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**CONTENTS**

# STUDY OF LYMPHODODAL CELL EVENTS IN THE WALKER-256 TUMOUR METASTATIC PROCESS

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***Abstract***

*Experimentation in the biomedical field is difficult and particularly complex. The extrapolation, in humans, of the experimental results remains a difficult problem and contested by some. Although there are differences between experimental graft tumours, due to the large number of years of maintenance, and spontaneous tumours (natural tumours), still, without experiments performed on these tumours, the number of cytostatic drugs used in cancer today would be very limited. In addition, with the help of metastasis models created with graft tumours, a series of pathogenesis and therapy problems (cytostatics and immunology) could be clarified, with beneficial effects in the correct direction of the treatments applied in the fight against cancer.*

*In this study, we managed to capture the entire film of the sequence of events specific to experimental loco regional lymph node metastases of Walker 256 tumour, ascitic form, intratesticular post-inoculation.*

*We exemplified this film through a rich cytomorphological imaging from the first moment, that of the intratesticular inoculation of the tumour cell population, until the illustration of the almost total "conquest" of the lymph node structure.*

**Keywords:** *metastasis, experimental tumour, asictic carcinosarcoma Walker 256*

## *Introduction*

Currently, cancer is considered a real scourge that affects plants, animals and humans, the oncosensitivity being different from one species to another, from one individual to another and from one tissue to another, correlating with nature, duration, dose of oncoinductive factors, with increased carcinogenic risk, but also with deficient homeostasis of the immune system.

Numerous studies attest to marked similarities of the appearance, progression but also of the paraneoplastic manifestations of cancer, which allowed the opening of new horizons towards the creation of targeted antineoplastic therapies.

Through this study, the authors wanted to highlight one of the essential and dreaded features of neoplastic disease, that of metastasis. Thus, the experimental model created by us tries to present the whole metastasis process, by capturing the migration of tumour cellularity from the original tissue to the afferent lymph node structures.

***Materials and methods***

The experimental study was performed on a group of 12 Wistar rats, outbred, male, with a body weight between 100–120 g. They were inoculated intratesticularly with 1 x $10^3$ tumour cells from the ascitic form of Walker 256 carcinosarcoma (Fig. 1).

The ascitic tumour line used is part of the experimental tumour base of the Oncological Institute "Prof. Dr. Alexandru Trestioreanu" and is maintained by serial passages, on the Wistar rat line.

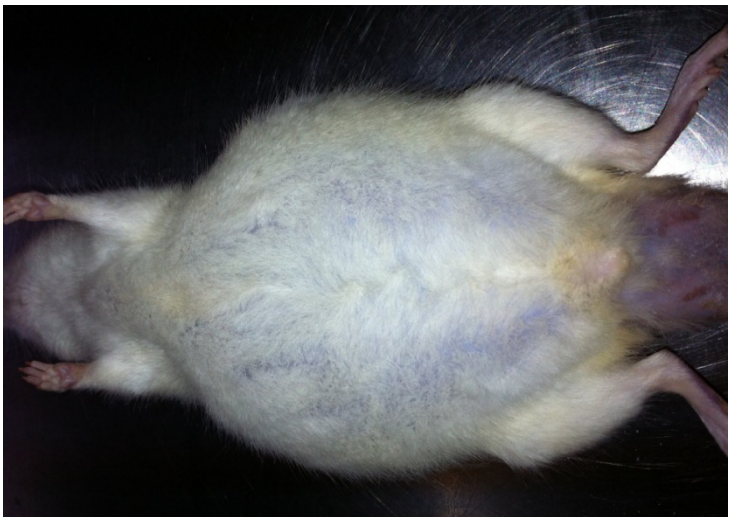

Fig. 1. *Rat, Walker 256 tumour – ascitic form, maintained by serial passages*

Intratesticular inoculation of the ascites tumour was done bilaterally, because we wanted the evolutionary process of the disease to be faster.

Inoculated animals were sacrificed at 24 hours, 3 days, 5 days, 7 days, 14 days, and 21 days postinoculation. From these, the testicles and the abdominal paraaortic lymph nodes were harvested for cytomorphological examination with the aim of capturing the metastatic process, specific to the neoplastic process.

From the collected samples, respectively the testicles and the paraaortic lymph nodes, smears were performed by scraping the section surface and displaying the secretion obtained on a slide.

In order to obtain correct, well-executed smears, we have taken into account some essential rules in performing this technique. Thus, we took into account the fact that the smear must be uniform, spread in a layer as thin as possible to ensure the distribution of cells in the monolayer, and its edges should be at a distance of a few millimetres from the edge of the glass blade on which it was displayed. Also, the end of the smear had a fringed and rounded end.

The smears thus obtained were stained panoptically using the May-Grunwald Giemsa staining technique and then examined under a microscope.

It should be mentioned that all the images used in this study is the property of the Oncological Institute "Prof. Dr. Alexandru Trestioreanu", Bucharest, because the entire experiment took place in this location, under the coordination of the responsible staff for creating the experimental models within the Cancer Biology Department.

## *Results and discussion*

### Macroscopic examination of the samples

Starting with the fourth post inoculation day, at the testicular level we observed their hypertrophy and ectasia of the blood vessels (fig. 2), and the paraaortic lymphadenopathy became visible starting with the 21st day after inoculation (fig. 3).

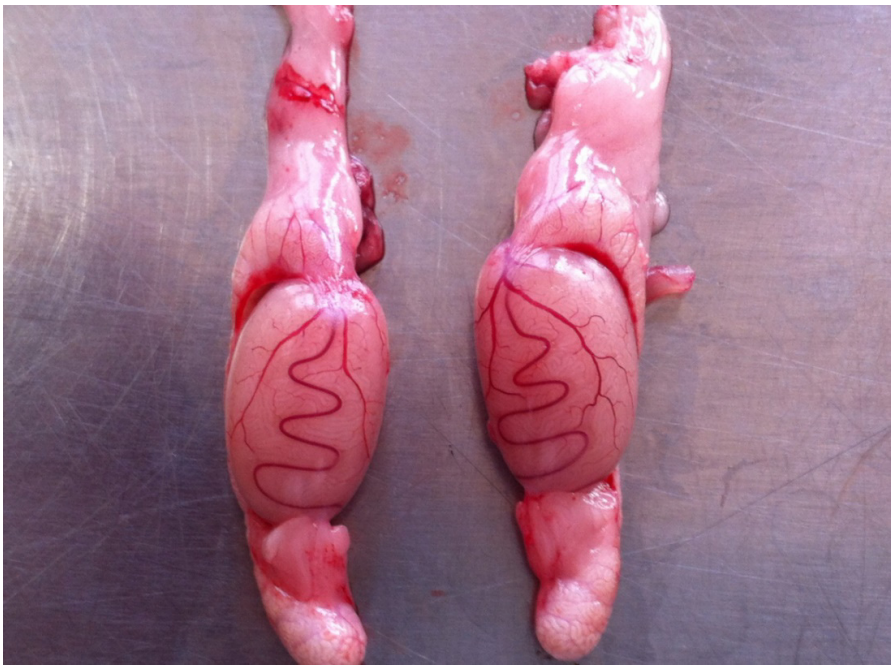

Fig. 2. *Rat testicles, ectasia of testicular vessels at 4 days after tumour inoculation*

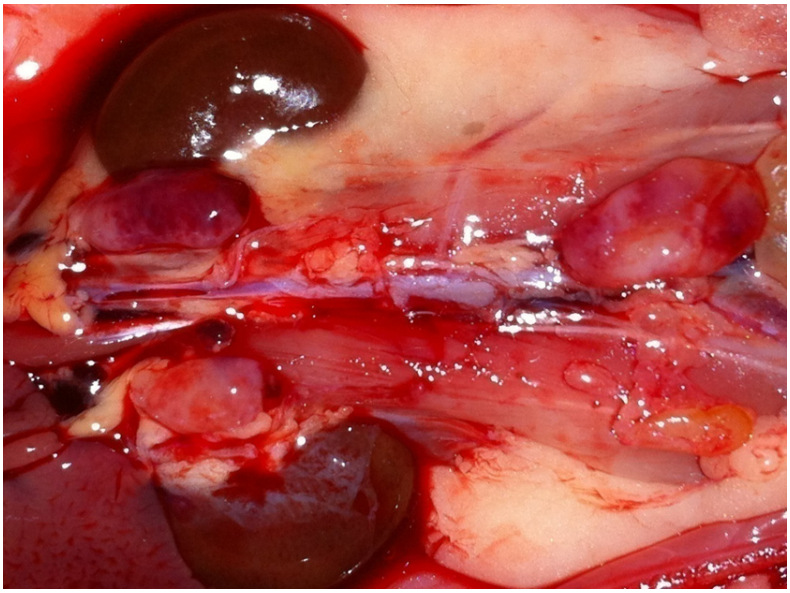

Fig. 3. *Rat lymph nodes, paraaortic lymphadenopathy*

**Microscopic examination**

The detected macroscopic changes were completed with microscopic investigations, by performing smears from the tumour (fig. 3), testicles (fig. 4, fig. 5, fig. 6) and from lymph nodes (fig. 7, fig. 8, fig. 9, fig. 10, fig. 11) to follow the metastatic evolution of Walker tumour 256.

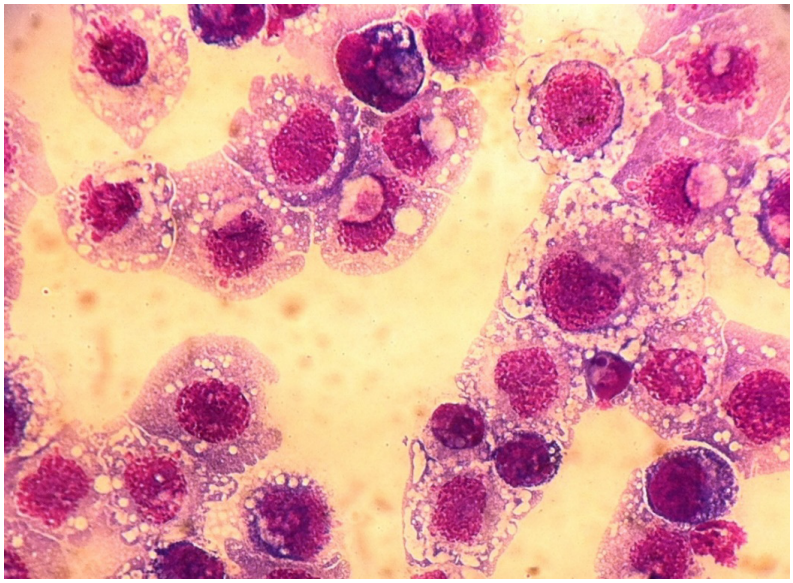

Fig. 3. *Walker 256 tumour, ascitic form – large tumour cells, with numerous intracytoplasmic vacuoles, with oval or lacy nucleus. MGG Col., ob. x100*

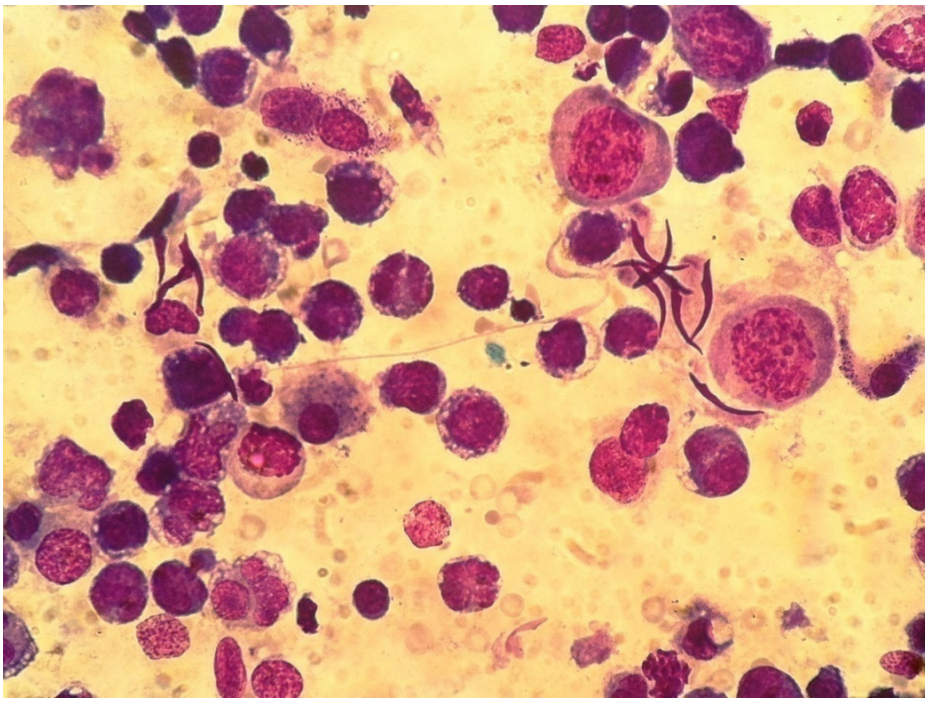

Fig. 4. *Rat testis – 24 hours after tumour inoculation, the presence of tumour cells among testicular tissue-specific cell populations, MGG Col., ob. x100*

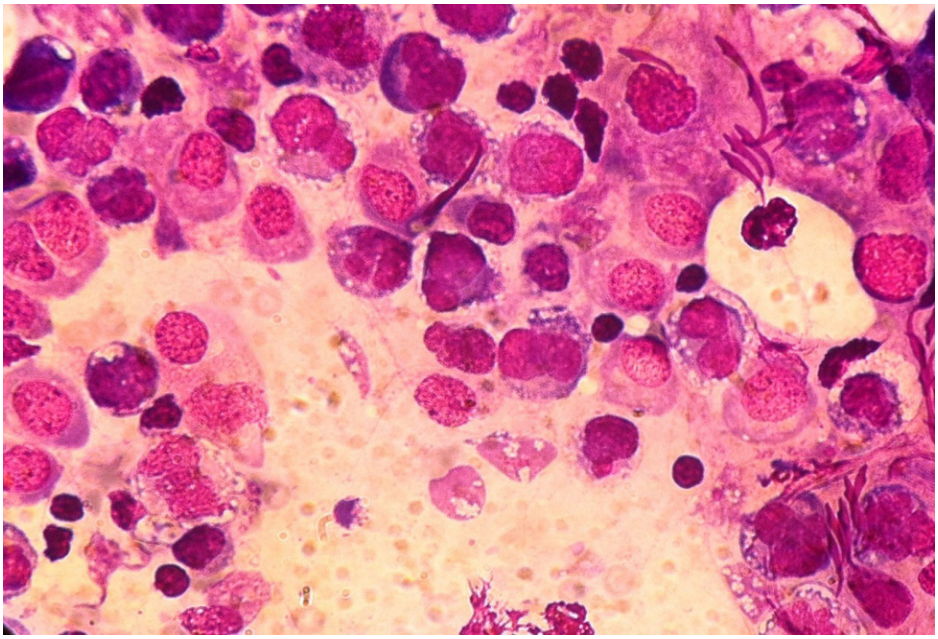

Fig. 5. *Rat testis – 5 days after tumour inoculation. Tumour cells, many mitoses, seminal cells are degenerated and necrotic, karyopyknosis and cytoplasmic vacuolation, numerous whole or partially degenerated sperm count, MGG Col., ob. x100*

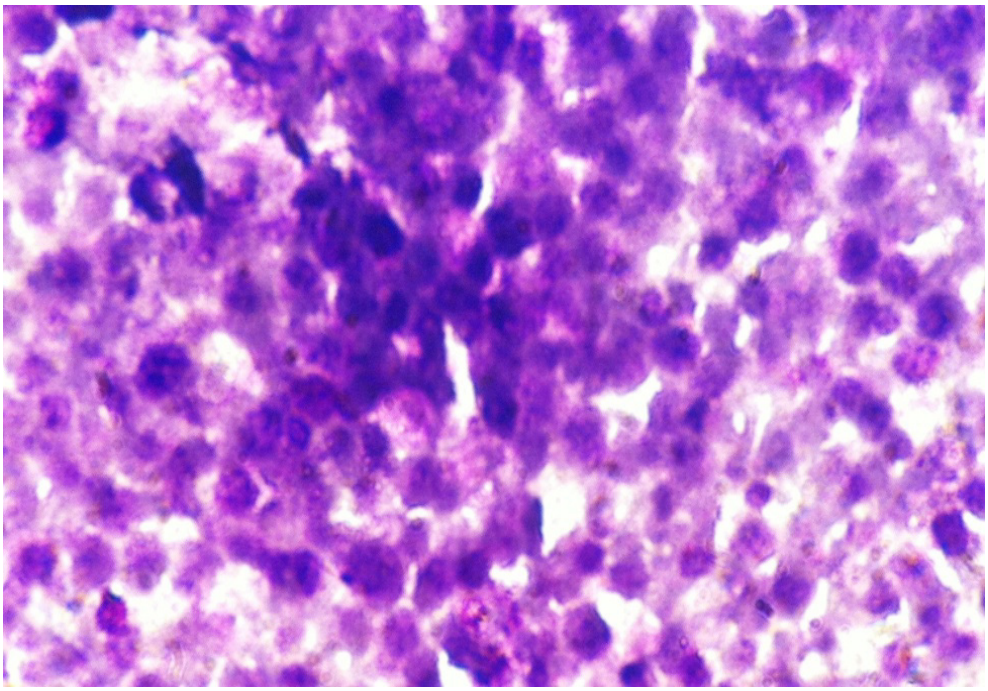

Fig. 6. *Rat testis – 21 days after tumour inoculation. Massive cell necrosis with detritus in the form of a homogeneous mass, MGG Col., ob. x100*

The testicular cytomorphological investigations, completed with the lymph node ones, showed us the existence of extremely large and deep rearrangements in their cyto-architecture. Thus, as seen in Figures 4, 5 and 6, the cell populations specific to the testis, respectively the cells of the germinal epithelium (spermatogonia, spermatocytes, spermatids) and cells with endocrine functions, hormone secretors, respectively Sertoli cells and Leydig cells suffer over time, due to the invasion of tumour cells, degeneration phenomena and necrobiosis, thus leading to the inhibition of spermatogenesis and implicitly to the installation of sterility.

At the lymph node level, cytomorphological phenomena have also been detected, attesting the phenomenon of tumour progression through the process of metastasis. Thus, during the experiment we harvested the paraaortic lymph nodes which are "stations" of lymph filtration from the testis, being able to detect, starting with the 5th day, the invasion of lymphatic tissue and its gradual replacement with tumour cells.

Also, at 72 hours post-tumour inoculation, we detected the intense reactivity of the immune system of the immunity (fig. 8) which is actively involved in preventing the spread of this disease.

Through the imaging that we will present, we try to create a suggestive film of this tumour progression by metastasis and of the reactivity of the host organism.

**Lymph node cytoarchitectural phenomena in Wistar rats during Walker 256 tumour metastasis**

In the physiological state, the lymph node station is composed dominant cytoarchitecturally of antigenically unexposed lymphocytes and recessively of antigenically exposed lymphocytes (fig. 7).

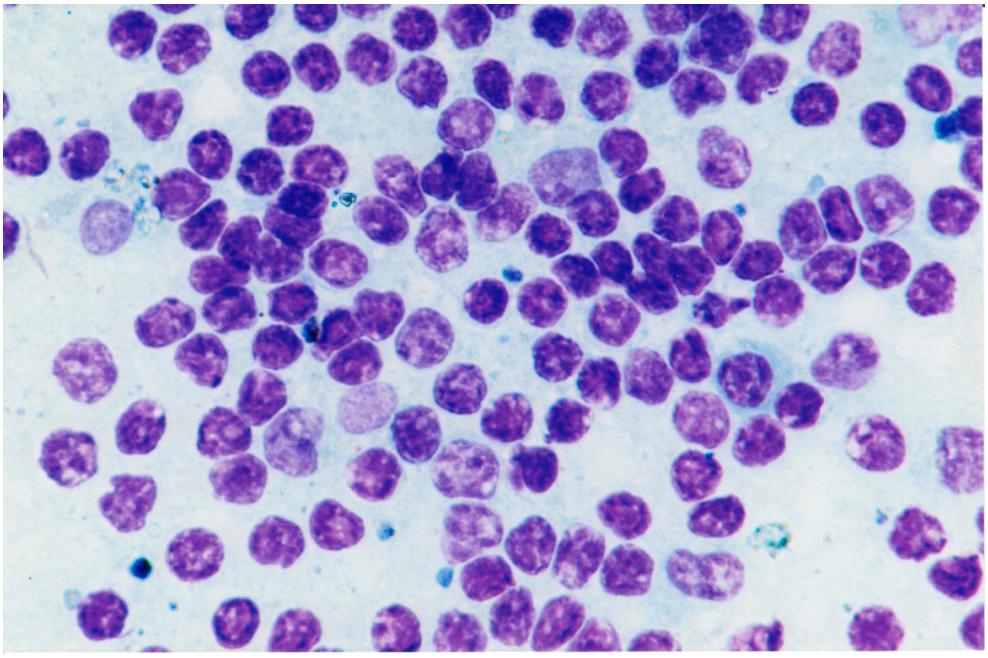

Fig. 7. *Rat lymph node, 24 hours after tumour inoculation. "Almost" normal appearance; cell dominance is represented by the unexposed antigenic adult lymphocyte, MGG Col., ob. x1000*

The individual cancer cells or in the microcolonies detach from the primary tumour and go exclusively by lymphatic route to the first lymph node locoregional station.

**Pre-invasion cell picture**

Even before the arrival of the first neoplastic cells in the lymph node subcapsular sinus, the dominant / recessive cell ratios are reversed in the sense that antigenically exposed lymphocytes are dominant and those that are not antigenically exposed become recessive.

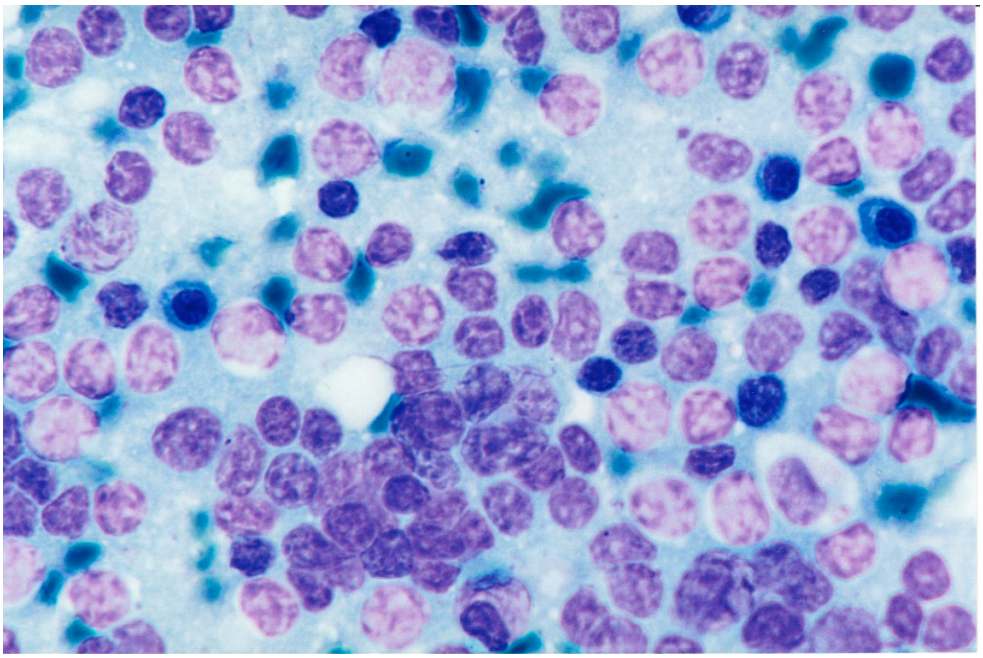

Fig. 8. *Rat lymph node, 72 hours post tumour inoculation.*
*The presence of a cellular "anxiety" caused by prelymphocyte dominants as a consequence of the exposure of the unexposed lymphocyte population, described above, to antigens produced by tumour cells, MGG Col., ob. x1000*

The consequence of this reversal is the preparation of the immune response (humoral and cellular) to the imminent landing of the neoplastic aggressor. This is done differently from the classical embodiment in the sense that a number of participating cellular elements are missing and replaced by others.

a. The classic dendritic cell – macrophage relationship is replaced by the dendritic cell – mast cell relationship (reported since 1998 by the N. Manolescu team).
b. Due to the lack of macrophages and NK cells, the killer type phenomenon (cell-mediated immune reaction) is achieved exclusively on account of TK lymphocytes, which in our opinion these cells alone develop a totally inefficient response in cancer.

The macroorganism (Wistar rat) attacked by the development of the primary tumour Walker 256 ascitic form prepares the future loco-regional lymph node metastatic invasion by alerting both the humorally mediated and the cellular immune system.

**Post-invasion cell picture**

Tumour cell structures detached from the primary tumour parenchyma destined for the first lymph node locoregional station travel exclusively by lymphatic route to the level of the landing station which is represented by the subcapsular sinus. These structures initially travel in the form of individual cancer cells (fig. 9) and then travel in the form of microcolonies (fig. 10).

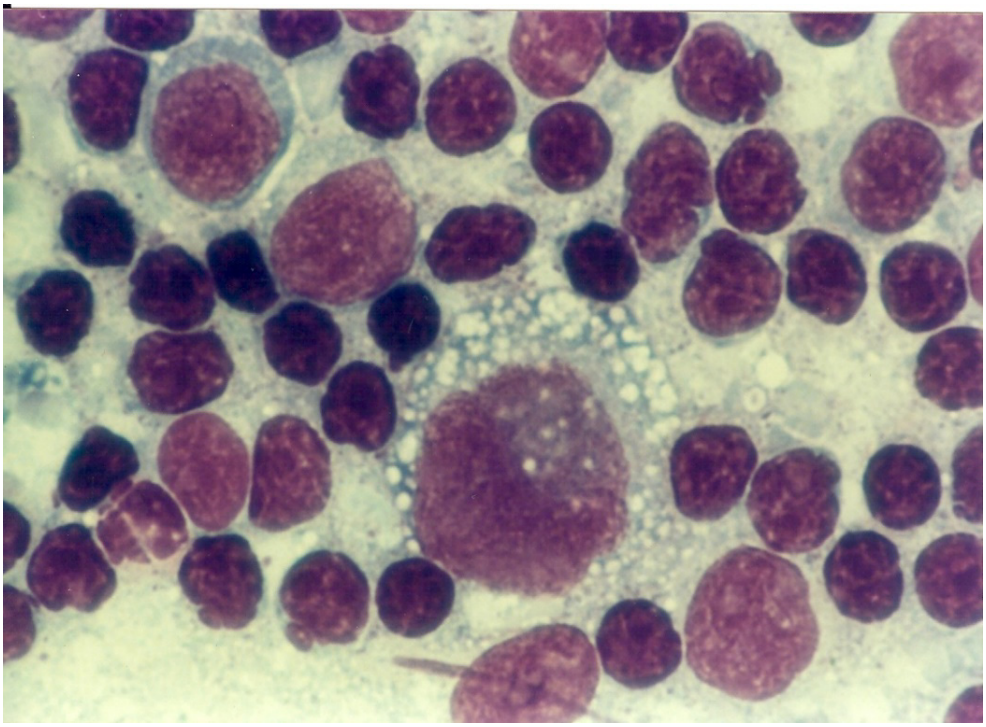

Fig. 9. *Rat lymph node, 5 days post-tumour inoculation. Invasion of tumour cells in lymph node cytoarchitecture, MGG Col., ob.x2000*

Also, in the loco regional lymph node structure we found numerous tumour cells in different stages of cell division (fig. 10, fig. 11) which attests to their intense proliferative capacity, so a marked aggressiveness of this type of tumour.

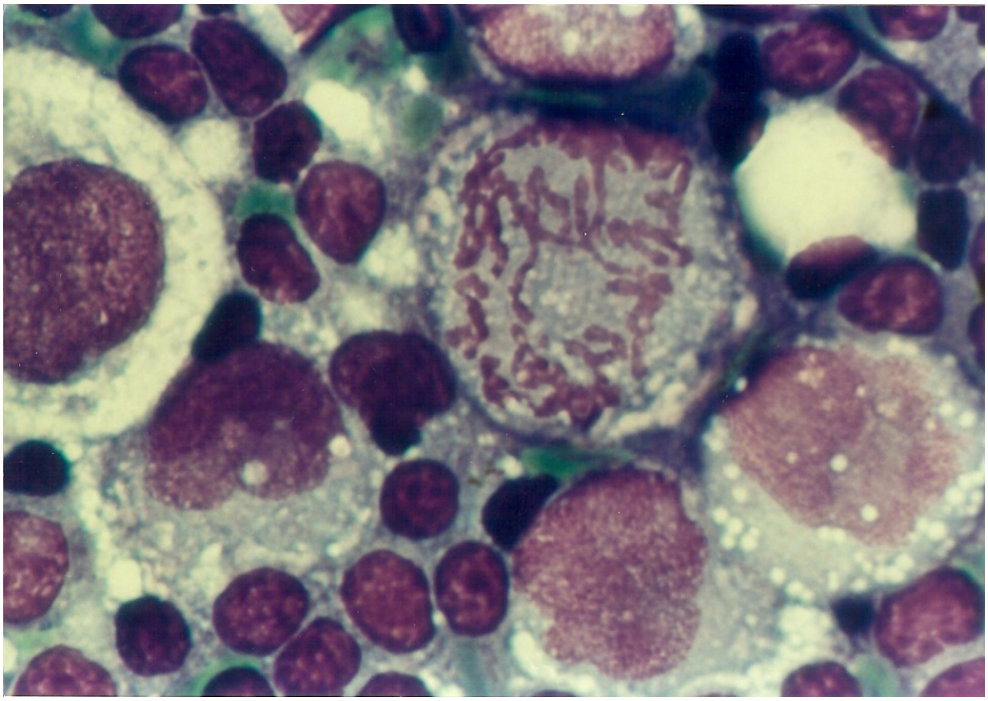

Fig. 10. *Rat lymph node, 14 days post-tumour inoculation.*
*Colonization of lymph node cellularity with tumour cells.*
*In the centre is observed a tumour cell in division, MGG Col., ob. x2000*

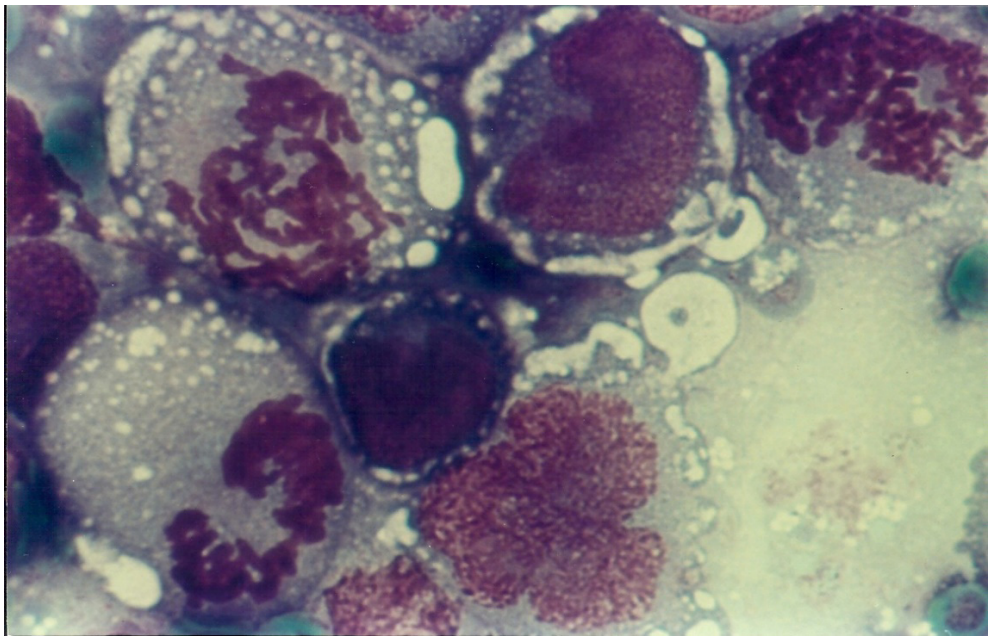

Fig. 11. *Rat lymph node, 14 days post-tumour inoculation. Numerous atypical tumour cells, in division, which attests to its aggressiveness, MGG Col., ob. x2000*

### *Conclusions*

Through this experiment we demonstrated, once again, the immense value of experimental oncology for both human oncology and veterinary oncology.

The time required for the primary tumour to invade the entire testis in rats is about 7 days, and 5 days are enough to achieve the first lymph node metastases.

Once the primary solid tumour Walker 256 in the testicle is made, the appearance of lymph node metastases in the natural way of the animal organism cannot be prevented. In the situation highlighted by us, the immune reaction of the host organism was triggered, but, subsequently, it became ineffective due to the reverse-killer phenomenon detected. The pathway of metastasis of Walker 256 tumour identified by us in this study was lymphatic.

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# RESEARCH ON THE USE OF HEPATOPROTECTIVE PREPARATIONS IN ADENOVIRAL INFECTIONS IN CHICKEN

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***Abstract***

*In two groups infected with hepatitis virus with inclusions, including hall 4 (L1) and hall 5 (L2) with 33,000 broiler chickens were given hepatoprotective preparations based on silymarin and artichoke extract compared to a control group also positive for adenoviral infection hall 6 (L3). The following were found: the decrease of the average body weight with values between 140 g and 250 g, the increase of the mortality percentage up to 7.3%, the reduction of the average daily increase between 3.33 g / day and 4.66 g / day for the entire period of study in the control group compared to the experimental groups.*

*In the control group, it was found: worsening of hepatic haemorrhages, hepatic steatosis, the appearance of erosions on the muscular stomach and the appearance of the anaemic syndrome.*

**Keywords:** *hepatitis including chickens, hepatoprotective preparations, mortality, hepatic haemorrhages, hepatic steatosis*

## *Introduction*

Inclusive body hepatitis has been described in chickens, parrots and other wild birds (3). In the etiology of the disease are involved 5 species of avian adenovirus (Fowl adenovirus A, B, C, D, E) (1,9) which have at least 11 adenovirus serotypes marked from 1 to 11 and which act singularly or synergistically (2, 4.5).

In chickens, the infection is more common in laying chicks and broilers, around the age of 4-9 weeks, but also in adult birds (6, 8). Clinically, the infection is externalized by a sudden increase in mortality over a period of about 3-4 days, with a percentage that varies between 2-10%, mortality may be higher if the herd is immunosuppressed. Birds show a slight state of deviation, loss of appetite and weight loss. Morbidity is relatively low. Naturally, the disease can also be transmitted vertically.

Macroscopically, avian adenovirus infection is characterized by enlarged liver and the presence of haemorrhages in the liver parenchyma (11).

Histologically, it is found the presence of a generalized diffuse hepatitis and the existence of the specific cytopathic effect, respectively of the intranuclear inclusion bodies in the hepatocytes.

These changes may be accompanied by hemorrhagic jaundice in various organs and tissues, in muscles and bone marrow; anaemic syndrome may also be present.

According to some researchers, hepatitis with inclusion bodies is constantly associated with the presence of other infections with viruses with immunosuppressive effect (avian infectious anaemia virus, infectious bursitis virus) (10).

***Materials and methods***

In order to determine the therapeutic efficacy of hepatoprotective preparations, an experimental model was created based on the collection of data from the farm and systematic determinations of serum antibodies against hepatitis including chickens, at the age of one day. For this, 3 halls (Hall 4, Hall 5, Hall 6) were chosen from a commercial farm with a staff of 33,000 heads in halls 4, 5 and 18,000 in Hall 6 – the ROSS 308 fast-growing breed.

- The broilers from hall 4, experimental group L1 received in the drinking water the hepatoprotective preparation containing silymarin (12);
- Chickens from hall 5 Experimental group L2 received in the drinking water the hepatoprotective preparation containing artichoke extract (12);
- The broilers from hall 6 did not receive any hepatoprotective preparation in the drinking water, being considered the control group.

The level of antiadenoviral antibodies, weight, mortality rate, average daily increase, the presence of anatomopathological changes in the investigated groups from the analysis bulletins, and from the existing records at the farm level were analyzed.

Blood samples were collected from the axillary vein in syringes without anticoagulant. After collection, the samples were identified and transported to the laboratory in isothermal crates where they were processed immediately by ELISA for the determination of serum antibodies against FAV type 1.

A total of 300 blood samples were collected from chickens, respectively 100 per shelter for the detection of antibodies against hepatitis including chickens.

The body weight of the broilers was established by performing a number of 500 control scales at the age of 0.7,14,21,28,35, 42 days of the lots under study. An electronic scale was used for this work

The mortality rate was determined by mathematical calculation by dividing the number of deaths daily by the number introduced.

The average daily increase was obtained by dividing the average delivery weight by the number of days of slaughtered chicks.

The presence of anatomopathological changes were made by performing necropsy examinations in puppies whose blood samples were collected for laboratory examinations.

***Results and discussions***

In the groups of broilers in halls 4 and 5 which were administered hepatoprotective preparations, there were significant changes in the values of the control scales compared to those in hall 6 of the control group.

**Table 1. Body weight dynamics in the investigated broiler flocks**

| No. | Age | Hall 4 (g) | Hall 5 (g) | Hall 6 (g) | Observations |
|---|---|---|---|---|---|
| 1 | 0-day | 42 | 42 | 42 | the batches had the same body weight. |
| 2 | 7 days | 190 | 175 | 165 | In the first week, significant weight differences were found between batches of 25 g less |
| 3 | 14 days | 487 | 430 | 402 | At the survey scale from week 2, significant weight differences were found between the studied groups. The weight differences between the lots were 28 g in hall 5 and 85 g respectively in hall 4 compared to hall 6 |
| 4 | 21 days | 960 | 910 | 880 | At the age of 21 days, the differences continued to increase, ranging between 30g (H5) g and 80 g (H4). |
| 5 | 28 days | 1480 | 1410 | 1390 | At the age of 28 days, the differences were between 20 g in hall 5 and 90 g in hall 4 compared to the weight in hall 6. |
| 6 | 35 days | 2040 | 1950 | 1870 | At the age of 35 days, the weight loss recorded was between 80 g and 170 g |
| 7 | 42 days | 2560 | 2450 | 2310 | At 42 days, a difference of 140 g and 250 g was found in the experimental lots (halls 5.4) compared to the one in hall 6 |

The mortality rates at the age of slaughter showed significant changes in the experimental groups in hall 4 (3.2%) and hall 5 (3.4%) compared to the control in hall 6 who were not given hepatopotective drugs (6, 1%).

**Table 2. Evolution of the cumulative mortality percentage for broiler flocks in halls 4, 5, 6**

| No. | week | Hall 4 (%) | Hall 5 (%) | Hall 6 (%) | Observations |
|---|---|---|---|---|---|
| 1 | 0-7 days | 0,6 | 0,7 | 0,9 | There are small differences in mortality rates between groups |
| 2 | 8-14 days | 1,2 | 1,3 | 1,8 | Significant differences in the cumulative mortality rate were found in chickens in hall 6 |
| 3 | 15-21 days | 1,7 | 1,8 | 4,1 | Halls 4 and 5, respectively, due to the administration of hepatoprotective substances, the mortality rates remained low. |
| 4 | 22-28 days | 2,2 | 2,4 | 5,1 | In halls 1.2 there were no significant differences in the mortality rate. In contrast, in hall 6 the percentage was almost 2.7% higher. |
| 5 | 29-35 days | 2,7 | 2,9 | 6,2 | Further to halls 6 and during this period there is an upward trend in the mortality rate of 6.2% |
| 6 | 36-42 days | 3,2 | 3,4 | 7,3 | At the end of the production cycle, a higher mortality rate was observed in the chickens of hall 6.7.3% compared to 3.4% in hall 5 and 3.2 in hall 4 |

From the analysis of the values presented in table 3 it is observed that the halls that benefited from the administration of hepatoprotective substances achieved good daily average increases of 65.7 g hall 4 and 71.42 g hall 5. The chickens that did not receive hepatoprotective substances in drinking water that is, those in hall 6 achieved an average daily increase of about 62.8 g.

**Table 3. Dynamics of the average daily increase in the number of broilers in halls 4, 5, 6**

| No. | week | Hall 4 (g) | Hall 5 (g) | Hall 6 (g) | Observations |
|---|---|---|---|---|---|
| 2 | 0-7 day | 21,14 | 19 | 17,5 | The lowest average daily increase of 17.5 g was recorded in chicken meat in hall 6. |
| 3 | 8-14 days | 42,42 | 36,42 | 33,85 | This week, hall 6, which did not benefit from the administration of hepatoprotectants, showed an average daily increase of 33.85 g. |
| 4 | 15-21 days | 67,57 | 68,57 | 68,2 | This week there were no significant differences in the average daily increase in halls 4,5,6. |
| 5 | 22-28 days | 74,28 | 71,42 | 72,8 | During the period 22-28 days, the 5.6 halls showed a slight decrease of the average daily increase. |
| 6 | 29-35 days | 80 | 77,14 | 68,5 | At the age of 35 days, halls 4.5 showed good values of the average daily increase and at hall 6 an average increase of 68.5 g was registered. |
| 7 | 36-42 days | 65,71 | 71,42 | 62,8 | At the slaughter of chickens, hall 6, which was not given hepatoprotectants, had the lowest average daily increase. |
| 8 | Total period | 59,66 | 58,33 | 55 | The lowest average daily increase was recorded |

From the analysis of the incidence of anatomopathological changes presented in table 4 it is observed that after administration of the preparation based on silymarin for 8 days in the period of 1-10 days a reduction in the presence of hepatic haemorrhages, enlarged liver, hepatic steatosis and disappearance of anaemic syndrome.

**Table 4. Incidence of anatomopathological changes in broilers in hall 4**

| No. | Tape of lesions | 0-7 days | 8-14 days | 15-21 days | 22-28 days | 29-35 days | 36-42 days |
|---|---|---|---|---|---|---|---|
| 1 | Liver enlarged in volume. | + | + | +++ | - | - | - |
| 2 | Hepatic bleeding | ++ | ++ | ++++ | - | - | - |
| 3 | Hepatic steatosis | ++ | ++ | +++ | + | + | + |
| 4 | Anaemic syndrome | + | - | +++ | - | - | - |
| 5 | Erosive lesions on the muscular stomach | - | - | +++ | - | - | - |
| 8 | Atrophy Fabricius bursitis | - | - | - | - | - | - |

At hall 5, which was administered the preparation based on artichoke extract for 8 days in the period of 1-10 days, there was a reduction in the presence of hepatic haemorrhages, enlarged liver, hepatosteatosis and the disappearance of anaemic syndrome (table 5 ).

**Table 5. Incidence of anatomopathological changes in broilers 5**

| No. | Tape of lesions | 0-7 days | 8-14 days | 15-21 days | 22-28 days | 29-35 days | 36-42 days |
|---|---|---|---|---|---|---|---|
| 1 | Liver enlarged in volume. | ++ | + | ++++ | - | + | + |
| 2 | Hepatic bleeding | ++ | ++ | +++++ | - | + | + |
| 3 | Hepatic steatosis | ++ | ++ | +++ | + | + | + |
| 4 | Anaemic syndrome | + | + | +++ | - | - | - |
| 5 | Erosive lesions on the muscular stomach | - | - | +++ | - | - | - |
| 8 | Atrophy Fabricius bursitis | - | - | - | - | - | - |

The analysis of the incidence of anatomopathological changes presented in table 18 shows an aggravation of the lesions in the control group in hall 6 as a result of the non-administration of liver protectors and the brutal evolution of hepatitis including chickens.

**Table 6. Incidence of anatomopathological changes in broilers in hall 6**

| No. | Tape of lesions | 0-7 days | 8-14 days | 15-21 days | 22-28 days | 29-35 days | 36-42 days |
|---|---|---|---|---|---|---|---|
| 1 | Liver enlarged in volume. | + | + | +++ | ++ | + | + |
| 2 | Hepatic bleeding | +++ | +++ | +++++ | ++ | ++ | +++++ |
| 3 | Hepatic steatosis | ++ | ++ | +++ | +++ | +++ | ++++++ |
| 4 | Anaemic syndrome | + | + | +++ | ++ | ++ | ++ |
| 5 | Erosive lesions on the muscular stomach | - | - | +++ | ++ | - | - |
| 8 | Atrophy Fabricius bursitis | - | - | - | - | - | - |

***Conclusions***

In chickens from the experimental group in halls 4 and 5 where hepatoprotective preparations based on silymarin and artichoke extract, respectively, the dynamic body weight was ascending, approaching the weights recommended by the hybrid manufacturer.

The experimental group in Hall 6, which was not given a hepatoprotective preparation, had a downward trend in weight due to virus-induced liver failure.

The mortality rate was 7.3% in broilers from the experimental group L3 In the rest of the birds, the number of deaths was included in the technological losses.

The average daily increase was affected by the evolution of the disease in the chicks of the control group, registering a decrease.

In broilers from experimental groups L1 and L2 who received hepatoprotective preparations, the average daily increase did not change significantly.

The anatomopathological changes were greatly diminished in the groups of broilers in halls 4 and 5 as a result of the administration of hepatoprotective preparations. They protected the hepatocyte from the aggressive action of the virus and supported major liver functions.

In the absence of liver protection in broiler chickens from hall 6, the virus acted aggressively by destroying the hepatocyte, affecting the synthesis, metabolic and antitoxic functions of the liver.

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# EPIDEMIOLOGICAL AND RADIOLOGICAL DATA OF LOCOMOTOR DISEASES IN DOGS

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***Abstract***

*The study targets a number of 73 dogs of different breeds that have had locomotor disorders. The diagnosis was suspected based on clinical signs and confirmed by general examination methods, supplemented by paraclinical examinations, especially by the use of radiological examination.*

*The aim was to update epidemiological data and to highlight the main locomotor disorders in dogs, related to breed, age and sex.*

*The main dog breeds were represented by mixed breed dogs (53.42%), followed by Bichon (12.33%) and German Shepherd (10.96%), aged between 6 months and 14 years. Most of the lesions had as etiology traumatic accidents (fractures – 64.38% and coxo-femoral dislocations – 12.33%), and the lesions with the lowest percentage were represented by bone agenesis and sacroiliac desmorexia (by 1.37% each).*

*Regarding the race / disease ratio (percentage related to the total number of patients), mixed breed (fracture 72.34%), Boxer (osteosarcoma 50%), German Shepherd (coxo-femoral dysplasia 40%), York Shire Terrier femoral 33.3%).*

*Consequently, we consider that there is no predisposition depending on the breed or age regarding the fractures, but there are breeds that have a high predisposition to neoplastic and dysplastic diseases (dysplasia and coxo-femoral dislocation).*

**Keywords**: *epidemiology, radiological examination, locomotor disorders, dog*

## *Introduction*

The main locomotor disorders are bone fractures, these are most often the result of trauma, although pathological fractures can be observed, having as substrate a basic bone disease (e.g. tumours, osteopenia). Fractures are seen radiologically as a continuity of the cortex, with radiolucent fracture lines. (Hollowa, & McConnell, 2016; Farow, 2003)

Fracture is a solution of bone continuity, which causes it to lose its normal shape and cause both damage to neighbouring soft tissue and locomotor functional disorders. The fracture occurs under the action of extrinsic and intrinsic factors. (Anson, 1993; Constantin, 2009).

The causes that determine the appearance of osteodystrophies are diverse: the disorders that appear are either of a metabolic nature (nutritional osteodystrophies) or of an endocrine nature (endocrine osteodystrophies) (Barzu, 1965; Bone, 1984).

Hip dysplasia occurs frequently in large breed dogs with a genetic predisposition (Trall, 2003).

Osteosarcoma is a malignant tumour that affects large and giant dog breeds. In dogs, it develops in the metaphyseal region of the long bones, then follows in descending order the radius and humerus bone (especially in boxer), and other locations are completely sporadic (in the bones of the head, spine, pelvis). (Baba, 2002; Garjoaba, 2009).

The radiological examination is the basic investigation in diagnosing the main bone diseases. (Trall, 2003; Farow, 2003)

***Materials and methods***

The research took place over 3 years at the Ortovet Bucharest veterinary clinic. During this period, 73 dogs were registered, belonging to several breeds, aged between 6 months and 13 years, with bone structure disorders.
All animals were subject to the following protocol:

- collecting anamnestic data,
- clinical examination (inspection, palpation, percussion),
- bone radiographic examination (at least two incidents were performed, latero-lateral and ventro-dorsal), using digital X-ray installation (Digivex 40),
- histopathological examination (bone tissue fragments collected after biopsy were fixed in 10% formaldehyde solution, processed by the classical histopathological method, with paraffin inclusion and microtome sectioning, and the dichromic hematoxylin-eosin method was used as staining methods (HE).

***Results and discussion***

Following the clinical examination of the 73 dogs with locomotor disorders, 8 breeds of different sizes were identified. The most affected dogs were the mixed breed (39 cases, representing 53.42%), followed by the Bichon dogs (9 cases, representing 12.33%) and the German Shepherd (8 cases, representing 10.96%), and the less affected were those of the Schnauzer breeds (2 cases, representing 2.74%) and Argentinean Dog (1 case, representing 1.37%) (fig. 1).

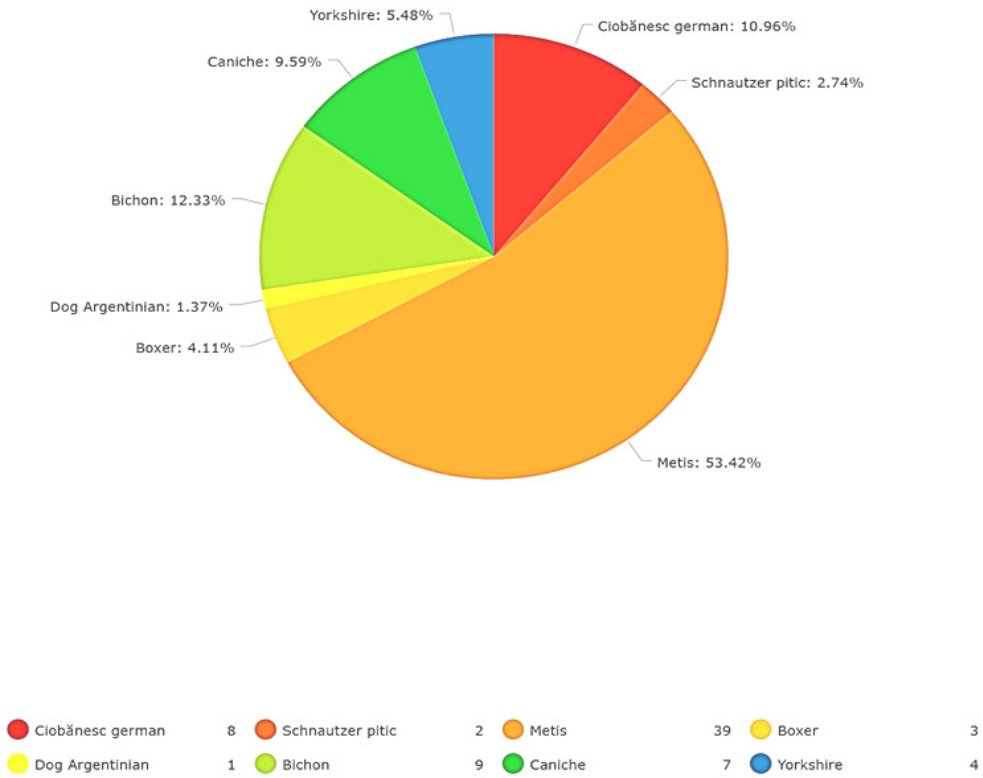


Fig. 1. *Graphical representation of locomotor disorders according to race*

The age of patients with locomotor osteopathies was between 6 months and 13 years, these being divided into age categories as follows:

– junior (6 months-2 years): 26 patients: 16 males, 10 females.
– adult (2 years-8 years): 39 patients: 20 males, 19 females.
– seniors (over 8 years): 8 patients: 5 males, 3 females.

Identifying 41 males and 32 females with distinct diseases depending on the age category (table number 1).

**Table 1. Informative data on the number of patients, age categories and locomotor disorders encountered in the study**

| Afecțiuni | Femele | Masculi | Juniori | Adulți | Seniori |
|---|---|---|---|---|---|
| Fractură | 31 | 16 | 12 | 28 | 7 |
| Osteosarcom | 2 | 0 | 1 | 1 | 0 |
| Displazie | 2 | 3 | 3 | 2 | 0 |
| Desmorexie | 1 | 0 | 0 | 1 | 0 |
| Coxartroză | 4 | 3 | 0 | 3 | 4 |
| Agenezie | 1 | 0 | 1 | 0 | 0 |
| Luxație coxo-femurală | 5 | 4 | 4 | 4 | 1 |
| Sindrom ulă scurtă | 1 | 0 | 1 | 0 | 0 |

Following the study, it is found that the most common form of bone disease is fracture (figure 4), representing 64.38% (47) of the total of 73 cases, followed by coxo-femoral dislocation 12.33% (9) (figure 6), coxarthrosis 9.59% (7), coxo-femoral dysplasia 6.85% (5), osteosarcoma 2.74% (2) (figure 5), bone agenesis 1.37% (1) (figure 7) and sacroiliac dysmorexia 1.37% (1) (Figures 2 and 3).

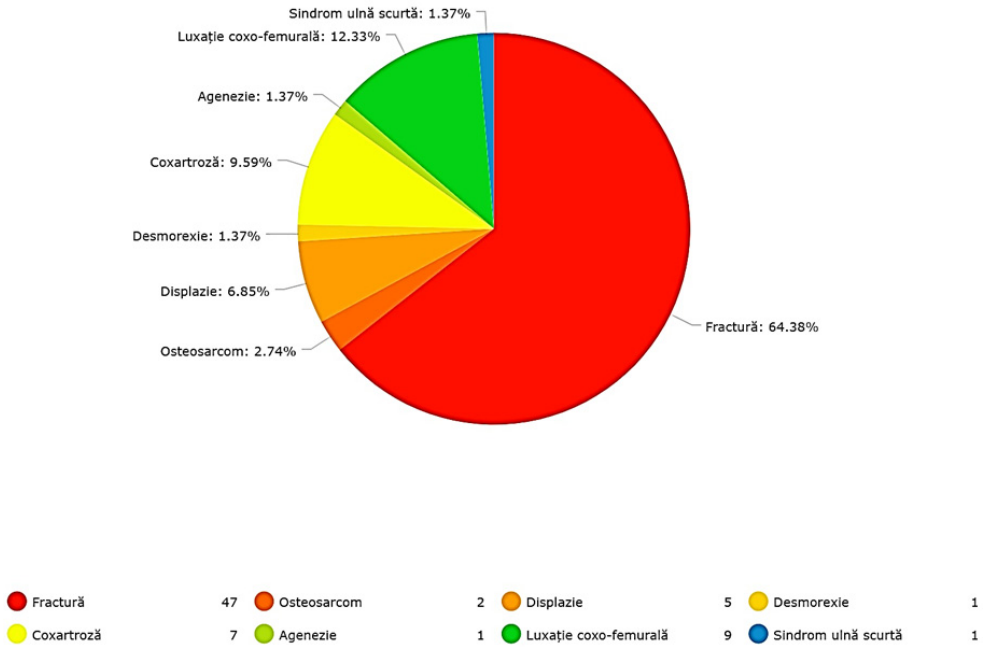


Fig. 2. *Graphical representation of locomotor disorders according to sex and age*

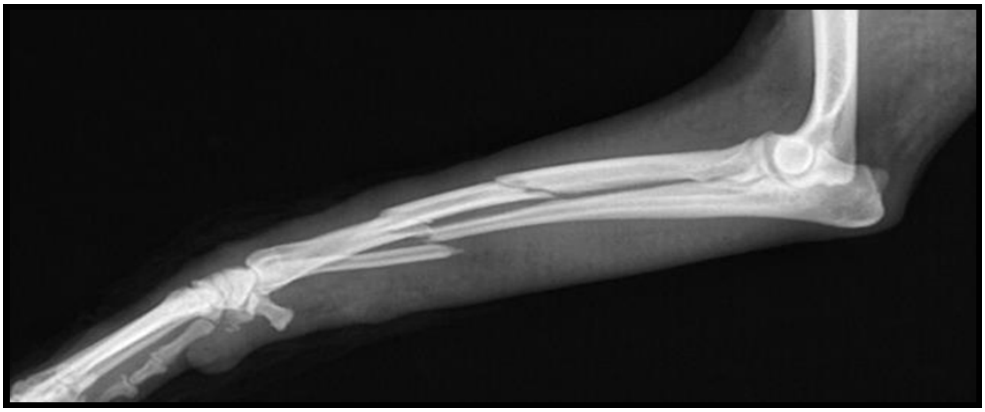

Fig. 3. *Medio-lateral radiograph of the right anterior limb, radius segmented fracture and oblique fracture with ulnar displacement*

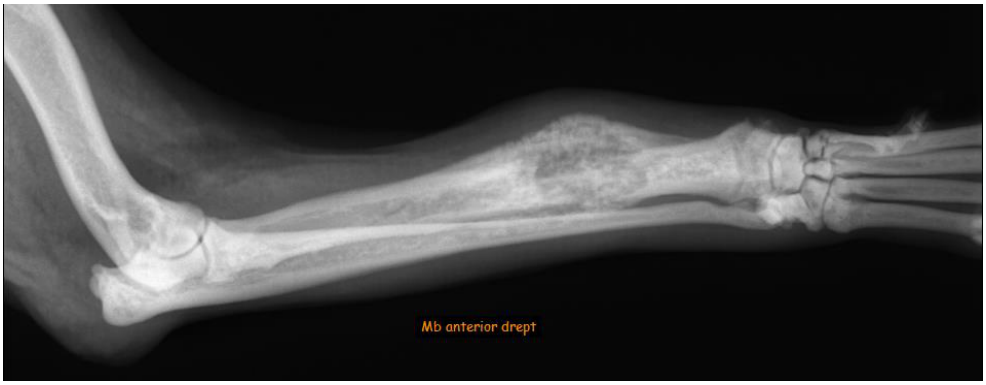


Fig. 4. *Medio-lateral radiography of the right anterior limb, in the distal portion of the radius, homogeneous radiolucency is identified, accompanied by bone deformation and fracture, the lesion being diagnosed histopathologically by fibromatous osteosarcoma with bone degeneration and osteonecrosis*

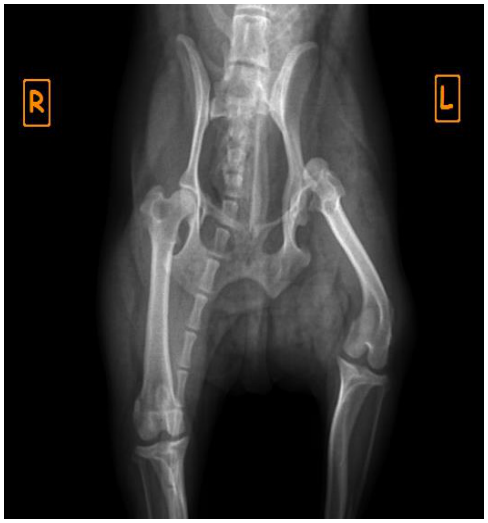


Fig. 5. *Pelvic radiograph, ventro-dorsal incidence, left coxo-femoral dislocation*

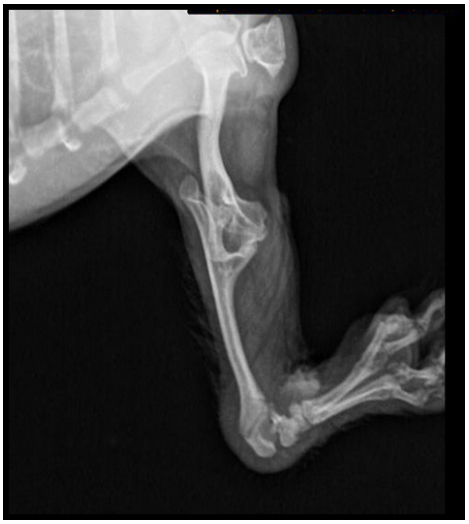

Fig. 6. *X-ray of the left forelimb, congenital radius agenesis*

***Conclusions***

More than half of the animals (53.42%) with locomotor disorders were found in half-breed dogs, due to the numerical increase of this category and uncontrolled matings.

Regarding locomotor disorders, traumatic injuries occupy the first places (fractures in 47 patients – 64.38%, coxo-femoral dislocations in 9 patients – 12.33%, sacroiliac desmorexia in 1 patient – 1.37%), being followed by

dysplastic processes (coxarthrosis in 7 patients – 9.59%, coxo-femoral dysplasia in 5 patients – 6.85%).

Regarding the locomotor disorders related to the age category, junior dogs (0-2 years) are mostly affected by 83.33% growth dysplasias (coxo-femoral dysplasia, bone agenesis and short ulna syndrome), adult dogs (2-8 years) are mostly affected by traumatic injuries 59.57% (fractures) and senior dogs (over 8 years) are affected by degenerative diseases 57.14% (hip osteoarthritis).

There is no specific race or age group with a predisposition to traumatic injuries (fractures, dislocations), which occur as a result of trauma.

Neoplastic diseases (osteosarcoma) were found in large breeds (2.74%) of all animals examined with osteopaths.

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# THE PROPHYLAXIS IMPORTANCE IN MYCOTOXIN CONTAMINATION OF ROMANIAN CEREAL FARMS

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***Abstract***

*The present paper aimed to determine the levels of mycotoxins, respectively, corn aflatoxin and wheat deoxynivalenol from a Romanian's farm. The technique used to perform mycotoxicological screening was based on lateral flow devices (LFD), using the RIDA QUICK Aflatoxin RQS and RIDA QUICK DON RQS ECO tests and interpreted with the RIDA QUICK SCAN.*

*Out of 20 samples performed for the determination of aflatoxin in maize, four samples (Sample 1, Sample 2, Sample 11, Sample 12) were included in the detection limit of the kit 2-75 µg/kg (ppb), respectively, Sample 1 with 4 ppb, Sample 2 with 16.5 ppb, Sample 11 with 3.4 ppb and Sample 12 with 13.5 ppb. Of these, two samples (Sample 2 and Sample 12) recorded values higher than the maximum permitted limit for mycotoxin levels in maize according to Regulation (EC) NO. 1881/2006, representing 10% of the total analyzed samples. The rest of the samples were below the minimum detection limit, respectively, 2 µg/kg (ppb). Of the 20 samples performed for the determination of deoxynivalenol in wheat, all samples were below the minimum detection limit of 0.25 mg/kg (ppm), respectively. Given the high incidence of aflatoxins and deoxynivalenol in cereals, as well as the hepatotoxic, teratogenic, genotoxic, oncogenic, nephrotoxic and immunomodulatory effects, an approach is needed from different perspectives, starting with the knowledge of the population about the existence of these mycotoxins and the diseases caused by them, up to the methods of determination and prevention.*

**Keywords***: aflatoxin, deoxynivalenol, mycotoxins, cereals, RIDA QUICK*

## *Introduction*

Mycotoxins are toxic compounds that are naturally produced by certain types of moulds (fungi). Fungi that can produce mycotoxins grow on many foodstuffs, such as cereals, dried fruit, nuts and spices. Mould growth can occur either before or after harvest, during storage and in food, often in warm and humid conditions. Most mycotoxins are chemically stable and survive food processing.

Several hundred different mycotoxins have been identified, but the most commonly observed ones that pose a threat to human and animal health include aflatoxins, ochratoxin A, patulin, fumonisins, zearalenone

and nivalenol/deoxynivalenol (Aycan Cinar, Elif Onbaşı, 2019). Mycotoxins occur in the food chain as a result of mould infection before harvest and after harvest. Exposure to mycotoxins can occur either directly through the consumption of infected food or indirectly from animals that are fed contaminated feed or its by-products (milk, eggs etc.) (Ahmad Alshannaq, Jae-Hyuk Yu, 2017).

Due to weather phenomena such as drought, heavy rainfall, sudden changes in temperature, which can vary from year to year, optimal conditions for mould growth can occur. A good way to prevent the occurrence of fungi and mycotoxins on a farm is to minimise the risk of contamination by following good agricultural practices and carrying out annual self-checks to monitor and improve the techniques applied.

Given the impact of technology on the medical field, new and advantageous techniques have been developed for the determination of mycotoxins in agricultural products. A good, practical and easy-to-use method without requiring high costs and specialised training is the immunochromatographic test based on the lateral flow principle (LFD), RIDA QUICK. With this test, mycotoxin levels in agricultural products can be identified directly on the farm.

***Materials and methods***

The study was conducted over a three-month period, February - April 2021, during which time samples of maize and wheat from different lots were collected and 40 toxicological determinations for mycotoxins, namely aflatoxin (B1,B2,G1,G2) and deoxynivalenol (DON), were performed.

Research materials:

- maize samples;
- wheat samples;
- RIDA QUICK Aflatoxin RQS and RIDA QUICK DON RQS ECO kits.

Sampling was carried out following the methodology of sampling for mycotoxicological examination in accordance with Regulation (EC) No 401/2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs.

The RIDA QUICK Aflatoxin RQS and RIDA QUICK DON RQS ECO were used for the qualitative and quantitative determination of aflatoxin residues in maize and deoxynivalenol in wheat. These are quantitative immunochromatographic band-type tests based on an antigen-antibody reaction. A specific anti-aflatoxin/anti-DON antibody detects aflatoxin/deoxynivalenol in the sample. During incubation of the test strip, a

band pattern is formed which is used to determine the concentration of the sample. The intensity of the test line depends on the aflatoxin/deoxynivalenol concentration of the sample. It increases with increasing aflatoxin/deoxynivalenol concentration. It must be possible to detect the control line after the reaction has taken place in order to check the functioning of the test. The control line weakens as the concentration of aflatoxin/deoxynivalenol in the sample increases. The test strip can be evaluated using the RIDA QUICK SCAN reader or the RIDA SMART APP, produced by R-Biopharm AG and marketed in Romania by Diamedix Diagnostica.

***Results and discussions***

Twenty RIDA QUICK Aflatoxin RQS tests were performed for the determination of aflatoxin (B1, B2, G1, G2) in maize. The detection range was between 2-75 µg/kg (ppb) (table 1).

**Table 1. Results of samples analyzed and reference level for aflatoxin**

| No. sample | Results (µg/kg; ppb) | Reference (µg/kg; ppb) |
|---|---|---|
| ***sample 1*** | ***4 ppb*** | 10 ppb |
| ***sample 2*** | *16.5 ppb* | 10 ppb |
| **sample 3** | <2 ppb | 10 ppb |
| **sample 4** | <2 ppb | 10 ppb |
| **sample 5** | <2 ppb | 10 ppb |
| **sample 6** | <2 ppb | 10 ppb |
| **sample 7** | <2 ppb | 10 ppb |
| **sample 8** | <2 ppb | 10 ppb |
| **sample 9** | <2 ppb | 10 ppb |
| **sample 10** | <2 ppb | 10 ppb |
| **sample *11*** | ***3.4 ppb*** | 10 ppb |
| **sample *12*** | ***13.5 ppb*** | 10 ppb |
| **sample 13** | <2 ppb | 10 ppb |
| **sample 14** | <2 ppb | 10 ppb |
| **sample 15** | <2 ppb | 10 ppb |
| **sample 16** | <2 ppb | 10 ppb |
| **sample 17** | <2 ppb | 10 ppb |
| **sample 18** | <2 ppb | 10 ppb |
| **sample 19** | <2 ppb | 10 ppb |
| **sample 20** | <2 ppb | 10 ppb |

According to the legislation in force (REGULATION (EC) No 1881/2006), the maximum permitted level for mycotoxins in maize and rice to be sorted or otherwise physically treated before human consumption or use as an ingredient in foodstuffs is 10 µg/kg (ppb).

Out of 20 samples performed, four samples (Sample 1, Sample 2, Sample 11, Sample 12) were within the kit detection limit 2-75 µg/kg, i.e. Sample 1 with 4 ppb, Sample 2 with 16.5 ppb, Sample 11 with 3.4 ppb and Sample 12 with 13.5 ppb. Of these, two samples (Sample 2 and Sample 12) were above the maximum limit for mycotoxin levels in maize according to Regulation (EC) No 1881/2006, representing 10% of all samples analyzed (Figure 1). The remaining samples were below the minimum detection limit of 2 µg/kg (ppb).

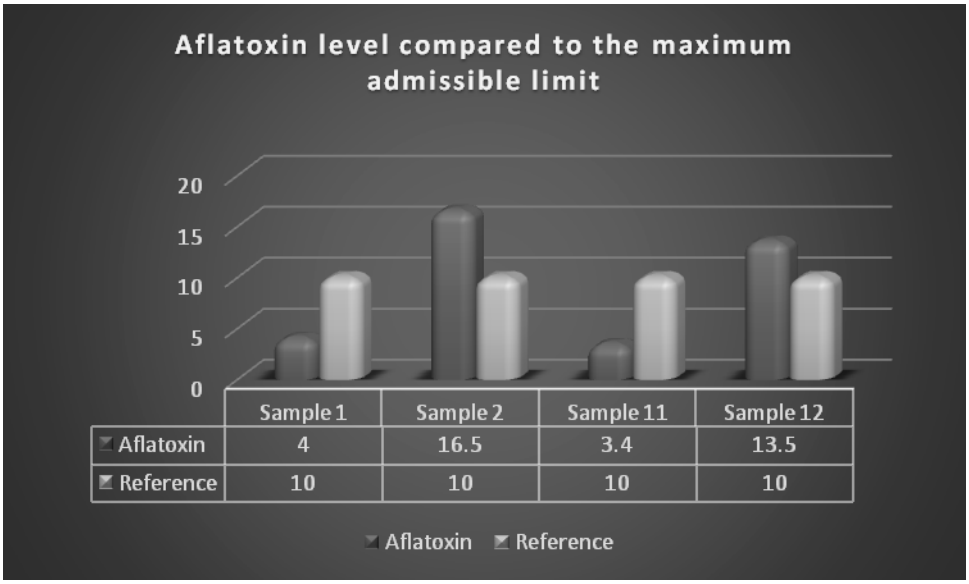


Fig. 1. *Aflatoxin level compared to the maximum admissible limit*

Although the incidence of aflatoxin occurrence in maize is high, according to the literature (No: 2017-1-RO01-KA202-037215 carried out under the Erasmus+ programme), the aflatoxin levels in the samples collected were harmless to animal health.

Considering that the **International Agency for Research on Cancer** has classified aflatoxins (B1, B2, G1, G2 and M1) as first degree carcinogens with increased risk of hepatocellular carcinoma, the importance of their determination is increased. Another aspect to be taken into account on contamination with aflatoxin-producing fungi (*Aspergillus flavus*, *Aspergillus parasiticus*) is their occurrence in the storage. Furthermore, if

plants are damaged by insects, weather conditions or mechanical farming practices, they become susceptible to infection. Critical factors for aflatoxin production for both *Aspergillus flavus* and *Aspergillus parasiticus* in storage are temperature and humidity. If beans are stored at temperatures above 20°C and humidity above 14% or if they are not properly dried beforehand, the risk of mould contamination is increased. Another factor influencing fungal growth and aflatoxin contamination is the presence of insects. Due to their metabolic process, insects can raise the temperature of cereals up to 57°C (Małgorzata Piotrowska and collab, 2013).

Twenty RIDA QUICK DON RQS ECO tests were carried out for the determination of deoxynivalenol in wheat. The detection range was between 0.25-7.5 mg/kg (ppm) (Table 2).

**Table 2. Results of analyzed samples and reference level for deoxynivalenol**

| No. sample | Results (mg/kg; ppm) | Reference (mg/kg; ppm) |
|---|---|---|
| **sample 1** | <0.25 ppm | 1.75 ppm |
| **sample 2** | <0.25 ppm | 1.75 ppm |
| **sample 3** | <0.25 ppm | 1.75 ppm |
| **sample 4** | <0.25 ppm | 1.75 ppm |
| **sample 5** | <0.25 ppm | 1.75 ppm |
| **sample 6** | <0.25 ppm | 1.75 ppm |
| **sample 7** | <0.25 ppm | 1.75 ppm |
| **sample 8** | <0.25 ppm | 1.75 ppm |
| **sample 9** | <0.25 ppm | 1.75 ppm |
| **sample 10** | <0.25 ppm | 1.75 ppm |
| **sample 11** | <0.25 ppm | 1.75 ppm |
| **sample 12** | <0.25 ppm | 1.75 ppm |
| **sample 13** | <0.25 ppm | 1.75 ppm |
| **sample 14** | <0.25 ppm | 1.75 ppm |
| **sample 15** | <0.25 ppm | 1.75 ppm |
| **sample 16** | <0.25 ppm | 1.75 ppm |
| **sample 17** | <0.25 ppm | 1.75 ppm |
| **sample 18** | <0.25 ppm | 1.75 ppm |
| **sample 19** | <0.25 ppm | 1.75 ppm |
| **sample 20** | <0.25 ppm | 1.75 ppm |

According to the legislation in force, the maximum permitted level for durum wheat and unprocessed oats is 1750 µg/kg or 1.75 mg/kg (ppm).

Out of 20 samples taken, all samples were below the minimum detection limit of 0.25 mg/kg (ppm) (Figure 2).

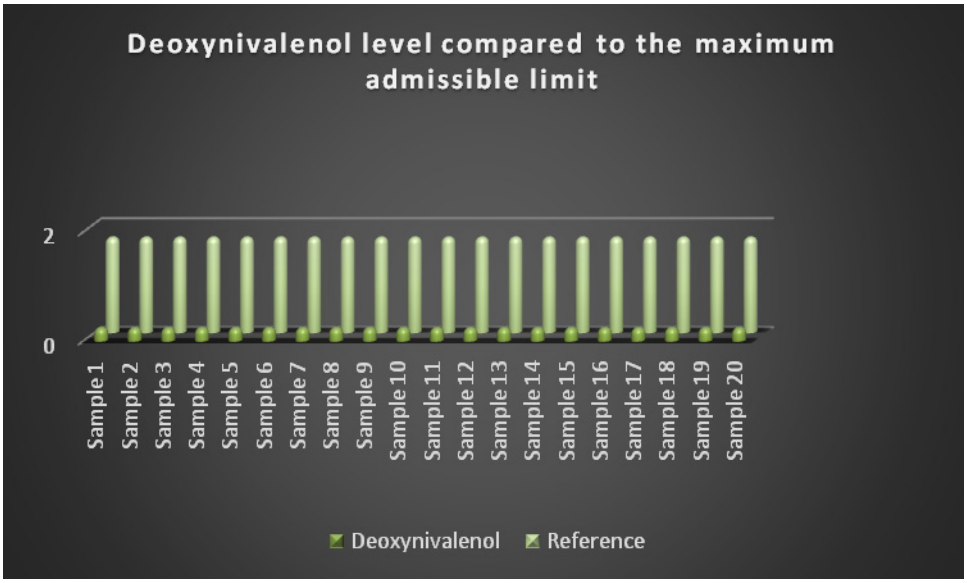


Fig. 2. *Deoxynivalenol level compared to the maximum admissible limit*

Although the incidence of deoxynivalenol occurrence in wheat is high, according to the literature (No: 2017-1-RO01-KA202-037215 conducted under the Erasmus+ programme), in the samples collected the level of deoxynivalenol was below the detection limit of the RIDA QUICK DON RQS ECO tests.

As deoxynivalenol is the most common of all trichothecenes with a predisposition in wheat and can cause adverse effects in animals such as nausea, vomiting, diarrhoea or weight loss, the International Agency for Research on Cancer has placed deoxynivalenol in group 3 carcinogens. Because of these effects, deoxynivalenol is also called vomitoxin. Deoxynivalenol is mainly produced by *Fusarium graminearum* and *Fusarium culmorum*, and they occur in wet, low-temperature conditions causing fusarium head blight in wheat. Another aspect to consider on contamination with deoxynivalenol-producing fungi (*Aspergillus flavus*, *Aspergillus parasiticus*) is their occurrence in storage. Moreover, if the humidity percentage in the warehouse is increased (>14%) there is a risk of increased carbon dioxide production, favouring the respiration and growth of moulds. Another factor influencing fungal growth and deoxynivalenol contamination is the presence of insects, which cause damage to the wheat grain and thus favour grain contamination.

The preventive actions used by the farmer on the crop are considered a possible cause of the results obtained from the 20 wheat samples (100% samples below detection limit) and 20 maize samples (90% samples below

detection limit) in this study, by using the latest products in the range of herbicides, fungicides and insecticides (Adengo 465 SC, Atlantis Flex, CREW ACE 4 SC, Pallas 75 WG, Orius 2 WS, Revystar Flex, Elatus Era, Falcon Pro, Aviator XPro 225 EC, Vantex 60 CS, Lamdex 5 EC).

The results demonstrate the importance of rigorous adherence to good agricultural practices, both in the field and during storage. According to the literature (No: 2017-1-RO01-KA202-037215 carried out under the Erasmus+ programme), the risks of contamination are in the field, during storage and during transport. In the field, these risks are mitigated by treatments applied to soil and planting material. During storage, maintaining optimal storage conditions such as temperature, humidity, aeration and pest control have a major impact in preventing mould growth. (Lindy J. Rose et al, 2018)

Because there is a lack of attention paid to products from a mycotoxicological point of view, and because livestock owners in the household system are not aware of the possible negative effects that feed may present, it is considered good agricultural practice for all farmers to perform annual self-monitoring tests. A good way, which is less expensive and does not require complex equipment, is the lateral flow device technique, i.e. RIDA QUICK kits from R-Biopharm AG. These give valid results in a short time and can be carried out directly on the farm.

***Conclusions***

The present work was carried out to assess the mycotoxin composition of aflatoxin (B1, B2, G1, G2) in maize and deoxynivalenol in wheat and to assess the risk of fungal contamination of cereals under storage and transport conditions.

The results obtained from mycotoxicological screening of aflatoxin in maize were 90% below the detection limit of the RIDA QUICK Aflatoxin RQS test (2 µg/kg), 5% were within the detection limit of the RIDA QUICK Aflatoxin RQS test (2 µg/kg) and below the maximum limit allowed by the legislation in force (10 µg/kg) and 5% were within the detection limit of the RIDA QUICK Aflatoxin RQS test but above the maximum limit allowed by the legislation in force (10 µg/kg).

The results obtained from mycotoxicological screening of deoxynivalenol in wheat were below the detection limit of the RIDA QUICK DON RQS ECO test (0.25 mg/kg) and below the maximum limit allowed by the legislation in force (1.75 mg/kg).

The risk of contamination is present throughout the technological flow, but prevention of product contamination is the most effective way to combat the occurrence of mycotoxin diseases in both humans and animals.

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