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HISTOLOGICAL STAINING AND MICROSCOPE EXAMINATION OF PERMANENT PARAFFIN SLIDES: A RING TRIAL OF THE ROMANIAN COUNTY SANITARY VETERINARY LABORATORIES' PROFICIENCY

COLORAȚIA HISTOLOGICĂ ȘI EXAMINAREA LA MICROSCOP A LAMELOR DE FRAGMENTE TISULARE INCLUSE ÎN BLOCURI DE PARAFINĂ: O TESTARE INTER-LABORATOARE A COMPETENȚEI LABORATOARELOR SANITARE VETERINARE JUDEȚENE DIN ROMÂNIA

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ABSTRACT | REZUMAT

In Romania, histological diagnosis is performed by 40 county sanitary veterinary laboratories under technical coordination and supervision of the Morphopathology Department from the Institute for Diagnosis and Animal Health, Bucharest.

To get reliable results, all laboratories have been implementing ISO/IEC 17025:2017 standard as part of a laboratory quality system. Quality assurance is done also by participation in interlaboratory ring trials.

In this respect Institute for Diagnosis and Animal Health is accredited on ISO/IEC 17043:2010 standard, as provider and organizer of proficiency-testing programs. Our paper presents the interlaboratory ring trial to evaluate 34 out of 40 County Sanitary Veterinary Laboratories for microscope examination and histology staining techniques of permanent paraffin slides. Sets of coded samples were prepared and distributed to each of the laboratories. The sample panel contained 4 permanent paraffin slides to be stained and after that examined to the microscope by attended laboratories. After receiving and interpreting the results, 61.76% of participants were qualified as "Very Well", 29.4% as "Well", and 5.88% -"Sufficient".

Keywords: interlaboratory ring trial, histological staining techniques, histopathology

În România, diagnosticul histologic este realizat de 40 de laboratoare sanitare veterinare județene, sub coordonarea tehnică și supervizarea Departamentului de Morfopatologie de la Institutul de Diagnostic și Sănătate Animală, București. Pentru a obține rezultate fiabile, în toate laboratoarele a fost implementat standardul ISO 17025: 2005 ca parte a sistemului de calitate al laboratorului. Asigurarea calității se face și prin participarea la testele inter-laboratoare. În acest sens, Institutul pentru Diagnostic și Sănătate Animală este acreditat pe standardul ISO 17043:2010, în calitate de furnizor și organizator al programelor de testare a competenței. Lucrarea noastră prezintă rezultatele testelor inter-laboratoare de evaluare a 34 din 40 de laboratoare sanitare veterinare județene, pentru examinarea microscopică și tehnicile de colorare histologică a lamelor de fragmente tisulare incluse în blocuri de parafină. În acest sens au fost pregătite seturi de eșantioane codate și distribuite fiecărui laborator. Panelul de eșantioane a fost constituit din patru lame histologice destinate colorării și apoi examinării la microscop de către laboratoarele participate. După primirea și interpretarea rezultatelor, 61,76% dintre participanți au obținut calificativul „Foarte bine", 29,4% „Bine" și 5,88% - „Suficient".

Cuvinte cheie: studiu interlaborator, tehnici de colorare histologice, histopatologie

A standardized, robust and reproducible histological diagnosis performed by County Sanitary Veterinary Laboratories under technical coordination and supervision of Morphopathology Department from Institute for Diagnosis and Animal Health is a need of the Romanian Sanitary Veterinary and Food Safety Authority.

Ring test (also called proficiency test or inter-laboratory ring trial) is an inter-laboratory test that allows evaluating the performance of testing laboratories and is based on the analysis of similar homogeneous samples. The aim is to enable laboratories to assess and improve their analysis performance. The ring test offers to laboratories the possibility of external quality assessment of the analytical results it produces. Any laboratory which needs independent assessment of the quality of analytical results it produces should participate in ring tests and take the opportunity to compare its performance with other laboratories. Ring tests can monitor the quality of analytical results and identify

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assays that need improvement through for example staff training, proper use of equipment, and suitability of methods respectively. Ring trials are an essential part of quality control measures to demon-strate competency to accreditation bodies, customers and other regulatory bodies, as ISO/IEC 17025:2017 standard requires (10). The Morphopathology Department from Institute for Diagnosis and Animal Health (IDAH), Bucharest is responsible for the technical evaluation and coordination of histological diagnosis in Romania. According to National Strategic Plan issued each year by Central Veterinary Authority (CVA) and based on Regulation (EC) No 625/2017 of the European Parliament and the Council, all sanitary veterinary laboratories involved in official controls must be accredited (8, 12). IDAH carried out an inter-laboratory comparison trial to evaluate the performance of the microscope examination and histology staining techniques of permanent paraffin slides used by County Sanitary Veterinary Laboratories (CSVL).

This paper describes the development of the ring test carried out by IDAH, from the production of the samples to the evaluation of the laboratories that participated in the trial. This study aims to contribute to the harmonization of reliable protocol for the histological diagnosis.

MATERIALS AND METHODS

Participants

In Romania, the histological diagnosis is performed by 40 county sanitary veterinary laboratories. Thirty-two out of 40 confirmed the participation in this trial. In addition to these, the other two private laboratories jointed the proficiency test. All of the laboratories involved remained anonymous to avoid any conflict among the participants.

Sample panel composition and distribution

The sample panel was prepared entirely in the Morphopathology Department from Institute for Diagnosis and Animal Health (IDAH), Bucharest and distributed to each participant laboratories. Sets of four permanent paraffin slides were prepared. Each set consisted of two liver bird permanent paraffin slides with Histomoniasis and two bovine lymph node permanent paraffin slides with Tuberculosis. Finally, all samples were encoded label, to blind the trial and kept at the room temperature until distribution to the participants.

Histology staining techniques

The participants had to evaluate the samples using two standard operational procedures, as follows: (1) Evidence of histological and topohistopathological structures by the Hematoxylin-Eosin (HE) staining method and (2) Evidence of pathognomonic histopathological changes in mycobacteriosis and alcohol-acid-resistant bacilli by modified Ziehl-Neelsen (ZN) staining method respectively. Both techniques were performed according to Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, OIE (18).

Participants' results analysis

The information about the ring trial, sending /reception forms, specific and technical instructions about reporting the results, were sent to all the laboratories at the same time with panels. The sample panels were picked up by each laboratory.

Evaluation of the laboratories had taken into account if the participants noticed at least one of the following data:

· HE staining: congestion /hemorrhage /keloids; diffuse necrotic hepatitis/vacuolar degeneration; cellular polymorph infiltrate (macrophages, lymphocytes, plasmocytes, heterophils); necrotic hepatitis in large outbreaks /histomoniasis; congestion, haemorrhage, ne*-crobiosis, necrotic hepatitis (9);

· ZN staining: granulomatous lymphoreticulitis (tuberculous); focal necrosis localizations with/without calcium deposition and epithelial and giant Langhans cells; infectious granuloma of tuberculous nature; infiltrating with epithelial and giant cells; epithelioid-giant-cellular reaction (7, 11, 13); presence/absence of alcohol - acid - resistant bacilli (Fig. 1)

The interlaboratory ring trial is a nominally qualitative scheme and the assigned value has been established as a known value, determined descriptively by following the fulfillment of the evaluation criteria mentioned above. If the participant met 100% of criteria, his performance is "Very Well", 80% "Well", 70% "Sufficient" and less than 60% means "Insufficient".

After the complete analysis of the results received from participants, the organizer issues the final report and send it to all attended laboratories.

RESULTS AND DISCUSSIONS

The key to manage the disease and to control and prevent is the rapid and accurate diagnosis (1, 2). The results obtained in this ring-test support the ability of the county sanitary veterinary laboratories to provide accurate results for the histopathological diagnosis in animals for accreditation purposes. Even more, knowing that the official diagnosis of tuberculosis is based mainly on the detection of the host cells response after infection, the ring-test provided an official inter-laboratory evaluation scheme that provides evidence of their proficiency to perform histopathological diagnosis of tuberculosis.

Thirty-four out of 40 CSVL attended to the inter-laboratory ring trial organized by IDAH. Twenty-one (61.76%) of participants were qualified as "Very Well", 10 (29.4%) as "Well", and 2 (5.88%) - "Sufficient" (Table 1). The inter-laboratory comparison showed a high value of understanding and implementation of

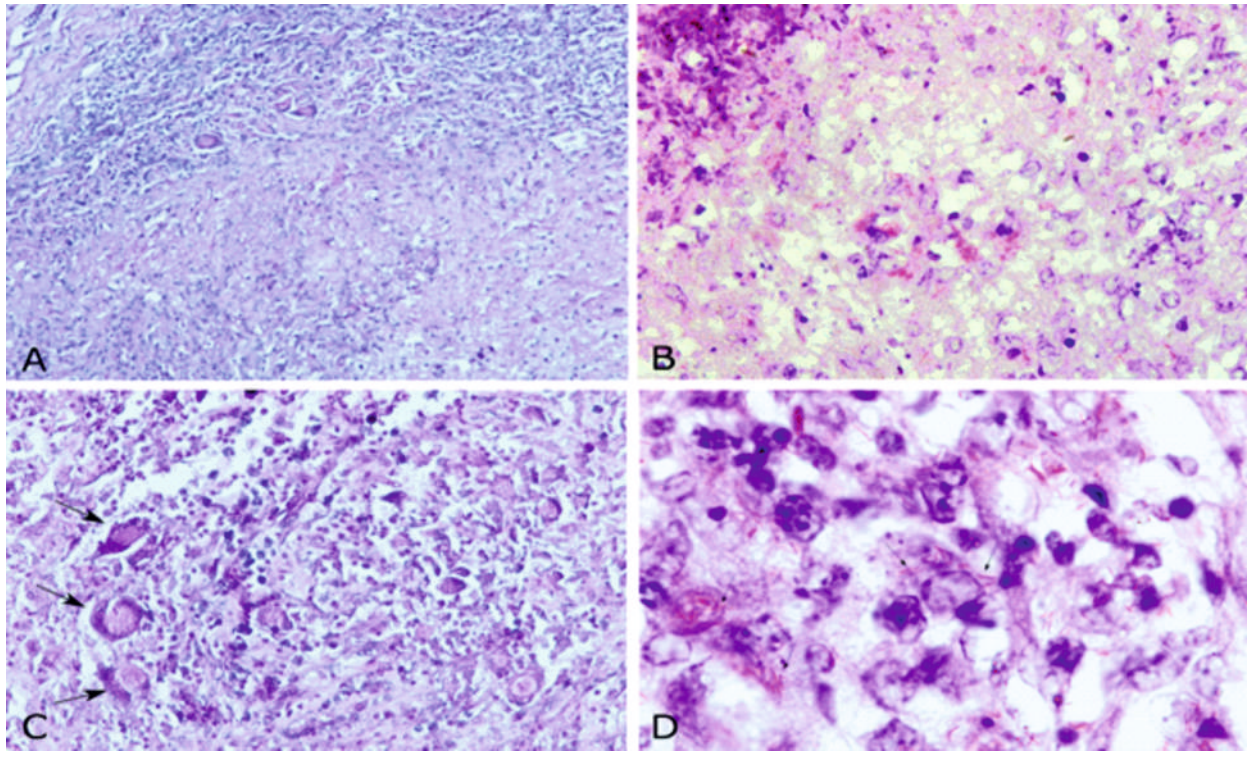

Fig. 1. Bovine lymph node. A - Multifocal necrosis area with peripheric multinucleated GL and epithelioid cells (ZNM, x40). B - Central necrosis area with alcohol - acid - resistant bacilli (ZNM, x400). C - Giant Langhans cells (arrows) (ZNM, x400). D - Intracitoplasmatic alcohol - acid - resistant bacilli (arrows) (ZNM, x1000)

Table 1

Performance of the participants

Crt. No.	Cod CSVL	Performance	Crt. No.	Cod CSVL	Performance	Crt. No.	Cod CSVL	Performance
1	101	Very well	13	116	Very well	25	134	Very well
2	102	Very well	14	117	**Sufficient**	26	135	**Sufficient**
3	104	Very well	15	118	Very well	27	136	Very well
4	105	Well	16	120	Very well	28	137	Very well
5	106	Very well	17	121	Very well	29	138	Very well
6	107	Very well	18	124	Very well	30	139	Well
7	108	Well	19	126	Well	31	140	Very well
8	109	Well	20	128	Well	32	143	Very well
9	110	Well	21	129	Very well	33	144	Very well
10	112	Very well	22	130	Well	34	103	Din not answer
11	113	Well	23	132	Very well			
12	114	Well	24	133	Very well			

methods besides the variability of the techniques. Therefore, the use of a standardized and generalized method is needed to ensure reliable results and inter-laboratory reproducibility.

Although the veterinary laboratory diagnosis has undergone a great evolution in recent years, and modern diagnostic techniques, like enzyme-linked immunosorbent assays and polymerase chain reaction techniques, are commonly used in the diagnosis of several animal diseases (1-4, 16, 17), the histological examination is still a vital pawn of the laboratory diagnosis, often classified as the "gold standard" test (5, 6, 14, 15). Considering tuberculosis, de definitive diagnostic suffer from a delay of timely results and is inadequate for rapid detection of infection, but it still cannot be completely replaced by a real-time polymerase chain reaction assay, with only 2 hours of turn-a-round time (2).

CONCLUSIONS

The ring-test proved "Very Well" proficiency of 61.76 % participants, "Well" proficiency of 29.4% participants and "Sufficient" proficiency of 5.88%. Also, County sanitary veterinary laboratories were able to provide a standardized, robust and reproducible histopathological diagnosis of tuberculosis under technical coordination and supervision of the Morphopathology Department from the Institute for Diagnosis and Animal Health.

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