostics and differential diagnosis of poultry's trematodoses: prosthogonimosis, echinostomatidoses and notocotylidoses

Class location– classroom, laboratory, museum of the department

Purpose of the lesson: To study the morphological and biological characteristics of pathogens of poultry's trematodoses the representetives of family *Prosthogonimidae (Prosthogonimus ovatus, P. cuneatus, P. macrorchis), Echinostomatidae (Echinostoma revolutum, Echinoparyphium recurvatum, Hypoderaeum conoideum), Notocotylidae (Notocotylus attenuatus*, *Catatropis verrucosa).* Their place in classification of parasitic worms. To master methods of life-time and post-mortem diagnosis and differential diagnosis. To get acquainted with anthelmintic preparations and with the peculiarities of their use in different types of animals. To study the eggs and larval stages of these parasites and draw them.

Task: To study the morphological features of pathogens of this family using macro- and micropreparations, to know the peculiarities of their development. To master features of diagnostics and differential diagnosis of these diseases. To study the samples of anthelmintic preparations, their use for therapeutic and preventive purposes. To master practically basic methods of laboratory diagnostics of a group of trematodous diseases of animals.

Independently prepare for classes using recommended books (1−4), lecture material and electronic files from the discipline «Veterinary Parasitology» at the «Distance learning portal (MOODLE) of SBTU».

Auditory work. To study and make a drawing or mark in pictures the basic diagnostic features of pathogents of these diseases using the museum material (macropreparations), temporary and permanent micropreparations. Carry out clinical and parasitological examination of animals, make a diagnostics and differential diagnosis, appoint treatment. Get acquainted with the arsenal of medicines recommended for control this group of diseases.

Task performance:

1. The place of pathogens of these diseases in the world animals system (classification):

Phylum _____

Class _____

Order _____

Family _____	Family _____	Family _____
Genus _____	Genus _____	Genus _____
	Genus _____	Genus _____
	Genus _____	

_____

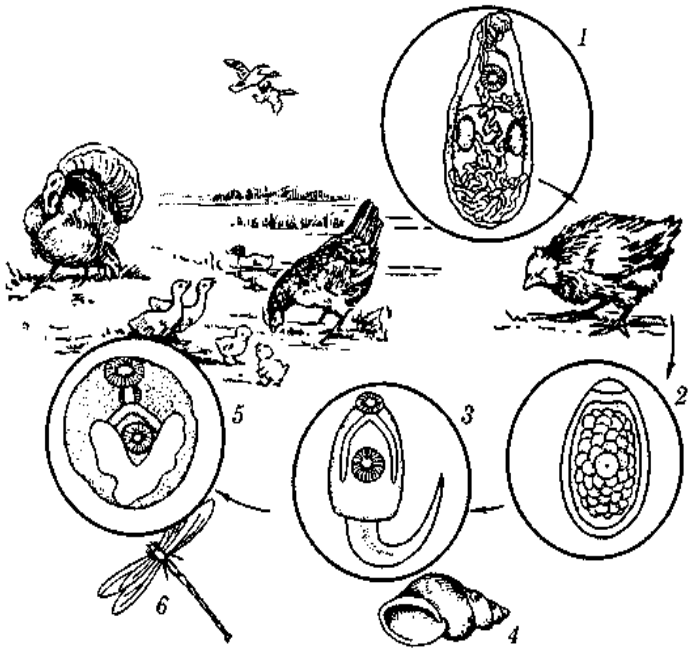
_____

_____

2. Morphological characteristics of pathogens of prosthogonimosis:

Prosthogonimus cuneatus

P. ovatus

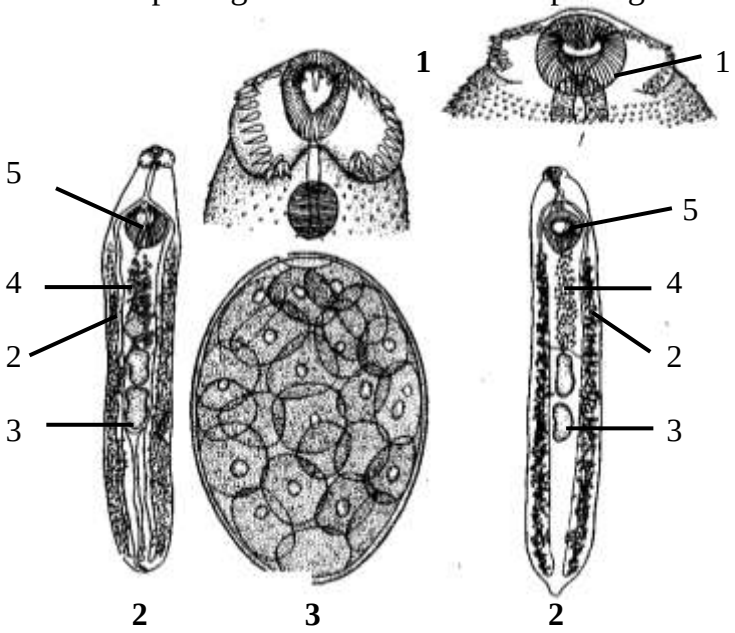


Life cycle of Prosthogonimus spp.:
1 – marita; 2 – egg; 3 – cercaria; 4 – intermediate host (pond snail); 5 – metacercaria; 6 – second intermediate host (dragonflies).

Definition: _____
_____
_____
_____
_____
_____

3. Sources and ways of invasion animals of this group of poultry's trematodoses: _____
_____
_____

4. Morphological characteristics of pathogens of echinostomatidoses:



1 – the head end of the parasite; **2** – marita; **3** – egg

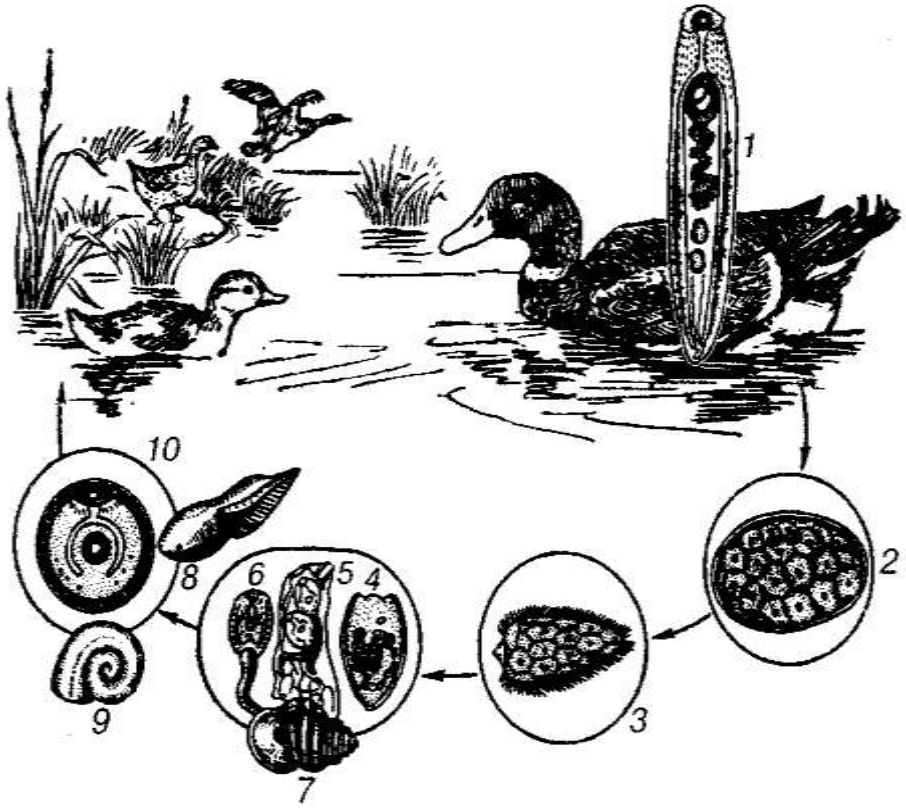
1 – _____

2 – _____

3 – _____

4 – _____

5 – _____



Life cycle of Echinostoma spp.:

1 – marita; 2 – egg; 3 – miracidium; 4 – sporocyst; 5 – redia;

6 – cercaria; 7 – pond snail; 8 – tadpole; 9 – orb snails; 10 – metacercaria

5. Features of life-time and post-mortem diagnostics, differential diagnosis of this group of trematodoses of poultry:

Clinical signs _____

_____

_____

_____

_____

Pathoanatomical changes _____

Special laboratory diagnostics _____

6. Measures of control and ways of prevention of such group of trematodoses in poultry. Samples of drugs.

Treatment _____

Prevention _____

Material and technical supply. Microscopes, magnifying glass, permanent macro preparations, temporary and permanent micro preparations, Intermediate hosts, Tables, schemes, Invasive poultry or freshly obtained faeces from them. Samples of drugs.

«____» _____ 20 p. Signatures: Student_____ Lecturer _____

LABORATORY CLASS № 11 **«____» _____ 20 p.**

TOPIC: Diagnostics and differential diagnosis of carnivorous' opisthorchidoses

Class location– classroom, laboratory, museum of the department

Purpose of the lesson: To study the morphological and biological characteristics of pathogens of representetives of family *Opisthorchidae* (*Opisthorchis tenuicollis (O. felineus), O. viverrini).* Their place in classification of parasitic worms. To master methods of life-time and post-mortem diagnosis and differential diagnosis. To get acquainted with anthelmintic preparations and with the peculiarities of their use in different types of animals. To study the eggs and larval stages of these parasites and draw them.

Task: To study the morphological features of pathogens of this family using macro- and micropreparations, to know the peculiarities of their development. To master features of diagnostics and differential diagnosis of these diseases. To study the samples of anthelmintic preparations, their use for therapeutic and preventive purposes. To master practically basic methods of laboratory diagnostics of a group of trematodous diseases of animals.

Independently prepare for classes using recommended books (1−4), lecture material and electronic files from the discipline «Veterinary Parasitology» at the «Distance learning portal (MOODLE) of SBTU».

Auditory work. To study and make a drawing or mark in pictures the basic diagnostic features of pathogents of these diseases using the museum material (macropreparations), temporary and permanent micropreparations. Carry out clinical and parasitological examination of animals, make a diagnostics and differential diagnosis, appoint treatment. Get acquainted with the arsenal of medicines recommended for control this group of diseases.

Task performance:

1. The place of pathogens of these diseases in the world animals system (classification):

Phylum _____ Order _____ Family _____

Class _____ Suborder _____ Genus _____

Definition:_____

_____

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2. Sources and ways of invasion carnivorous by trematodoses _____

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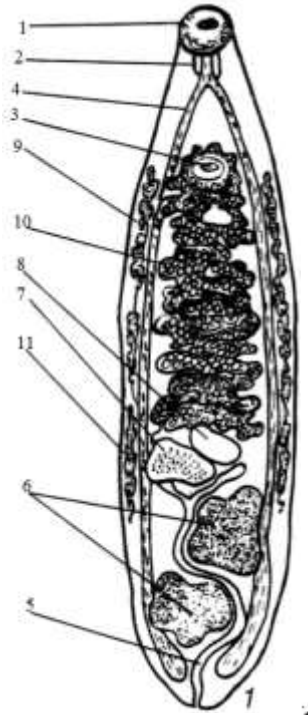
_____

3. Morphological characteristics of genus *Opisthorchis*:



1 – _____

2 – _____

3 – _____

4 – _____

5 – _____

6 – _____

7 – _____

8 – _____

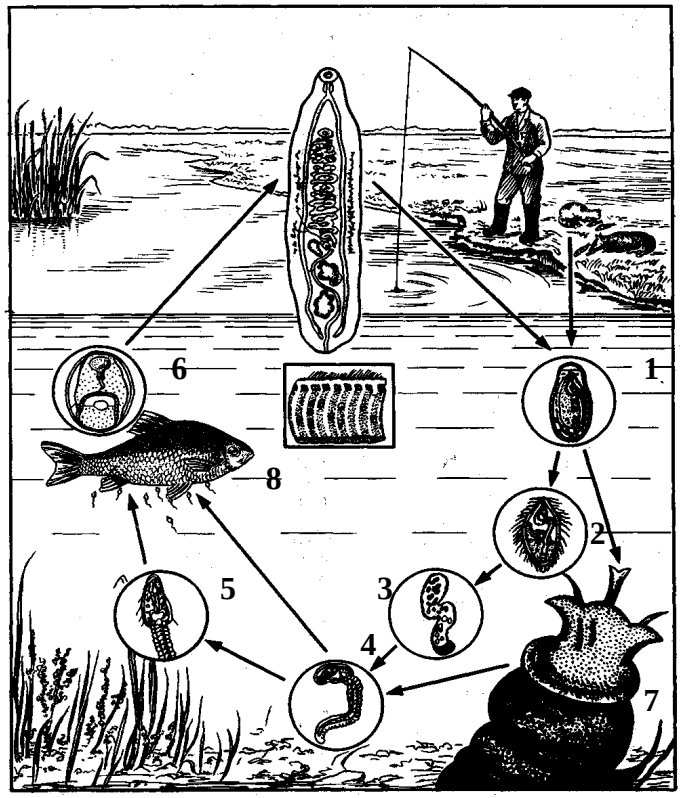
9 – _____

10 – _____

11 – _____

Second intermediate host

Intermediate host



Life cycle of *Opisthorchis felineus*:
1 – egg; 2 – miracidium; 3 – sporocyst; 4 – redia;
5 – cercaria; 6 – metacercaria; 7 – intermediate host;
8 – second intermediate host.

4. Features of life-time and post-mortem diagnostics, differential diagnosis of trematodesof carnivorous:

Clinical signs _____

_____

_____

_____

_____

Pathoanatomical changes _____

Special laboratory diagnostics _____

5. Measures of control and ways of prevention of trematodoses of carnivorous. Therapeutic drugs.

Treatment _____

Prevention _____

Material and technical supply. Microscopes, permanent macro preparations, temporary and permanent micro preparations. Tables, schemes, photoes. Freshly obtained faeces from carnivorous (fisheaten) animals, all that is necessary for carrying out a coproscopic study by the sedimantation method. Samples of drugs.

«____» _____ 20 p. Signatures: Student_____ Lecturer _____

LABORATORY CLASS № 12 **«____» _____ 20 p.**

TOPIC: Testing equipment of lifetime and post-mortem diagnostics of helminthous invasions of animals
Content module II. «Veterinary trematodology and trematodoses of animals»

Class location– classroom, laboratory, museum of the department

Purpose of the lesson: To learn the basic methods of life-time and post-mortem laboratory diagnostics and differential diagnosis of helminth invasions of animals.

Task: To workout the methodology and to know the features of special diagnostics of helminth invasions.

Independently prepare for classes using recommended books (1−4), lecture material and electronic files from the discipline «Veterinary Parasitology» at the «Distance learning portal (MOODLE) of SBTU».

Auditory work. To conduct a clinical and parasitological examination of a sick animal or a freshly obtained faecas from it and to diagnose.

Task performance:

COLLECTION OF FAECES

Faecal samples should preferably be collected from the rectum and examined fresh. If it is difficult to take rectal samples, then fresh faeces can be collected from the field or floor. A plastic glove is suitable for collection, the glove being turned inside out to act as the receptacle. For small pets a thermometer or glass rod may be used.

Ideally, about 5 g of faeces should be collected, since this amount is required for some of the concentration methods of examination.

Since eggs embryonate rapidly the faeces should be stored in the refrigerator unless examination is carried out within a day. For samples sent through the post the addition of twice the faecal volume of 10 % formalin to the faeces will minimize development and hatching.

METHODS OF EXAMINATION OF FAECES

Several methods are available for preparing faeces for microscopic examination to detect the presence of eggs or larvae. However, whatever method of preparation is used, the slides should first be examined under low power since most eggs can be detected at this magnification. If necessary, higher magnification can then be employed for measurement of the eggs or more detailed morphological differentiation. An eyepiece micrometer is very useful for sizing populations of eggs or larvae.

Direct smear method. A few drops of water plus an equivalent amount of faeces are mixed on a microscope slide. Tilting the slide then allows the lighter eggs to flow away from the heavier debris, a cover slip is placed on the fluid and the preparation is then examined microscopically. It is possible to detect most eggs or larvae by this method, but due to the small amount of faeces used it may only detect relatively heavy infections.

Flotation methods. The basis of any flotation method is that when worm eggs are suspended in a liquid with a specific gravity higher than that of the eggs, the latter will float up to the surface. Nematode and cestode eggs float in a liquid with a specific gravity of between 1.10 and 1.20; tremalode eggs, which are much heavier, require a specific gravity of 1.30-1.35.

The flotation solutions used for nematode and cestode ova are mainly based on sodium chloride or sometimes magnesium sulphate. A saturated solution of these is prepared and stored for a few days and the specific gravity checked prior to usage. In some laboratories a sugar solution of density 1.2 is preferred. For trematode eggs, saturated solutions of zinc chloride or zinc sulphate are widely used. Some laboratories use the more expensive and toxic potassium mercury iodine solution.

Whatever solutions are employed the specific gravity should be checked regularly and examination of the solution containing the eggs or larvae made rapidly, otherwise distortion may take place.

Direct flotation. A small amount of fresh faeces, say 2.0 g, is added to 10 ml of the flotation solution and following thorough mixing the suspension is poured into a test tube and more flotation solution added to fill the tube to the top. A cover glass is then placed on top of the surface of the liquid and the tube and coverslip left standing for 10 to 15 minutes. The cover slip is then removed vertically and placed on a slide and examined under the microscope. If a centrifuge is available the flotation of the eggs in the flotation solution may be accelerated by centrifugation.

McMaster method

This quantitative technique is used where it is desirable to count the number of eggs or larvae per gramme of faeces. The method is as follows:

- Weigh 3.0g of faeces or, if faeces are diarrhoeic, 3 teaspoonfuls.
- Break up thoroughly in 42 ml of water in a plastic container. This can be done using a homogenizer if available or in a stoppered bottle containing glass beads.
- Pour through a fine mesh sieve (aperture 205/tm, or 100 to 1 inch)
- Collect filtrate, agitate, and till a 15ml test tube.
- Centrifuge at 2000rpm for 2 minutes.
- Pour off supernatant, agitate sediment and fill tube to previous level with flotation solution,
- Invert tube six times and remove fluid with pipette to fill both chambers of McMaster slide (Fig. 1). Leave no fluid in the pipette or else pipette rapidly, since the eggs will rise quickly in the flotation fluid.
- Examine one chamber and multiply number of eggs or larvae under one etched area by 100, or two chambers and multiply by 50, to arrive al the number of eggs per gram of faeces (epg):

If 3 g of faeces are dissolved in 42ml
Total volume is 45ml
Therefore lg 15 ml

The volume under etched area is 0.15 ml Therefore the number of eggs is multiplied by 100. If two chambers are examined, multiply by 50.

An abbreviated version of this technique is to homogenize the 3g of faeces in 42 ml of salt solution, sieve, and pipette the filtrate directly into the McMaster slide. Although a faster process the slide contents are more difficult to 'read' because of their dark colour.

It is impossible to calculate from the epg the actual worm population of the host, since many factors influence egg production of worms and the number of eggs also varies with the species. Nevertheless, egg counts in excess of 1000 are generally considered indicative of heavy infections and those over 500 of moderate infection. However, a low epg is not necessarily indicative of very low infections, since patency may just be newly established; alternatively, the epg may be affected by developing immunity. The eggs of some species, such as certain ascarids, *Sirongyioides*, *Oxyuris, Trichuris* and *Capillaria,* can be easily recognized morphologically. However, with the exception of *Nematodirus* spp., the common trichostrongyle eggs require measurement for differentiation.

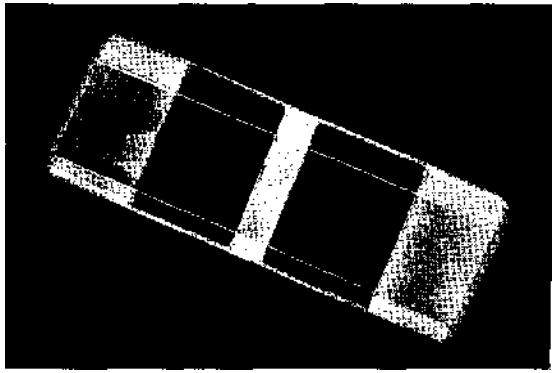


Fig. 1. McMaster slide for estimating numbers of nematode eggs in faeces

While this technique will detect the eggs and larvae of most nematodes, cestodes, and coccidia, it will not demonstrate trematode eggs which have a higher specific density. For these, a flotation fluid of higher specific gravity such as a saturated solution of zinc sulphate lias to be used or a sedimentation method employed as described below.

Sedimentation methods.

For trematode eggs: Homogenize 3 g of faeces with water and pass the suspension through a coarse mesh sieve (250/ml). Thoroughly wash the material retained on this screen using a fine water jet and discard the debris.

Transfer the filtrate to a conical flask and allow to stand for 2 minutes, remove the supernatant and transfer the remainder (approximately 12-15 ml) to a flat-bottomed tube. After sedimentation for a further 2 minutes the supernatant is again drawn off, a few drops of 5% methylene blue added and the sediment screened using a low power stereomicroscope. Any trematode eggs are readily visible

against the pale blue background.

For lungworm larvae: The Baerman apparatus may be used. This consists of a glass funnel held in a retort stand. A rubber tube attached to the bottom of the funnel is constricted by a clip. A sieve (aperture 250/ml) is placed in the wide neck of the funnel, which has been partially filled with water, and a double layer of gauze is placed on top of the sieve. Faeces are placed on the gauze and the funnel is slowly filled with water until the faeces are immersed. Alternatively, faeces are spread on a filter paper which is then inverted and placed on the sieve (Fig. 1). The apparatus is left overnight at room temperature during which the larvae migrate out of the faeces and through the sieve to sediment in the neck of the funnel. The clip on the rubber is then removed and the water in the neck of the funnel collected in a small beaker for microscopic examination in a petri dish.

A simple adaptation of the above method is to suspend the faeces enclosed in gauze in a urine glass filled with water and leave overnight. The larvae will leave the faeces, migrate through the gauze and settle at the bottom of the glass. After siphoning off the supernatant, the sediment is examined under the low power of the microscope as above.

CULTIVATION AND IDENTIFICATION OF LARVAE

Two techniques are widely used for the cultivation of infective larvae from nematode eggs.

In the first, faeces are placed in a jar with a lid and stored in the dark at a temperature of 21 to 24° C. The lid should be lined with moist filter paper and should not be tightly attached. After seven days incubation, the jar is filled with water and allowed to stand for 2 to 3 hours. The larvae will migrate into the water and the latter is poured into a cylinder for sedimentation. The larval suspension can be cleaned and concentrated by using the Baerman apparatus as described above and then killed by adding a few drops of Lugol's iodine and examined microscopically. An alternative method is to spread the faeces on the middle tWrd of a filter paper placed in a moistened petri dish. After storage at 21 to 24°C for 7 to 10 days, the dish is flooded with water and the larvae harvested as before.

The identification of infective larvae is a specialist technique and.

RECOVERY OF ALIMENTARY NEMATODES

Details are given below of a technique for the collection, counting and identification of the alimentary nematodes of ruminants. The procedure is similar for other host species, information on identification being available in the text.

(1) As soon as possible after removing the alimentary tract from the body cavity, the abomasal/duodenal junction should be ligatured to prevent transfer of parasites from one site to the other.

(2) Separate the abomasum, small intestine and large intestine.

(3) Open abomasum along the side of the greater curvature, wash contents into a bucket under running water and make the total volume up to 2 to 4 litres.

(4) After thorough mixing transfer duplicate 200 ml samples to suitably labelled containers and preserve in 10 % formalin.

(5) Scrape off the abomasal mucosa and digest in a pepsin/HCl mixture at 42°C for 6 hours; 200 g of mucosa wilt require 1 litre of mixture. Make digest up to a volume of 2 or 4 litres with cold water and again take duplicate 200 ml samples.

Alternatively, the Williams technique may be used. In this, the washed abomasum is placed, mucosal surface down, in a bucket containing several litres of normal saline and maintained at 40°C for 4 hours. Subsequently, the abomasum is gently rubbed in a second bucket of warm saline. The saline from both buckets is poured through a sieve (aperture 38/rm, about 600 to 1 inch) and the residue examined.

(6) Open the small intestine along its entire length and wash contents into a bucket. Treat as for the abomasal contents, but digestion of mucosal scrapings is unnecessary.

(7) The contents of the large intestine are washed into a bucket, passed through a coarse mesh sieve (aperture 2-3mm) and any parasites present collected and formalized.

Method of scraping from the skin of anal folds. Females of the nematode suborder oxyurate lay eggs of on the skin of the animal's anus. Using a matchstick or a wooden stick with a hook on the end, after moistening with a water-glycerol mixture (1:1), scrapers are made from the skin of prynal folds, from the inner surface of the root of the tail and from the perineum. Part of the scraper is placed in a water-glycerol drop on slide, covered with a cover and microscopic with a slight magnificant in the presence of eggs oxyuride.

In small animals, scotch tape is used. It is recommended that a scotch tape is used for sampling for the study of oxyurosis. It is pressed to the body of the animal in the same areas: to the anus folds, the inner surface of the tail and the perineum. Then they are glued to the slides and examined under a microscope.

Method of cultivation of larvae. In the gastrointestinal tract, ruminant and horse parasitic helminths of suborder Strongylata species and eggs of these worms are identical by size and structure. So using helminthic methods could only to diagnose in common – strongilatosis. Differential diagnosis of strongylatf by the structure of invasive larvae, which have specific morphological features and size, their internal structure for each genus and even species.

For the cultivation of the larvae take 20-30 g of freshly obtained faeces, placed in a glass or jar. Dishes with faeces samples are closed with gauze or glass, put in a warm place or thermostat at 25-27° C for 7 days or at room temperature for 10-12 days. During this period, the faeces are periodically moistened with water. After the cultivation the feces is investigated by Berman’s method. Selected invasive larvae immobilize, count 100-200 larvae and build a larvogram.

Urine testing is practiced to detect nematode eggs. Diagnosis of invasive diseases, parasites of which are parasitized in the urinary organs (capillary disease, dioctophimosis, etc.), is carried out by urinating for 5-10 minutes. Then the top layer is drained or collected with a syringe, and the precipitate is examined with a microscope. Sometimes the urine is diluted with water (1:1), centrifuged for 2-3 minutes, and the precipitate is examined with a microscope.

Blood tests. To detect microfilariae, blood is taken from a jugular vein into a test tube or from the tip of the ear or from the affected skin on a glass slide. A drop of blood is mixed thoroughly on a slide with one or two drops of a 50 % glycerol solution (native smear), covered with a glass, and examined under a microscope (eyepiece ×10, lens ×8). Two strokes can be made on one glass. At least 2-3 smears are examined from one animal (this method can be used in addition to other methods).

Modified Knott's technique and direct blood smear

The modified Knott's method is used for the concentration and identification of microfilariae, specifically the heartworm Dirofilaria immitis. It must be differentiated from the non-pathogenic microfilaria of Dipetalonema reconditum ("Dipet" for short).

A direct blood smear can be done at the same time. It is a quick, non-concentration test for microfilaria. Dirofilaria and Dipet are differentiated by their motility pattern. These tests should always be done whenever a patient tests heartworm antigen positive, or there is any reason to suspect the dog currently has or has had heartworm disease in the past. The presence of Dirofilaria will impact the course and/or choice of treatment. The Knott's test can be done without doing a direct smear, but never do a direct smear without doing a Knott's test. The Knott's is a more sensitive test because it concentrates the microfilaria so they are less likely to be missed during microscopic examination.

Procedure for direct smear: Simple. Place a drop of blood, preferably from an EDTA tube or heparinized syringe, in the center of a slide. Drop a cover slip over top and examine under the microscope on 10x power. Dirofilaria will have a stationary, writhing movement; Dipetalonema will exhibit a more rapid, directional movement and will often shoot right off of the field.

Procedure for the modified Knott's technique: Ensure you are wearing gloves for protection. Using a 15ml centrifuge tube, add approximately 10ml of 2 % formalin (this can be purchased commercially) to 1 ml of anticoagulated blood. Place your thumb over the top of the capped tube and invert several times to thoroughly mix. Centrifuge for 5 minutes at 1000 to 1500 rpms.

You should be able to visualize a whitish plug at the bottom of the tube after spinning. Discard the supernatant in the proper hazardous waste container. Using a long glass or plastic pipette, add a drop of new methylene blue stain. Use the pipette to mix the stain with the sediment. Add a drop of this mix to the center of a slide. Drop a cover slip on top. Examine on the microscope under 10x power. Unlike with the direct smear, the microfilaria will present as stationary. Dirofilaria must be distinguished from Dipetalonema based on size and shape. Use the following table to help you differentiate:

Parameter	D. immitus	D. reconditum
Length (microns)	286-340	258-292
Width (microns)	5-7.5	4.5-5.5
Shape (anterior end)	Tapered	blunt
Shape (body)	Straight	curved
Shape (tail)	Straight	Button hook
Number present	Few to many	few
Movement	Stationary	Moves across slide

METHODS OF POST-MORTEM DIAGNOSTICS OF ANIMALS' HELMINTHOSES

Post-mortem diagnosis of helminthoses is carried out: at the opening of the carcasses killed animals and dead bodies of animals and the detection of parasitic worms in the affected organs and tissues against the background of certain pathoanatomical changes. Many invasive

diseases caused by small parasites can be diagnosed by helminthological autopsy of animals. Their most advanced technique was suggested by academician K.I. Scriabin. There are incomplete and complete helminthological sections.

1. At full helminthological opening the inspection of all without exception organs and tissues of animals is supposed with the purpose of detection and collecting of parasitic worms at different stages of their development. This is the most effective method because it enables both quantitative and qualitative accounting of all helminths that have been infected with animals, but is used mainly for scientific purposes.

2. The method of complete helminthological dissection of individual organs is used when it is necessary to have data on the degree of invasiveness of individual organs by certain types of parasites. For example, in the case of fasciolosis, only the liver is examined, in the case of dictiocaulosis – the lungs, and in the small bowel – the small intestine. With the complete helminthological dissection method, it is mandatory to use the compressor method. The organ or part thereof is crushed under a compressor or between two glass plates and examined under a magnifying glass or microscope. Using this method, the helminthological section of small animals, as well as intermediate, additional and reservoir hosts is carried out. The digestive system is carefully isolated and placed in a separate vessel is the esophagus, stomach, small and large intestines, liver, pancreas. Then they are cut with scissors. After their inspection, scrapings from the mucous membrane are selected and examined by the compressor method. The stomach is dissected by a large curvature, and its contents are placed in the cylinder and washed several times with water. The precipitate is examined macroscopically and microscopically. From the mucous membrane take a scraper and examine it. The small intestine is released from intestinal fat and cut laterally. The contents are transferred to the cylinder and examined by sequential washing. A deep scraping of their mucous membrane is also investigated by this method. Similarly, examine the large intestine. The liver is placed in a white pike, separating the gallbladder, cut it and washed several times with water. Cut the liver with scissors in the course of the bile ducts, and then grind with your hands. Washed in water and explored the precipitate on a white background. The pancreas is crushed by hands, washed in water, and the precipitate examined under a magnifying glass.

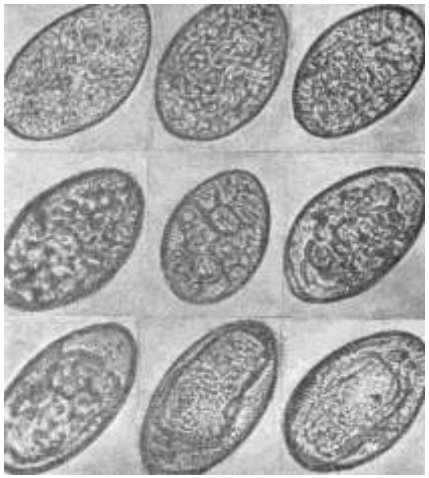
The lung parenchyma is crushed by hands and examined by a compressor method. The contents of the genitals are examined by sequential washing, and scraping of the mucous membranes – by the compressor method. The kidneys and bladder are cut with scissors, take deep scrapes from the mucous membrane of the bladder, ureter, examined by the compressor method, and the urine is defended, centrifuged and examined under a microscope. The eyes, along with the surrounding tissues, are removed from the orbital eye, examine the inner eyelids, conjunctival sacs, dissect the ducts of the lacrimal glands, and examine by sequential lavage. Both the brain and spinal cord are examined by the compressor method. The heart and large blood vessels are dissected in saline and examined by sequential washing, the contents of the chest and abdominal cavities as well. Detected helminths are collected with preparatory needles or tweezers, washed, fixed and labeled.

3. Incomplete helminthology dissection – a common pathoanatomical method of autopsy, in the process of which the largest helminths are found in the organs and tissues: moniesia, pathogens, ascaridia, etc.

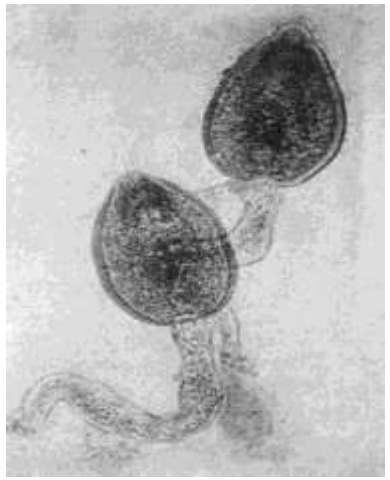
Material and technical supply. Microscopes, magnifying glass, permanent macro preparations, temporary and permanent micro preparations. Tables, schemes. Invasive animals, Freshly obtained faeces from them, all that is necessary for carrying out a coproscopic study.

«____» _____ 20 p. Signatures: Student_____ Lecturer _____

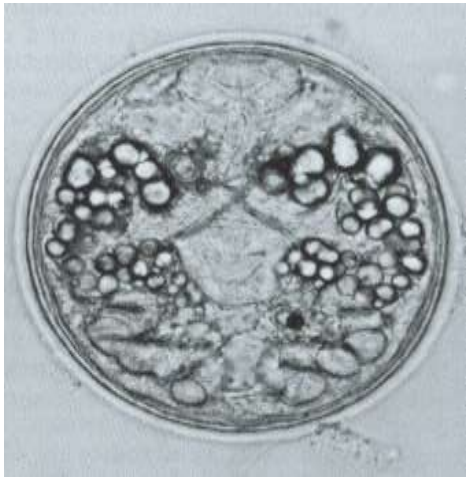
PICTURES FOR SECTION «VETERINARY TREMATODOLOGY AND TREMATODOSES OF ANIMALS»



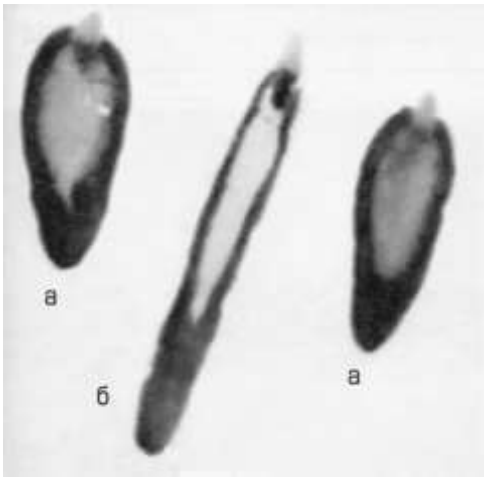
1. Eggs of Fasciola hepatica on different stages of development



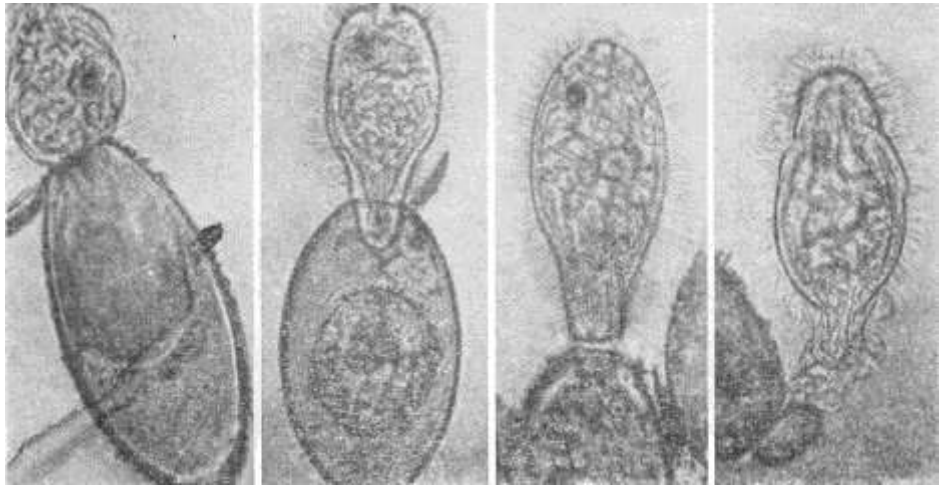
2. Cercarias of Fasciola hepatica.



3. Metacercaria of *Fasciola hepatica.*



4. а – *Fasciola hepatica,*
б – *Fasciola gigantica.*



5. Miracidium exit from the mature egg of the fasciola.

6. Marita of *Fasciola hepatica*:
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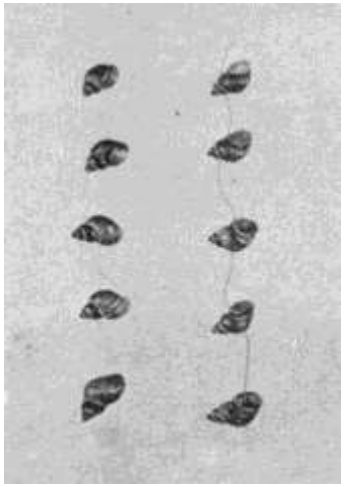
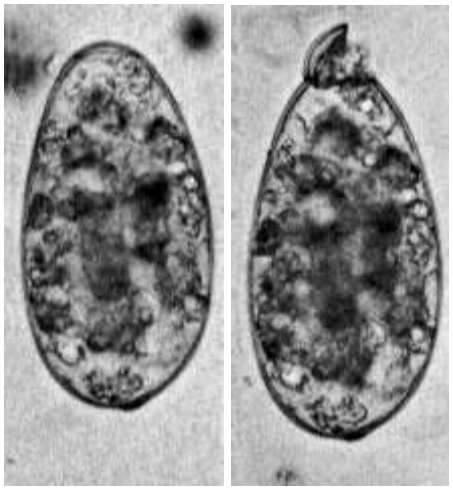


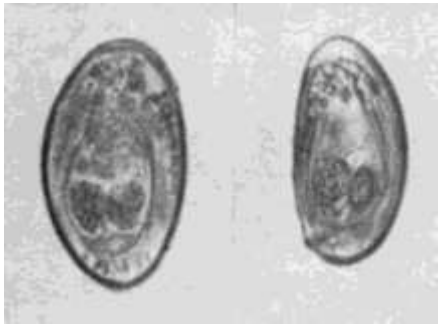
7. Snails of *Limnaea truncatula* – intermediate hosts of Fasciola.



8. Eggs of Paramphistomum

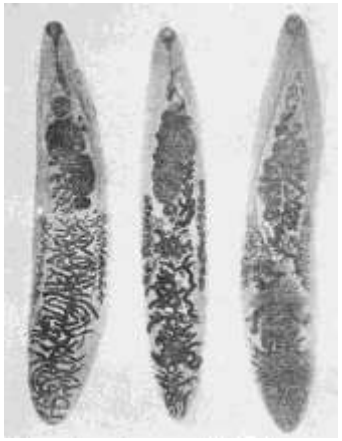
9. Cut through a suction cup of Paramphistomum in rumen of cattle.

10. Paramphostomum in rument of ruminant.

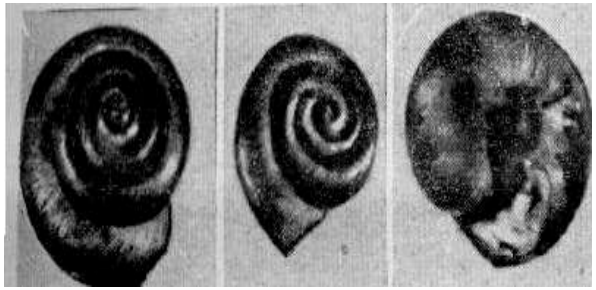


11. Mature eggs of Dicrocoelium

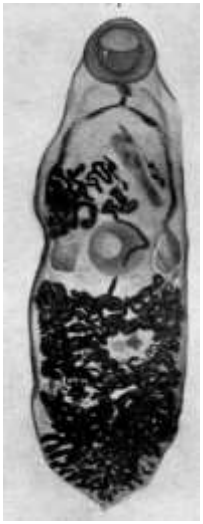
12. Sporocyst of Dicrocoelium with mature cercarias.



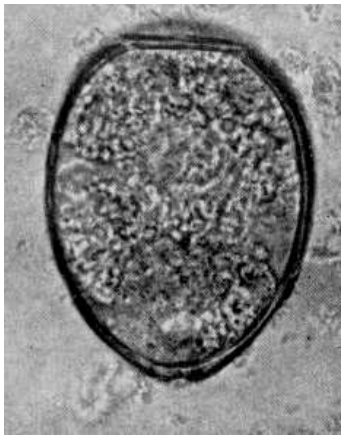
13. Dicrocoelium lanceatum.



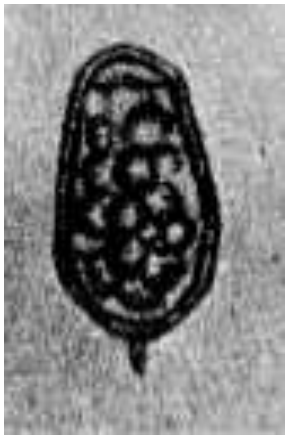
14. Orb snails *Monacha rubiginosa*



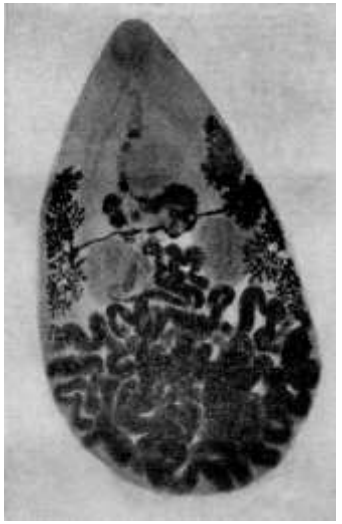
15. *Eurytrema pancreaticum.*



16. Egg of *Eurytrema* .

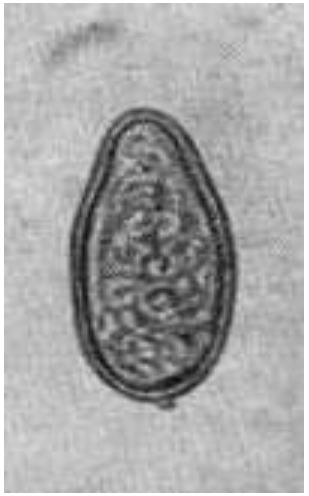


17. Egg *P. ovatus.*

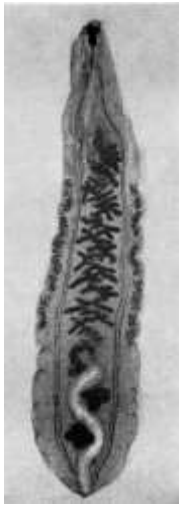


18. *Prosthogonimus cuneatus.*

19. *Echinostoma revolutum.*



20. Egg of *Opisthorchis felineus.*



21. *O. felineus.*