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**ORIGINAL RESEARCH ARTICLE**

# The prevalence of lymph node hyperplasia in animals diagnosed with echinococcosis from Prahova County, Romania

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**Abstract.** Echinococcosis is a zoonotic parasitosis, produced by the larval form of *Echinococcus granulosus*. During this parasitic infection, a series of complex modifications appear in the host's body, which mostly depends on the intensity of the infection.

Considering that Echinococcosis is an important disease in Romania, this study aimed to investigate the histopathological modifications during *E. granulosus* infection and to determine the occurrence of lymph node hyperplasia in horses, cattle, sheep, and swine.

Overall, 435 animals originating from Prahova county, Romania were submitted to postmortem and cytomorphological examinations. The results indicated that adenopathies (lymphoreticular hyperplasia, malignant pre-lymphoma) were present in 147 ovines, 38 bovines, 82 equines, and 168 swine, although only 101 ovines, 11 bovines, 17 equines, and 4 swine had echinococcosis specific lesions.

In Prahova county, lymphoreticular hyperplasias were predominantly identified in ovines and bovines. Moreover, malignant pre-lymphomas were present in all the tested animal species, with the exception of bovines.

**Keywords:** Echinococcosis; Lymph node hyperplasia; Malignant pre-lymphoma.

**Prevalenţa hiperplaziei limfonodale la animalele diagnosticate cu echinococcoză din judeţul Prahova, România**

**Rezumat.** Echinococcoza este o parazitoză zoonotică, produsă de forma larvară a cestodului *Echinococcus granulosus*. În timpul acestei infestatii parazitare, o serie de modificări complexe apar în corpul gazdelor, are depind în mare parte de intensitatea infestatiei.

Având în vedere că echinococoza este o afectiune des intalnită în România, obiectivele acestui studiu au fost de a investiga modificările histopatologice în timpul infestaţiei cu *E. granulosus* şi de a determina apariţia hiperplaziei limfonodale la cabaline, bovine, ovine şi suine.

În total, 435 de animale, provenite din judeţul Prahova, România au fost supuse examinărilor postmortem şi citomorfologice. Rezultatele au indicat faptul că adenopatiile (hiperplazia limforeticulară, pre-limfomul malign) au fost prezente la 147 ovine, 38 bovine, 82 ecvidee şi 168 suine, deşi doar 101 ovine, 11 bovine, 17 ecvidee şi 4 suine au avut leziuni specifice echinococcozei.

În judeţul Prahova, hiperplaziile limforeticulare au fost identificate predominant la ovine şi bovine. Mai mult, pre-limfoamele maligne au fost prezente la toate speciile de animale testate, cu excepţia bovinelor.

**Cuvinte cheie:** Echinococoză; Hiperplazie limfonodală; Pre-limfoame maligne.

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## Introduction

Cystic echinococcosis (CE) is a parasitosis caused by *Echinococcus granulosus* cestode, which has several potential intermediate (sheep and other domestic livestock, omnivores, and lagomorphs) and definitive (carnivores, usual dogs) hosts (Shaikenov et al., 2004).

Parasitic diseases, under favorable conditions, can cause chronic inflammation in which the cell population of lymphoid organs reacts in different ways (Mesrobeanu and Berceanu, 1968; Yazdanbakhsh and Sasks, 2010; Kumar et al., 2015). However, a lot of these responses are not protective, and some are even harmful to the host (Davidson, 1985).

A better understanding of host-parasite relationship, and the elucidation of specific events that lead to echinococcosis, are very important for the development of the right control protocols (Prisacari et al., 2012; Gottstein et al., 2017).

Reactive hyperplastic adenopathies should be taken into account when thinking about clinical disorders in echinococcosis (Micu, 1973). *E granulosus* has a remarkable influence on the host's immune response. Recent studies have shown that during human infection different molecules are released. These molecules can modulate the host's immune system and can induce a strong anti-inflammatory response (Grubor et al., 2017).

Considering that previous studies in Romania have shown the presence of *E. granulosus* in various animal species (Seres et al., 2009), this study aimed to investigate the histopathological modifications during *E. granulosus* infection and to determine the occurrence of nodal lymph hyperplasia in horses, cattle, sheep, and swine.

## Materials and methods

*The subjects of the study*

The current study was carried out on a total of 435 animals (horses, cattle, sheep, and swine), slaughtered in a single authorized slaughterhouse. The animals included in the study originated from both the extensive (ovines, bovines, equines) and the intensive (swine) husbandry systems from Prahova county, Romania.

*Postmortem examination*

A routine necropsy examination was carried out for each animal carcass. Palpation and several incisions were carried out through each liver, lung, and lymph nodes. Mesenteric or traheobronhic adenopathies, livers, and lungs were examined by applying the routine meat inspection procedures (Abo-Aziza, 2019; Ayele et al., 2019).

*Cytomorphological examination*

Smears from the lymph nodes were obtained by sectioning the areas with lesions and displaying on microscopic slides the excreted liquid. The obtained smears were colored by using May-Grünwald – Giemsa (MGG) stain (Piaton et al., 2015) for general microscopic examination.

## Results and discussions

The results indicate that from 147 ovines, 38 bovines, 168 swine, and 82 equines with adenopathy, only 101 ovines, 11 bovines, 4 swine, and 17 equines had *E. granulosus* infection (table 1). The prevalence of echinococcosis is significantly higher in animals raised in an extensive system compared to animals raised in the intensive system, whose living environment is controlled (Solanki and Purohit, 2019). Thus, ovines revealed the highest degree of the infestation (68.70%), while swine had the lowest infestation (2.38%).

Lymph node (figure 1) pathology with a long-lasting evolution, due to chronic infestation with *E. granulosus*, made possible the classification of the lesions that occur at this level, namely: simple lymphoreticular hyperplasia, aggravated lymphoreticular-hyperplasia, and malignant pre-lymphoma that appear as a reaction to persistent antigenic stimulation of the lymphoid tissue.

The cytomorphological spectrum of the simple lymphoreticular hyperplasia (SH) does not raise problems regarding the health status of the animal, although the following two successive stages, namely aggravated lymphoreticular hyperplasia (AH) (figures 2, 3) and malignant pre-lymphoma (MP) (figure 4) could lead to the appearance of cellular gigantisms, nucleolar gigantism, nucleo-cytoplasmic degenerative phenomena and atypical mitoses specific to malignant lesions (Micu et al., 1964; Dema et al., 2019). Not all animals diagnosed with echinococcosis had adenopathies such as hyperplasia or malignant pre-lymphoma, as seen in table 2 and charts 1-4.

Helminths can be a major cause of morbidity and mortality, especially in developing countries (Colley et al., 2001). In hydatidosis, we can observe, an immune granulomatous reaction with mononuclear cell infiltration and intensive pericystic fibrosis (Sakamoto and Cabrera, 2003). Despite the extensive complexity, in the majority of cases, the immune responses of the hosts to the helminthic infection are remarkably similar, being Th2-like with the production of significant quantities of interleukin-4 (IL-4), IL-5, IL-9, IL-10, and IL-13 and consequently the development of strong immunoglobulin E (IgE), eosinophil, and mast cell responses (MacDonald et al., 2002). During this parasitic infestation antigens (Ag) released can induce a marked Th2 response that orchestrates the development of granulomatous lesions usually in the liver (Cheever et al., 2000). Cellular and humoral response to infestation with *E. granulosus* is characterized by the release of cytokines. These cytokines play a key role in the regulation of the production of antibody isotypes IL4 cytokine regulates IgE and IgG synthesis (Candolfi et al., 1985).

Jenkins et al. (1986) observed that subcutaneous inoculation of *E. granulosus* protoscolices caused a blastic transformation in local lymph node cells. Swiss mice infested intraperitoneally with 1,500 protoscolices of *E. granulosus* yielded both viable and degenerated hydatid cysts. Sections of spleens and lymph nodes after three months post-infestation showed hyperplastic follicles and blastoid cells in thymus-dependent areas (Ali-Khan, 1978). *E. granulosus* protoscolex (PSC) infestation in BALB/c mice led, after 4 days to B cells activation, this was accompanied by secretion of immunoglobulin (Cox et al., 1989).

The staging of the cytomorphological changes detected in reactive lymph nodes, which are induced by chronic parasitism, can be used to elaborate an epidemiological study. This study would contain the incidence of the stages of lymph node transformation related to the type of animal husbandry system and their reactivity to the *E. granulosus* parasite (Jubb, 2012).

**Table 1.** The prevalence of echinococcosis in relation to the animal species and to the husbandry system

| | Husbandry system | Animals with adenopathies | Animals with echinococcosis | Prevalence of echinococcosis |
|---|---|---|---|---|
| **Ovines** | Extensive | 147 | 101 | 68,70% |
| **Bovines** | Extensive | 38 | 11 | 28,95% |
| **Equines** | Extensive | 82 | 17 | 20,73% |
| **Swine** | Intensive | 168 | 4 | 2,38% |

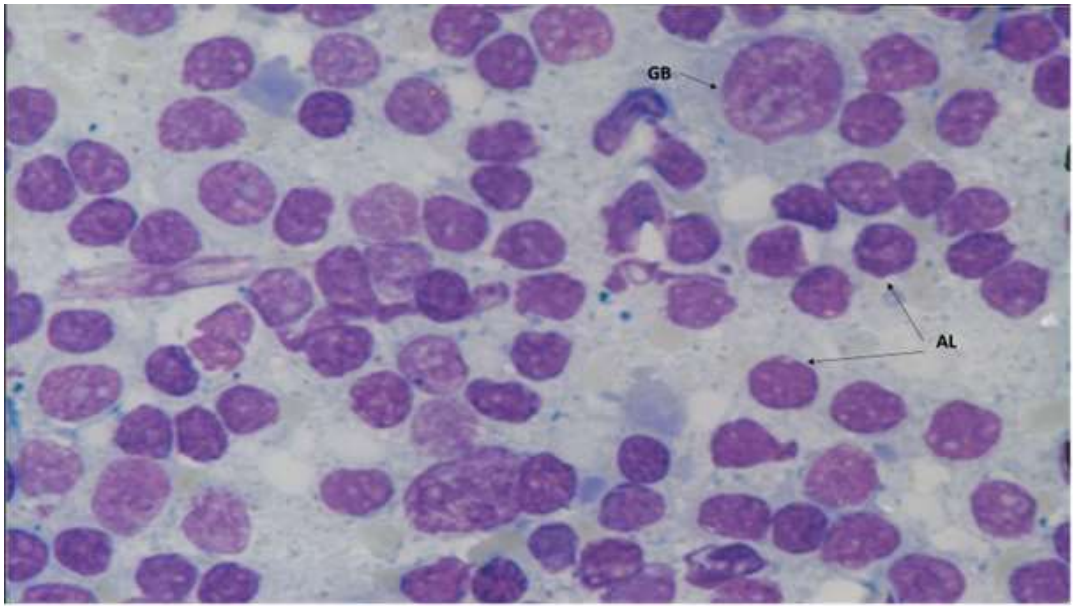


**Figure 1.** Bovine lymph node, quasi-normal status, presence of adult lymphocytes (AL) and germinoblast cells (GB), MGGX1000

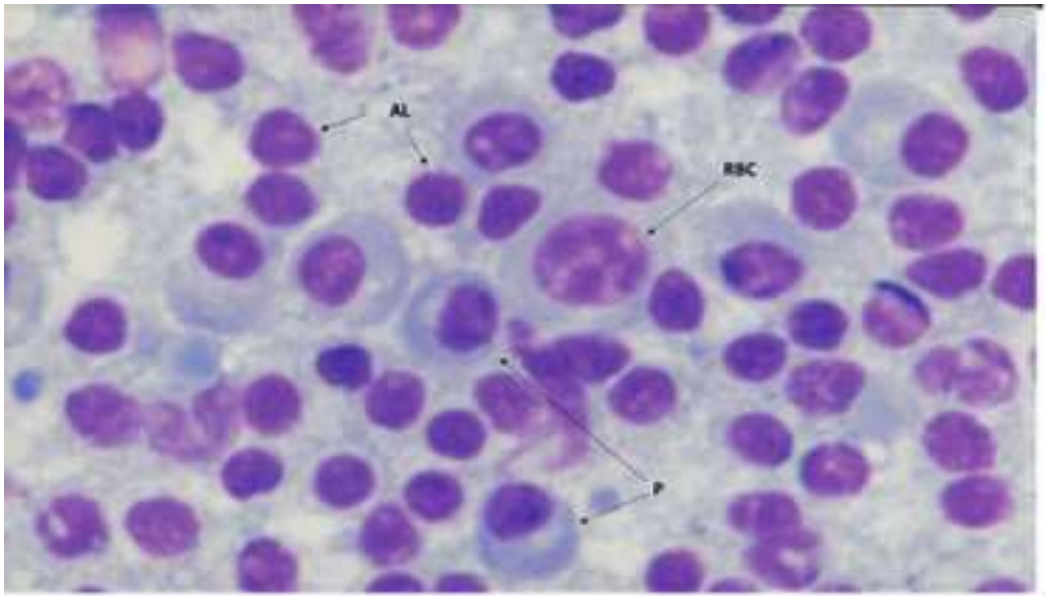


**Figure 2.** Swine lymph node, simple lymph-reticular-plasmacytic hyperplasia; the presence of adult lymphocytes (AL), plasmocytes (P) and reticuloblast cells (RBC), MGGX1000

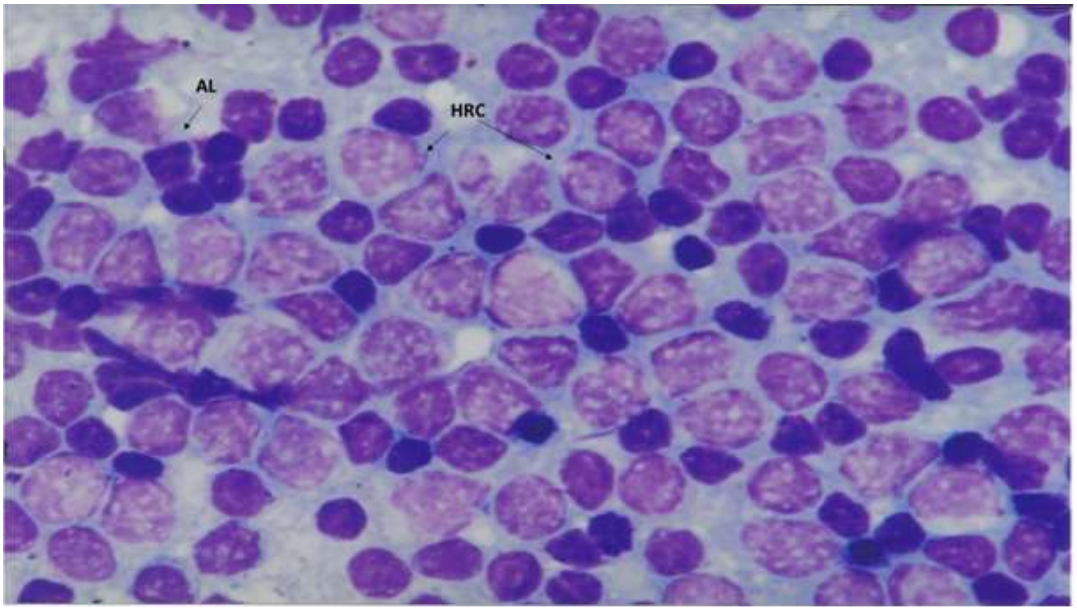


**Figure 3.** Swine lymph node, aggravated lymphoreticular hyperplasia, the mass of adult lymphocytes (AL) alongside histioid reticular cells (HRC), MGGX1000

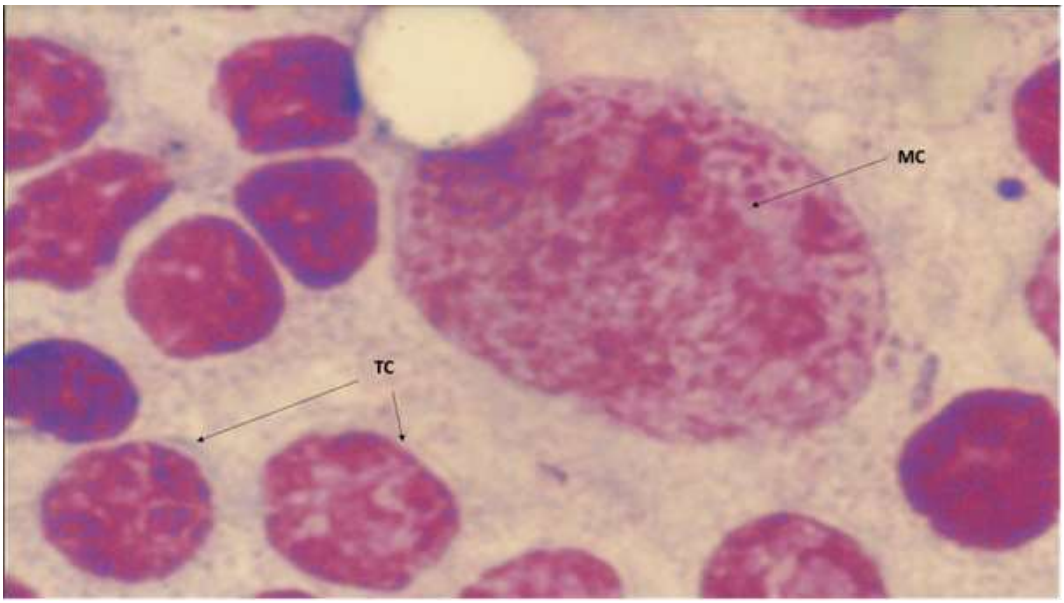


**Figure 4.** Bovine lymph node, malignant pre-lymphoma, the presence of a monstrous cell (MC) alongside of malignant cells from lymphoreticular origins (TC), MGGX2000

**Table 2.** The number of cases in which hyperplastic lymph node lesions or malignant pre-lymphoma have been identified. N- Lymph nodes with lesions outside the hyperplastic sphere; SH - Simple lymphoreticular hyperplasia; AH - Aggravated lymphoreticular hyperplasia; MP - Malignant pre-lymphoma

| | Animals with adenopathies | N | % | SH | % | AH | % | MP | % |
|---|---|---|---|---|---|---|---|---|---|
| **Ovines** | 147 | 93 | 63,26 | 23 | 15,64 | 19 | 12,92 | 12 | 8,16 |
| **Bovines** | 38 | 28 | 73,68 | 6 | 15,78 | 4 | 10,52 | 0 | 0 |
| **Equines** | 82 | 76 | 92,68 | 3 | 3,65 | 2 | 2,43 | 1 | 1,21 |
| **Swine** | 168 | 164 | 97,61 | 2 | 1,19 | 1 | 0,59 | 1 | 0,59 |

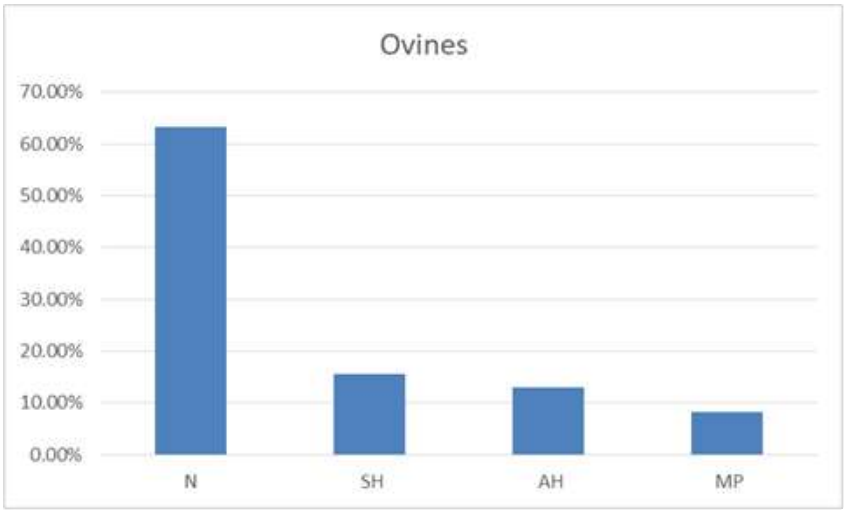


**Chart 1**. Prevalence of hyperplasia in ovines. N- Lymph nodes with lesions outside the hyperplastic sphere; SH - Simple lymphoreticular hyperplasia; AH - Aggravated lymphoreticular hyperplasia; MP - Malignant pre-lymphoma

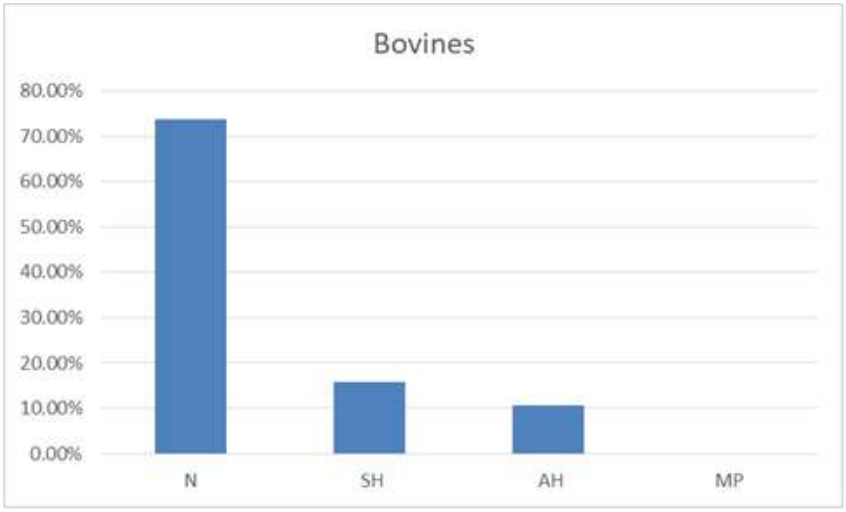


**Chart 2**. Prevalence of hyperplasia in bovines. N- Lymph nodes with lesions outside the hyperplastic sphere; SH - Simple lymphoreticular hyperplasia; AH - Aggravated lymphoreticular hyperplasia; MP - Malignant pre-lymphoma

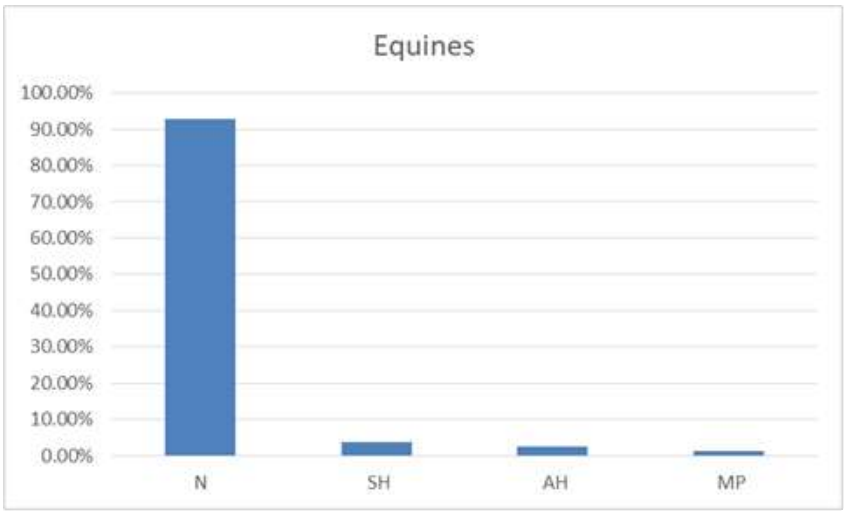


**Chart 3.** Prevalence of hyperplasia in equines. N- Lymph nodes with lesions outside the hyperplastic sphere; SH - Simple lymphoreticular hyperplasia; AH - Aggravated lymphoreticular hyperplasia; MP - Malignant pre-lymphoma

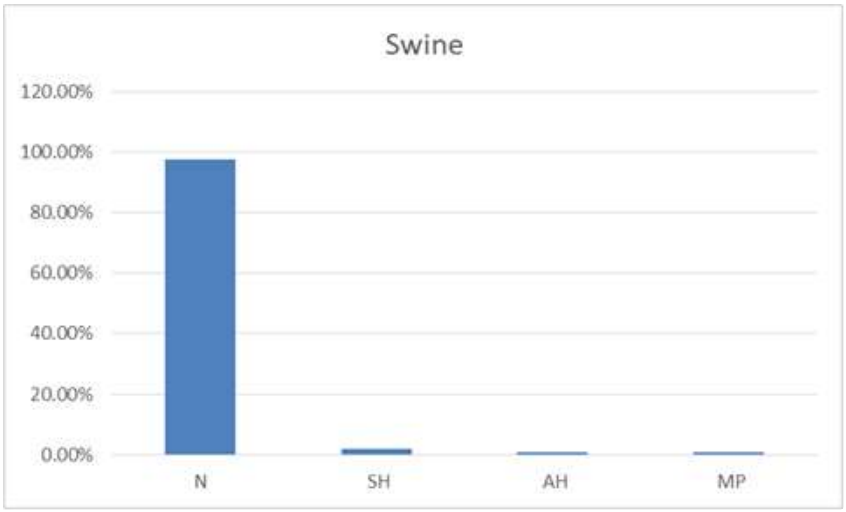


**Chart 4.** Prevalence of hyperplasia in swine. N- Lymph nodes with lesions outside the hyperplastic sphere; SH - Simple lymphoreticular hyperplasia; AH - Aggravated lymphoreticular hyperplasia; MP - Malignant pre-lymphoma

## Conclusions

The present paper shows that ovines followed by bovines have the highest lymph node reactivity to *E. granulosus* infestation. The simple lymphoreticular hyperplasia was predominantly found in bovines and, the aggravated form was predominantly present in ovines. Malignant pre-lymphoma was also detected in ovines, equines and swine but it was absent in bovines.

## References

Abo-Aziza F.A.M., Oda S.S., Aboelsoued D., Farag T.K., Almuzaini A.M. 2019. Variabilities of hydatidosis in domestic animals slaughtered at Cairo and Giza abattoirs Egypt. Vet. World 12(7):998-1007.

Ali-Khan Z. 1978. Pathological changes in the lymphoreticular tissues of Swiss mice infected with *Echinococcus granulosus* cysts. Z. Parasitenk. 58(1):47-54.

Ayele A., Gezaw E., Birhan M. 2019. Prevalence and associated risk factors of cysticechinococcosis in pigs slaughtered at addis ababa abattoir enterprise. Online J. Anim. Feed Res. 9(6):225-232.

Candolfi E., Kien T., Chaker E., Fourati M., Benyounes A. 1985. Interet de l'antigenemie et des anticorps IgG, IgM, IgA et IgE dans l'immunologie du kyste hydatique. Resultats de l'immuno-enzymologie. Bull. Soc. Pathol. Exot. 78:700-706.

Cheever A.W., Hoffmann K.F., Wynn T.A. 2000. Immunopathology of schistosomiasis mansoni in mice and men. Immunol. Today 21:465-466.

Colley D.G., LoVerde P.T., Savioli L. 2001. Medical helminthology in the 21st century. Science 293:1437-1438.

Cox D.A., Marshall-Clarke S., Dixon J.B. 1989. Activation of normal murine B cells by *Echinococcus granulosus*. Immunology 67(1):16.

Davidson R.A. 1985. Immunology of parasitic infections. Med. Clin. North Am. 69(4):751-758.

Dema A., Cornianu M., Lazureanu C., Vaduva A., Jurescu A., Mihai I., Lupu V., Taban S., Muresan A., Cornea R., Vita O., Gheju A., Olteanu E. 2019. General morphopathology (Morfopatologie generală). Victor Babeş Publishing House, Timişoara, pp. 23-24.

Gottstein B., Soboslay P., Ortona E., Wang J., Siracusano A., Vuitton D.A. 2017. Advances in parasitology. vol. 96, Edited by R.C.A. Thompson, P. Deplazes, A.J. Lymbery, pp. 1-54.

Grubor N.M., Jovanova-Nesic K.D., Shoenfeld Y. 2017. Liver cystic echinococcosis and human host immune and autoimmune follow-up: A review. World J. Hepatol. 9(30):1176-1189.

Jenkins P., Dixon J.B., Ross G., Cox D.A. 1986. *Echinococcus granulosus*: changes in the transformational behavior of murine lymph node cells during early infection. Ann. Trop. Med. Parasit. 80(1):43-47.

Jubb K.V.F., Kennedy P.K., Palmer N. 2012. Pathology of domestic animals. 4th edition, volume 3, Academic Press Publishing House, p. 113.

Kumar V., Abbas A., Aster J.C. 2015. Patologia Robbins: Bazele morfologice şi fiziopatologice ale bolilor. ediţia a 9-a [Robbins Pathology: Morphological and pathophysiological basis of diseases. 9th edition] [in Romanian]. Callisto Medical Publishing House, Bucharest, pp. 428-429.

MacDonald A.S., Araujo M.I., Pearce E.J. 2002. Immunology of parasitic helminth infections. Infect. Immun. 70(2):427-433.

Mesrobeanu I., Berceanu S. 1968. Imunologie şi imunopatologie [Immunology and immunopathology] [in Romanian]. Medical Publishing House, Bucharest, pp. 340-347.

Micu D. 1964. Puncţia ganglionară în clinică [Lymph-node puncture in clinic]. Publishing House of the Academy of the Socialist Republic of Romania, pp. 35-42.

Micu D. 1973. Citologia organelor limfoide [Cytology of lymphoid organs]. Publishing House of the Academy of the Socialist Republic of Romania, pp. 138-154.

Piaton E., Fabre M., Goubin-Versini I., Bretz-Grenier M.F., Courtade-Saïdi M., Vincent S., Belleannée G., Thivolet F., Boutonnat J., Debaque H., Fleury-Feith J., Vielh P., Cochand-Priollet B. 2015. Technical recommendations and best practice guidelines for May-Grünwald-Giemsa staining: literature review and insights from the quality assurance. Ann Pathol. 35(4):294-305.

Prisacari V., Pantea V., Lungu V., Iaravoi P. 2012. Echinococoza (hidatidoza): etiologie, patogenie, tabloul clinic, diagnostic, tratament, epidemiologie şi profilaxie (Indicaţii metodice) [Echinococcosis (hydatidosis): etiology, pathogenesis, clinical picture, diagnosis, treatment, epidemiology and prophylaxis (Methodical indications)]. Ministry of Health of the Republic of Moldova, Chisinau.

Sakamoto T., Cabrera P.A. 2003. Immunohistochemical observations on cellular response in unilocular hydatid lesions and lymph nodes of cattle. Acta Trop. 85(2):271-279.

Seres S., Iovu A., Junie M., Cozma V. 2009. Prevalence of *Echinococcus granulosus* in Cluj county, Romania, revealed by PCR. Sci. Parasitol. 10(1-2):68-71.

Shaikenov B.S., Rysmukhambetova A.T., Massenov B., Deplazes P., Mathis, A., Torgerson P.R. 2004. The use of a polymerase chain reaction to detect *Echinococcus granulosus* (G1 strain) eggs in soil samples. Am. J. Trop. Med. Hyg. 71:441-443.

Solanki S., Purohit K. 2019. Case report: A heavily infected camel with both Cystic echinococcosis and *Oestrus ovis* larva. J. Entomol. Zool. Stud. 7(4): 312-314.

Yazdanbakhsh M., Sacks D. 2010. Why does immunity to parasites take so long to develop? Nat. Rev. Immunol. 10(2):80-81.