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A SIMPLE CULTIVATION METHOD FOR FIELD DIAGNOSIS OF AVIAN TRYPANOSOMES

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Abstract. A description is given of a cultivation method for detecting trypanosomes in the peripheral blood of birds. A modified medium SNB-9 is used as culture medium. The cultivation is effected in small penicillin bottles (PEN method) and the blood is collected and antibiotics diluted by means of plastic syringes with a single use each. The method was tested in 125 passerines from Bohemia and trypanosomes were found in 24 of them.

Trypanosomes are abundant parasites of birds in Czechoslovakia, but studies on their incidence have run across certain methodical difficulties (Kučera 1978). The most current method of demonstrating avian blood parasites are smears made from a drop of peripheral blood. However, only small numbers of avian trypanosomes occur in the peripheral blood of hosts and are therefore very rarely found in blood smears.

Another widely used method for detecting avian trypanosomes is the cultivation from blood and bone marrow on blood agars. Most species of these parasites readily grow on simple culture media. The disadvantage of previously used cultivation techniques consisted in the fact that the examined birds either had to be killed (to take their bone marrow) or were at risk of being harmed (while puncturing the heart or bone marrow). In addition, a possible contamination of cultures with other microorganisms in classical culture tubes restricted the use of such techniques in the field.

MATERIAL AND METHODS

Culture medium. A modified medium SNB-9 after Diamond and Herman (1954) was used. Rabbit blood was substituted by human blood from the transfusion station blood jars and to prevent blood coagulation sodium citrate was added to the liquid medium. Blood agar was prepared by autoclaving 0.6 g of sodium chloride, 2.0 g of Neopepton Difco, 2.0 g of Bacto-agar Difco, 100 ml of distilled water, to which 25 ml of sterile blood was added after cooling to 50 °C. While still warm the agar was aseptically dispensed in 1-ml amounts in small (5 ml) penicillin bottles which were aseptically stoppered with special rubber plugs covered with aluminum foil and secured with elastic. The agar was allowed to harden in moderately slanted bottles.

Citrate SNB-9 medium (0.6 g of sodium chloride, 2.0 g of Neopepton Difco, 1.5 g of sodium citrate, 100 ml of distilled water) was employed as liquid medium. All ingredients were dissolved by brief boiling and the medium was dispensed in 5-ml amounts in small (penicillin) rubber-stoppered bottles, with plugs secured by metal bands. The medium was then sterilized by autoclaving.

Both, the blood agar and medium were stored in refrigerator at 3 °C. One bottle with citrate SNB-9 medium (Citrate SNB) was enough for 10 bottles with blood agar (PEN bottles) when the material was collected.

Admixture of antibiotics. Penicillin G Spofa (1,000,000 units per bottle) and Streptomycin (Pharmachim 1 g per bottle) were used as antibiotics. Each day of field work fresh diluted antibiotics were used. Antibiotics were diluted by means of sterile plastic syringes with a single use each (Amefa, GDR) (Fig. 1). The distilled water for dilution was previously sterilized by autoclaving in larger (20 ml) penicillin bottles. The resulting concentration of antibiotics in citrate SNB is approximately 1,000 to 2,000 units of penicillin and 0.5 to 1.0 mg of streptomycin per 1 ml of medium and is harmless to trypanosomes.

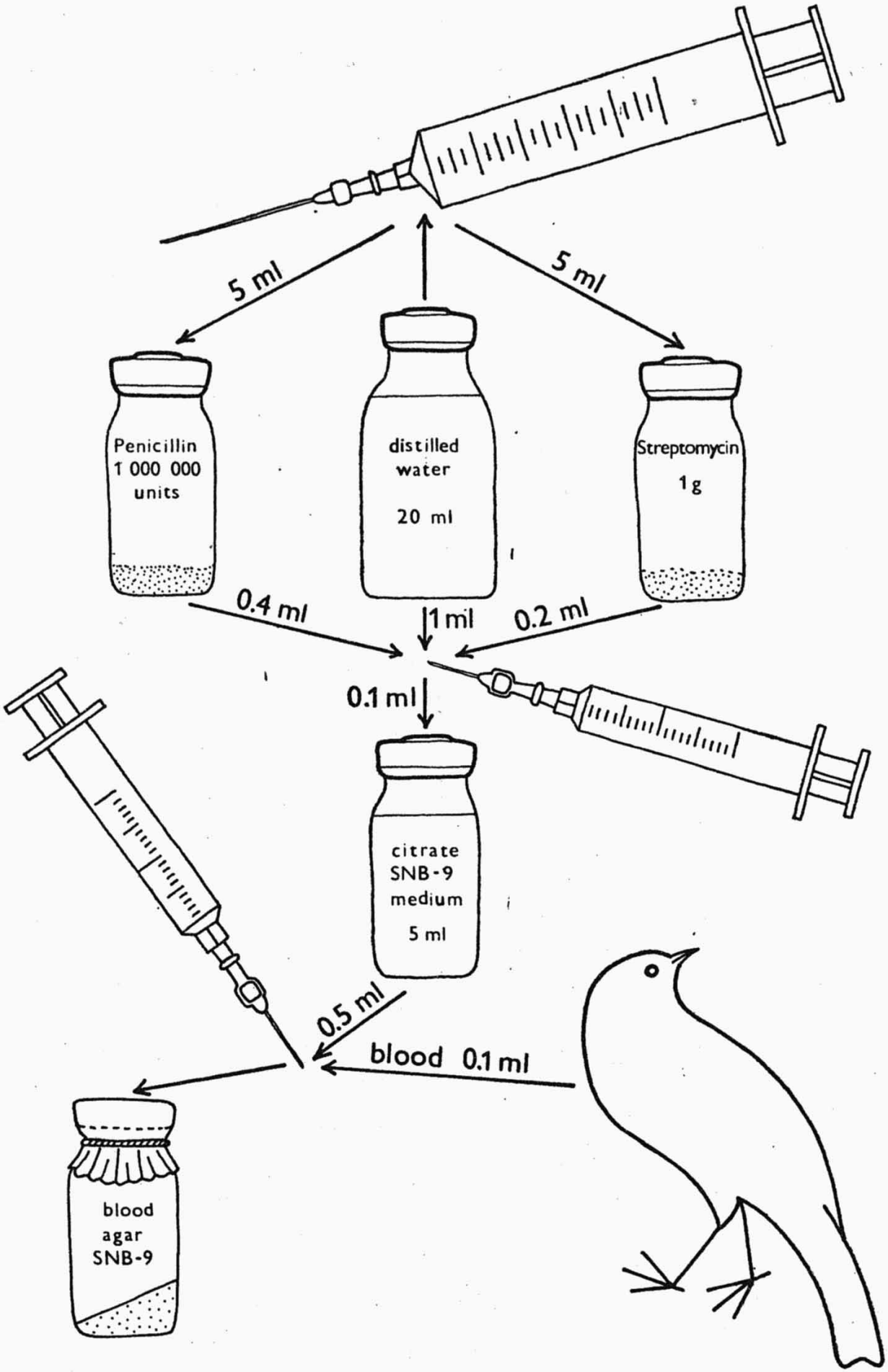

Fig. 1. Scheme of the PEN method.

Blood collection from birds and inoculation of media. Each bird from which the blood was collected was placed on its back with one wing stretched and its vena cutanea ulnaris well exposed. The vein was punctured with a sharp hypodermic needle using the same technique as in the preparation of blood smears (see details Bennet 1970a). The site of puncture as well as the needle were thoroughly sterilized with 96% ethylalcohol. The blood was drawn up (about 0.1 ml) in a syringe previously filled up with 0.5 ml of citrate SNB and this suspension was inoculated into PEN bottle with blood agar. Simultaneously blood smears were made and the blood in the wound stopped with a tampon or spray plastic dressing (Akutol, Spofa).

Incubation of media. Inoculated PEN bottles were incubated for 14 days at 20 to 25 °C. Cultures experimentally cooled to as low as 3 °C proved to be harmless to trypanosomes, only slowed down their multiplication. After 14 days the contents of the bottles were microscopically examined on the presence of trypanosomes.

RESULTS AND DISCUSSION

The first results obtained by the PEN method are given in Table 1. A total of 125 passerines from several localities in Bohemia were investigated. Trypanosomes were found in 24 birds. The table shows that the percentage of positive findings considerably varies in different localities and at different periods. Sometimes no trypanosomes were found at all, while at some other time a high percentage of infected birds was demonstrated. This is probably due to the fact that the incidence of trypanosomes in peripheral blood of birds would manifest similar seasonal and diurnal changes as the incidence of blood phases of other blood parasites (see e.g. Bennet 1970b, Beaudoin et al. 1971, Noblet and Noblet 1977).

It has been demonstrated previously (Baker 1976) that some particular species of avian trypanosomes accumulate in the marrow of long bones where they may be detected more frequently than in peripheral blood. By bone marrow culture on SNB-9 medium trypanosomes were found in 33 % of birds in Bohemia (Kučera 1978). The bone marrow, however, could be obtained only from killed birds. Only with large birds a technique of puncturing bone marrow of tibiotarsus was elaborated (Diamond and Herman

Table 1. Incidence of trypanosomes in birds in Bohemia detected by PEN cultivation method

Date	Locality	Number of birds		
		examined	infected*)	percentage of infected
20. 4. 78	Praha—Křeslice	3	0	0 %
30. 4. 78	Praha—Počernice	5	0	0 %
4. 5. 78	Praha—Křeslice	11	3	28 %
24. 5. 78	Praha—Křeslice	5	0	0 %
27. 5. 78	Veselí n. Lužnicí	24	0	0 %
28. 5. 78	Veselí n. Lužnicí	3	0	0 %
21. 6. 78	Krkonoše—Rýchory	11	5	46 %
22. 6. 78	Krkonoše—Rýchory	20	6	30 %
23. 6. 78	Krkonoše—Rýchory	17	8	52 %
18. 9. 78	Krkonoše—Vosecká b.	15	0	0 %
19. 9. 78	Krkonoše—Vosecká b.	11	2	18 %
Total		125	24	19 %

*) Positive birds: *Acanthis flammea* (1), *Anthus trivialis* (2), *Fringilla coelebs* (9), *Loxia curvirostra* (1), *Motacilla cinerea* (1), *Phylloscopus bonelli* (1), *Phylloscopus trochilus* (1), *Prunella modularis* (3), *Sitta europaea* (1), *Sylvia curruca* (1), *Turdus merula* (3).

1954). Some authors (Nieschulz 1922, Baker 1956 etc) used heart punctures of live birds for blood collection. This method, however, involves a risk of contamination with other microorganisms, because the needle usually also punctures unsterile air pockets in sternum (Baker 1976) and moreover the high mortality of birds thus investigated restricts the use of such technique in the field. The painstakingly carried out PEN method considerably eliminates the possibility of contamination of cultures with other microorganisms despite the fact that the birds are investigated in considerably aseptic conditions in the open air. No contamination with bacteria was recorded by the author and only 4 PEN bottles were contaminated by fungi (they were not included in the number of birds investigated). Other methods used in diagnosing avian trypanosomes such as e.g. microscopic examination of fresh blood and xenodiagnosis (Baker 1976) and hematocrit method after Bennett (1962) are restricted only to laboratory examination and are not suitable for field work.

In conclusion, the advantages of the PEN method may be summed up in the following items:

1. Easy implementation of the method and simplicity in blood collecting without inflicting any harm to birds makes this method applicable in the field investigation of a large number of birds captured e.g. when they are ringed during ornithological activities.
2. The method considerably eliminates contamination of cultures with bacteria and fungi by adding freshly mixed antibiotics and by using the system of syringe—penicillin bottle.
3. Quick reading of results in microscopic examination and possibility of further procedure with strains of trypanosomes obtained.
4. Small expenditures and easy preparation of the method. The only inconvenience of this method consists in the fact that it reveals only the incidence of trypanosomes in peripheral blood of birds, while trypanosomes may be present in the bone marrow and internal organs without being detected in peripheral blood.

ПРОСТОЙ МЕТОД КУЛЬТИВАЦИИ ДЛЯ ДИАГНОСТИКИ ПТИЧЬИХ ТРИПАНОЗОМ В ПОЛЕВЫХ УСЛОВИЯХ

Я. Кучера

Резюме. Дано описание метода культивации для выявления трипанозом в периферической крови птиц. В качестве питательной среды использована модифицированная питательная среда SNB-9. Культивирование проводят в небольших пенициллиновых бутылках (PEN метод) и взятие крови и разведение антибиотиков выполняют с помощью шприца из пластмассы в один прием. Метод проверили у 125 воробьиных на территории Чехии и трипанозомы обнаружены у 24 из них.

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A NEW NEMATODE OF THE GENUS PETROWOSPIRURA (SPIRUROIDEA) FROM THE CAT, FELIS DOMESTICA FROM INDIA

During July, 1976 a cat was collected from Ummed Chowk, Jodhpur, India and examined for nematode infection. Ten worms, four males and six females belonging to the spirurid genus *Petrowospirura* Matschulsky, 1952 were received. The worms differ from other known species and appear to constitute a new species.

Petrowospirura barusi sp.n. Figs. 1—2

Host: *Felis domestica.*
Location: Stomach. Locality: Ummed Chowk, Jodhpur, India.
Type specimens: Holotype male, NJ 18 (a); deposited with the Zoology Museum, University of Jodhpur, Jodhpur, India. Allotype female No. NJ 18 (b): other data as for holotype. Paratypes No. NJ 18 (c); other data as for holotype. (All measurements are given in millimeters).

Worms soft, long and whitish, cuticular striations well developed, 0.007—0.008 and 0.006 to 0.008 apart in male and female respectively. Mouth with two lobes. Each lobe with three lips and two pairs of cephalic papillae. Buccal capsule funnel-shaped with heavily chitinized walls and twelve longitudinal ridges extending throughout its length. Buccal capsule 0.07—0.12 and 0.06—0.10 long in male and female respectively. Diameter of buccal capsule at anterior

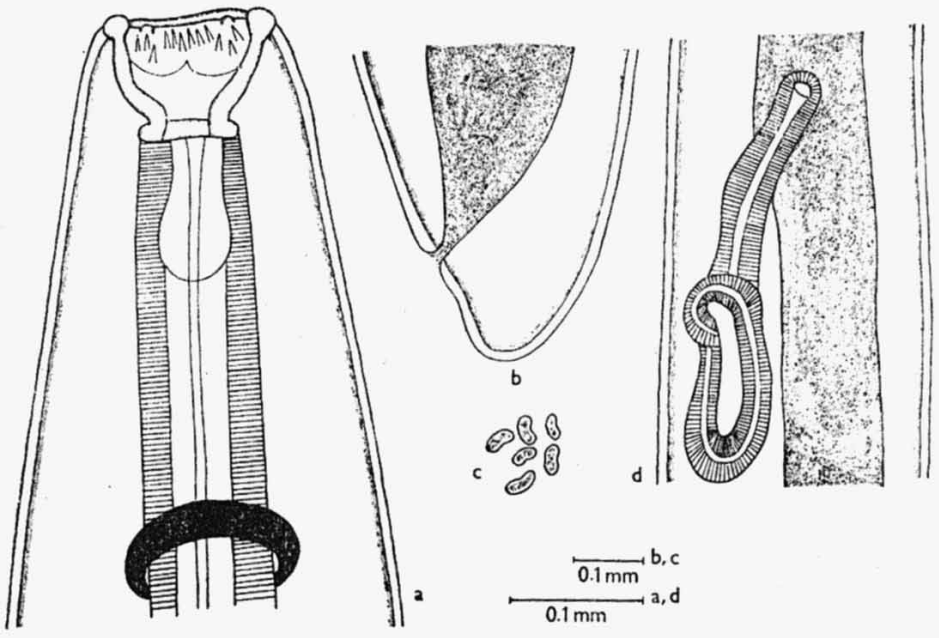

Fig. 1. *Petrowospirura barusi* sp.n. a — Anterior extremity of female, b — Posterior extremity of female, c — Vulvar region, d — Eggs.