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Radiological study of lung tissue in rats with bleomycin-induced fibrosis

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Abstract. The increasing incidence of pulmonary fibrosis in animals caused by toxic exposure necessitates a detailed analysis of radiological changes in lung tissue. This is essential for assessing the progression of fibrosis and developing effective therapeutic approaches. In this context, the study aimed to utilise radiological methods to identify time-dependent changes in the lungs of rats subjected to a model of bleomycin-induced pulmonary fibrosis, contributing to the improvement of diagnostic techniques for this pathological condition. Pulmonary fibrosis was experimentally induced in laboratory rats by administering bleomycin. Radiological examinations were conducted

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on days 7, 14, 30, and 45 to monitor the condition of lung tissue and detect progressive changes. Particular attention was given to identifying characteristic radiological signs of fibrosis, such as lung tissue consolidation and reduced transparency. The findings revealed a progressive nature of interstitial lesions. It was established that as early as seven days after bleomycin administration, signs of inflammation appeared in the lungs, accompanied by reduced transparency of the pulmonary fields. By day 14, initial signs of fibrosis were observed, marked by increased lung tissue density and the presence of infiltrates. By day 30 of pathology progression, significant fibrosis development was evident, characterised by more pronounced linear inclusions and reduced transparency. By day 45, scar tissue formation occurred, leading to substantial structural deterioration of the lungs. The findings highlighted the importance of radiological examination as an informative method for diagnosing and monitoring the progression of pulmonary fibrosis. The radiological changes observed at different stages of fibrosis development enable a more detailed characterisation of the disease pathogenesis, which is crucial for evaluating the effectiveness of therapeutic interventions. Accordingly, the study results could be utilised to establish new diagnostic criteria and enhance the monitoring of patients with pulmonary fibrosis

Keywords: pulmonary fibrosis; radiographic imaging; interstitial lung disease; experimental model; laboratory rats; diagnosis

Introduction

Pulmonary fibrosis is a pathological condition characterised by the progressive replacement of normal lung tissue with fibrous tissue. As a result, pulmonary fibrosis leads to a decrease in lung elasticity, impaired gas exchange, and ultimately, respiratory failure. The global literature has increasingly focused on the diagnosis of pulmonary fibrosis using various imaging techniques, including radiography, computed tomography, and ultrasound. Radiographic examination is particularly noted for its effectiveness, allowing for the diagnosis of the development of this pathological process at the early stages of the disease, which is relevant to veterinary medicine. B. Ayilya *et al.* (2023) indicated that the use of bleomycin to induce pulmonary fibrosis in animal models is one of the most common methods for studying the pathophysiology of the disease, as well as for evaluating the effectiveness of various therapeutic approaches.

A. Abu Qubo *et al.* (2022) and E. Ortiz-Zapater *et al.* (2022) investigated the possibility of using radiography to monitor changes in lung tissue in cases of progressive fibrosis, which is especially important for detecting early changes that have not yet affected clinical manifestations. According to recent research by T. Hoffman *et al.* (2022), radiography allows for the highly accurate detection of diffuse changes in lung tissue, particularly in experimental models of pulmonary fibrosis in rodents and dogs, where fibrosis is induced using bleomycin. Furthermore, radiographic signs, such as decreased lung field transparency, the appearance of reticular changes, and subpleural densities, allow the correlation of the degree of lung tissue damage with the patient's clinical condition, as suggested by S. Scharm *et al.* (2022).

The use of radiography in the diagnosis of pulmonary fibrosis in veterinary medicine significantly expands the possibilities for detecting and monitoring the progression of the disease. T. Hoffman *et al.* (2022) indicated that particular attention should be paid to early radiographic manifestations that precede pronounced fibrosis. In this context, S. Stanel & P. Rivera-Ortega (2023) demonstrated the importance of systematic radiographic monitoring for assessing the dynamics of changes in lung tissue. Thus, the analysis of radiographic images becomes a necessary tool not only in diagnosis but also in evaluating the effectiveness of therapeutic interventions.

T. Hoffman *et al.* (2022) demonstrated that pulmonary fibrosis is a complex disease often associated with numerous diagnostic challenges, significantly impacting the timely recognition and treatment of this pathology. The average interval from the onset of the first clinical manifestations to the establishment of a definitive diagnosis is approximately 24 months, highlighting the need for more effective diagnostic methods. The authors noted that chest radiography can reveal interstitial abnormalities in over 50% of patients with idiopathic pulmonary fibrosis, even before the onset of significant clinical symptoms, indicating the potential for early diagnosis. Although the time required to establish a diagnosis does not directly impact patient survival, early detection of pulmonary fibrosis facilitates timely treatment, which can potentially improve clinical outcomes. This was emphasised by T. Hoffman *et al.* (2022), who indicated the need to improve existing approaches to early diagnosis and apply a comprehensive approach to assessing the patient's condition, including both radiography and other imaging modalities, such as high-resolution computed tomography.

This study aimed to evaluate radiographic changes in the lung tissue of animals with bleomycin-induced pulmonary fibrosis, as well as to determine the possibilities of its early diagnosis and monitoring of disease progression. The research objectives included: analysis of radiographic changes in the lungs at different stages of fibrosis; comparison of radiographic data with clinical manifestations; and evaluation of the effectiveness of radiography as a method for early diagnosis of pulmonary fibrosis in animals.

Literature Review

According to F. Cony *et al.* (2019), although radiography has limited ability to determine the specific aetiology of a disease, it remains an indispensable method for assessing the dynamics of a pathological process. In particular, radiography helps monitor the progression of fibrosis and allows the detection of potential acute complications such as infiltrates or pleural effusions, which are of significant clinical importance for adjusting treatment. For this reason, regular radiographic examination is recommended for long-term monitoring of patients with pulmonary fibrosis. In their study, the scientists noted that characteristic radiographic changes in pulmonary fibrosis include the presence of diffuse ground-glass opacity, which is predominantly localised in the region of the cardiac shadow and the basal segments of the lungs. Compensatory emphysema, which predominates in the peripheral and apical zones of the lungs, is also often observed, which can further complicate differential diagnosis. Given these changes, analysis of radiographs requires a high level of expertise, as the timely detection of structural changes in lung tissue is important for preventing complications and disease progression.

Pulmonary fibrosis in dogs is a progressive pathological condition characterised by the gradual development of dyspnoea, decreased exercise tolerance, and the presence of characteristic inspiratory crackles, as reported by H. Mattoo & S. Pillai (2021). These clinical manifestations are a result of respiratory system damage and represent important diagnostic criteria for this disease, as indicated by A. Ferrazza & P. Baldassarri (2020). Radiographic examination revealed diffuse changes in lung structure, specifically an interstitial pattern accompanied by variable bronchial changes, which are characteristic of this pathological condition.

A significant breakthrough in the diagnosis of pulmonary fibrosis in dogs is associated with the introduction of computed tomography (CT). This method, as emphasised by scientists F. Thierry *et al.* (2017), allows for accurate determination of the stages of disease development and assessment of the degree of lung tissue damage. Computed tomography provides visualisation of several changes indicative of the severity of the disease. Characteristic findings that reflected the altered state of lung tissue included areas of "ground-glass opacity" and reticular-nodular patterns. Moreover, there is a positive correlation between the degree of visualised changes on CT and the clinical manifestations of fibrosis. In particular, researchers have stated that more severe forms of damage have a negative impact on animal lifespan.

A. Ferrazza & P. Baldassarri (2020) conducted histopathological analyses, which allowed for a more detailed examination of morphological changes in pulmonary fibrosis, including thickening of the alveolar septa, accompanied by interstitial fibrosis and hyperplasia of pneumocytes. Although fibrosis can develop in dogs of various breeds, H. Laurila & M. Rajamäki (2020) noted the greatest susceptibility to this disease in West Highland White Terriers.

In cats, pulmonary fibrosis manifests with radiographic features including a mixed bronchiolointerstitial and alveolar pattern, the presence of pleural effusion, and the formation of various pulmonary nodules. As reported by F. Cony *et al.* (2019), radiographic changes are often characterised by increased lung tissue density, which progresses from bronchiolointerstitial to alveolar structures. The scientists proved that dense areas or diffuse interstitial infiltrates of the bronchiolar and alveolar type are common features of this disease. Histopathological studies conducted by the authors of the aforementioned research established that pulmonary fibrosis in cats is characterised by multifocal fibrosis, hyperplasia of type II pneumocytes, and hypertrophy or hyperplasia of smooth muscles. Fibrosis was most often observed in middle-aged and older cats, accompanied by symptoms of respiratory distress and cough. The difficulty in diagnosing fibrosis in cats is due to the fact that radiographic manifestations can be similar to asthma, pneumonia, or even neoplasia. As the authors noted, the prognosis for cats with pulmonary fibrosis is poor, as even with active treatment, most animals die within a few days or months.

X. Wang (1992) studied pulmonary fibrosis induced by bleomycin administration in rats. W. Mohr (1988) demonstrated that the initial pathological changes were characterised by the detachment of type I pneumocytes, exposure of the basement membrane, and endothelial swelling, which are likely caused by the effects of reactive oxygen species. I. Persson *et al.* (2020) found that the progression of fibrosis can be tracked using various imaging methods such as magnetic resonance imaging (MRI) and CT, revealing an increase in the volume of affected tissue and a decrease in lung volume. According to W. Mohr (1988), in cattle, this disease can manifest as diffuse fibrosing alveolitis with clinical signs such as a dry cough, tachycardia, and tachypnoea. The researcher noted that the aetiology of fibrosis in cattle remains unknown and may be associated with the ingestion of toxic substances. Confirmation of the diagnosis is usually performed post-mortem, through histological examination, which reveals fibrosis of the alveolar septa and cellular infiltrates.

C. Easton-Jones *et al.* (2020) demonstrated through radiographic examination that in horses, pulmonary fibrosis manifests as interstitial, bronchiolointerstitial, and nodular changes in the lungs. The scientists observed primary clinical symptoms such as weight loss, decreased exercise tolerance, and respiratory distress. C. Easton-Jones *et al.* (2020) and A. Craven *et al.* (2024) linked multinodular pulmonary fibrosis in horses to equine herpesvirus type 5, which carries an unfavourable prognosis. The scientists found that imaging modalities can identify nodular interstitial lesions and superficial changes in the lungs of horses. One of the prognostic indicators for fibrosis in horses is the respiratory rate, the ratio of lymphocytes to neutrophils in bronchoalveolar lavage, and the level of lymphocytes in the blood.

Materials and Methods

The experimental study was conducted during 2022-2023 in the educational and research laboratory "Centre of Cell Technologies in Veterinary Medicine", operating under the Department of Veterinary Surgery named after Academician I.O. Povazhenko at the National University of Life and Environmental Sciences of Ukraine (NULES), Kyiv, Ukraine. The trials were performed on female *Wistar* rats, aged four months, with an average weight of 269.6 ± 1.80 g. The housing conditions of the experimental animals complied with the provisions of Directive 2010/63/EU of the European Parliament and the Council (2010) and the Law of Ukraine No. 3447-IV "On Protection of Animals from Cruel Treatment" (2006). The rats were kept in a vivarium with a stable 12-hour light-dark cycle (12 hours of light and 12 hours of darkness), a temperature range of 20-23°C, and unrestricted access to water and standard feed. The study was approved by the NULES local bioethics committee (Protocol No. 31-1, dated 27 October 2020).

An experimental model of pulmonary fibrosis in rats was developed through a single administration of bleomycin hydrochloride solution directly into the lung tissue. The dose was 1.0 mg of the drug per 100 g of body weight, delivered in a 0.3 mL volume of saline. Administration was performed via transthoracic instillation, ensuring direct contact of the drug with lung tissue (Ayilya *et al.,* 2023). Observations of fibrosis progression were conducted for 45 days following the administration, focusing on clinical signs of the disease and morphofunctional changes in lung tissues. To objectively assess the pathological process, radiological examinations of the thoracic organs were performed on days 7, 14, 30, and 45 of the experiment, enabling the monitoring of dynamic changes.

Radiological assessments were conducted using the VATEL-1 digital veterinary system (Ukraine) following the manufacturer's recommendations. Images were taken in right lateral and direct dorsoventral projections, which required sedation of the test animals. For this purpose, Medison 0.1% (BrovaPharma, Ukraine) was administered intramuscularly at a dose of 0.25 mg/kg (Plumb, 2008). These selected projections visualised changes located in any region of the lung tissue, including areas that were difficult to access or entirely non-visualised. The use of these two projections was justified by the specific localisation of the pathological process under investigation.

The selection of right lateral and dorsoventral projections was based on the need to visualise structural changes in different regions of the lung tissue. This combination of projections enabled the acquisition of more accurate data on morphological changes, particularly in cases where the pathological process was locally confined and either difficult to access or entirely invisible when only a single projection was used. Consequently, the integration of two orthogonal views provided comprehensive information about the structural condition of the lung tissue and facilitated a more precise assessment of pathological changes, which is critical in the study of pulmonary fibrosis.

Results and Discussion

Bleomycin is one of the drugs that induces fibrotic changes in lung tissue, and its use in experimental models contributes to a deeper understanding of the mechanisms of developing this disease and assessing potential treatment methods. A crucial stage of the study involved conducting a radiographic analysis, which allowed for the evaluation of structural changes in the lungs at various stages of fibrosis progression. In clinically healthy animals, lung tissue had a homogeneous structure without noticeable shadows or inclusions, indicating its normal physiological state. The lungs were characterised by a dark colouration, indicating their air-filled state. The heart was located in the central part of the thoracic cavity, which is typical for this species of animal (Fig. 1).

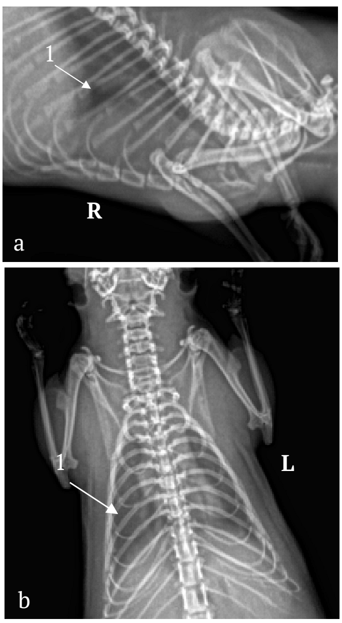

Figure 1. Typical radiograph of a clinically healthy rat

Note: *radiograph of the lungs in the right lateral (a) and dorsoventral (b) projections: 1 – lungs*

Source: *authors' photo*

These results align with the data presented in the studies of F. Gassert *et al.* (2021), who indicated that in healthy animals, lung tissue has a high level of radiographic transparency due to the absence of fibrotic or infiltrative changes. The researchers demonstrated that the homogeneity of the lung parenchyma structure is a key marker of a healthy organ state. Moreover, they noted that the dark colouration of the lung fields on a radiograph reflects a high signal intensity, air filling, and is normal for healthy animals. E. Doğan *et al.* (2022) also established that the localisation of the heart in the central part of the thoracic cavity in small laboratory animals indicates the absence of pathological displacement of organs, which could be due to a mass, effusion, or other pathologies.

The absence of pathological changes in the lungs, such as shadows, inclusions, or areas of increased density, allows the obtained data to be used as a control group for comparison with animals in which pulmonary fibrosis has been modelled. This underscores the importance of baseline radiographic assessment for the diagnosis and monitoring of changes in the lungs. For example, F. Gassert *et al.* (2021) noted that establishing the baseline transparency of the lung fields in healthy animals is critical for assessing the progression of pathological changes.

Since radiographic evaluation of lung tissue in clinically healthy animals justifies the absence of fibrotic or inflammatory changes, it forms the basis for further comparative assessments of pathological states. To deepen the understanding of pathological progression in lung tissue, a comparative radiographic evaluation of the lungs in experimental animals was performed and changes in the lung tissue of rats were revealed. On the 7th day after the administration of bleomycin to the animals, early signs of lung tissue damage were observed. The lung fields partially lost their transparency, and the first signs of spotted areas of increased density appeared, indicating the initial stage of tissue infiltration (Fig. 2). The echogenicity of the tissues increased compared to the control group, indicating initial inflammatory changes.

Figure 2. Typical radiograph of the lungs in a test rat on the 7th day of bleomycin-induced pulmonary fibrosis modelling

Note: *radiograph of the lungs in the right lateral (a) and dorsoventral (b) projections: 1 – increased tissue density*

Source: *authors' photo*

Additionally, a small amount of pleural effusion was detected in the pleural cavity, which did not reach clinically significant volumes but served as confirmation of the onset of an inflammatory process. P. Kolb *et al.* (2020) demonstrated that in the early stages of bleomycin-induced fibrosis, an inflammatory process occurs. This is confirmed by the present study, namely the moderate accumulation of fluid in the pleural cavity as early as 7 days of observation. According to the research of P. Kolb *et al.* (2020), early signs of tissue infiltration reflect the initial activation of the immune response, which stimulates the development of fibrosis. I. Persson *et al.* (2020) confirmed that in the early stages of bleomycin-induced damage, moderately dense areas form in the lungs, corresponding to interstitial oedema and initial inflammatory processes. These changes are clearly reflected in the form of increased density and decreased transparency of the fields, as recorded in this study.

Although at this stage the structure of the lungs still retains its basic architecture, according to C. Malinczak *et al.* (2024), such early changes can lead to a decrease in the diffusion capacity of the alveoli, which is a precursor of later respiratory function impairments. The observations made in this study confirm this trend, as early structural changes were accompanied by a gradual change in field density, which may affect gas exchange in the future. By day 14 post-induction of bleomycin-induced pulmonary fibrosis in the experimental rats, significant changes were recorded, reflecting the onset of the pathological process (Fig. 3).

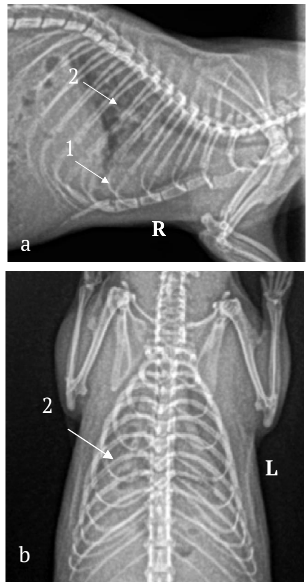

Figure 3. Typical radiograph of the lungs in an experimental rat on the 14th day of bleomycin-induced pulmonary fibrosis modelling

Note: *radiograph of the lungs in the right lateral (a) and dorsoventral (b) projections: 1 – free fluid; 2 – increased tissue density*

Source: *authors' photo*

The mottled areas of increased density detected on the radiograph (Fig. 3) can be associated with tissue oedema, inflammatory infiltration, and early fibrosis. The established pathological process was confirmed by the detected light areas in the parenchyma of the lung tissue, indicating increased echogenicity caused by the accumulation of inflammatory cells, exudate proteins, and altered extracellular matrix. F. Ruscitti *et al*. (2020) indicated that in the early stages of bleomycin-induced fibrosis in rodents, changes that can be detected radiographically as foci of increased density predominate. These areas correspond to localised processes of inflammation and oedema, which form the basis for the subsequent formation of fibrous tissue. Similar results were obtained by G. Soldati *et al.* (2020), who noted that areas with increased echogenicity on ultrasound or decreased transparency on radiographs can be used as early markers of pathology in modelling interstitial lung diseases.

The radiographic changes observed in this study were further confirmed by a post-mortem examination. The presence of effusion in the caudal regions of the pleural cavity is consistent with the observations of Y. Xiong *et al.* (2021), who reported that in bleomycin-induced fibrosis, an inflammatory process is activated in the early stages with exudation of fluid into the pleural cavity. This is a result of damage to the vascular endothelium and increased permeability caused by inflammatory cytokines such as IL-6 and TNF-α. The importance of radiographic studies in the early detection of pathological changes was emphasised by S. Scharm *et al.* (2022), who noted that even with minimal clinical manifestations, radiographic imaging can reveal the first signs of the disease. In particular, the authors indicated that mottled areas of increased density reduce the transparency of the lung fields, which is a marker for monitoring disease progression. The changes found in this study on day 14 of the experiment were consistent with the conclusions of S. Scharm *et al.* (2022) regarding the sensitivity of the method. In addition to radiographic changes, the presence of inflammation in the pleural cavity reflects the systemic response of the organism to the administration of bleomycin. This corresponds to the studies of H. Mattoo & S. Pillai (2021), who suggested that even with localised lesions of the lung tissue, a systemic inflammatory response plays an important role in the pathogenesis of fibrosis. At the same time, A. Hjerpe *et al.* (2020) additionally indicated that the early presence of effusion can be a sign not only of inflammation but also of the initial stages of pleural remodelling.

By day 30 post-induction of bleomycin-induced pulmonary fibrosis, further progression of pathological changes in the rat lungs was observed. The mottled areas of increased density became more pronounced and widespread. The first diffuse linear inclusions appeared, indicating the beginning of structural remodelling of the lung parenchyma (Fig. 4).

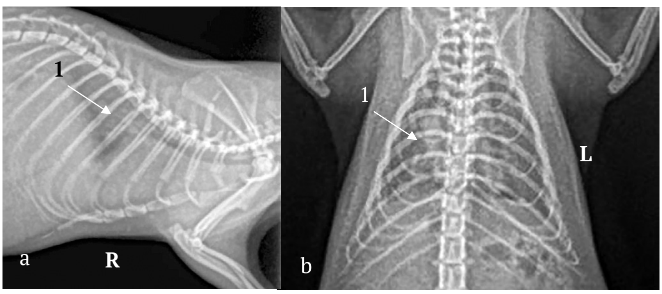

Figure 4. Typical radiograph of the lungs in an experimental rat on the 30th day of bleomycin-induced pulmonary fibrosis modelling

Note: *radiograph of the lungs in the right lateral (a) and dorsoventral (b) projections: 1 – increased tissue density*
Source: *authors' photo*

The lungs lost some of their transparency, indicating the presence of moderate fibrosis. Y. Ishida *et al.* (2023) demonstrated that 30 days after modelling bleomycin-induced fibrosis, signs of tissue remodelling were observed, manifested as linear inclusions, with the formation of fibrous septa between areas of alveolar destruction, which clearly coincides with the results of this study. N. Tanabe *et al.* (2020) noted that the progression of fibrosis is accompanied by an increase in tissue density and a decrease in lung volume, which corresponds to the changes recorded in this study on day 30. They emphasised that lesions of this nature may be accompanied by a partial decrease in lung elasticity, which begins to manifest clinically.

The results obtained on day 30 allowed the identification of the intermediate stage of fibrosis, characterised by structural remodelling of the lung tissue. The linear inclusions that appeared at this stage indicate the beginning of active fibrotic reconstruction of the parenchyma. These data are consistent with the results of S. Libório-Ramos *et al.* (2023), who indicated that such structural changes are important markers of progressive fibrosis.

By day 45 post-induction of bleomycin-induced pulmonary fibrosis in rats, a significant progression of pathological changes in the lung tissue was observed. The lungs acquired a matte appearance, reflecting a significant decrease in transparency, typical of developed fibrosis. In addition, the number of diffuse areas of increased density increased, taking on the appearance of elongated (linear) inclusions. Such structural changes indicate the remodelling of the parenchyma and the development of scar formations (Fig. 5).

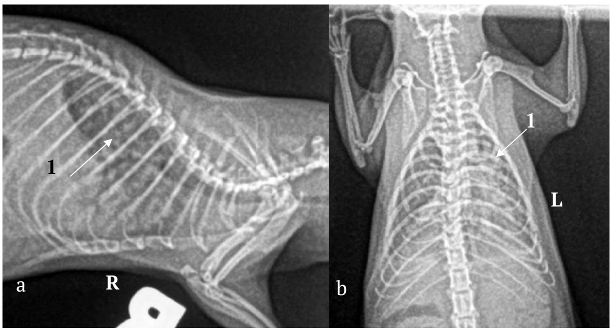

Figure 5. Typical radiograph of the lungs in an experimental rat on the 45th day of bleomycin-induced pulmonary fibrosis modelling

Note: *radiograph of the lungs in the right lateral (a) and dorsoventral (b) projections: 1 – increased tissue density*

Source: *authors' photo*

According to the research by Y. Ishida *et al.* (2023), linear inclusions in the late stages of bleomycin-induced fibrosis correspond to the formation of organised fibrous structures that replace normal lung parenchyma. Their study indicates that these changes are the result of the activation of myofibroblasts and excessive collagen production, which is intensified in the late stages of the disease. The results obtained in the conducted research confirm this pathogenetic mechanism, as a similar picture was observed in the lung tissue on day 45 of the experiment. K. Lee *et al.* (2023) indicated that the matte appearance of the lungs in combination with linear inclusions on radiographs is the main radiographic sign of developed fibrosis.

They emphasised that these changes are associated with a disruption of the alveolar structure, leading to a decrease in gas exchange and the formation of stable scar tissue.

The morphological changes in the lungs, recorded in this study, are also reflected in the functional state of the lungs. A. He *et al.* (2024) noted that in the late stages of fibrosis modelling in animals, a significant decrease in lung volume and a deterioration of respiratory function are observed. According to their data, similar structural changes, including linear inclusions, are accompanied by a decrease in lung elasticity and progressive restriction of ventilation.

The radiographic changes identified in this study were confirmed by post-mortem examination with subsequent histological examination and described in a previously published article (Mazurkevych & Surtaieva, 2023). The lung tissue at 45 days had a dense, inelastic structure, corresponding to the late stages of fibrosis. The established pathological signs correlate with the data of D. Zakaria *et al.* (2020), who reported similar pathological features, such as consolidation of lung tissue and an increase in collagen fibres in rats with bleomycin-induced fibrosis.

Research into bleomycin-induced pulmonary fibrosis is a crucial area in veterinary and medical science. This disease has a complex pathogenesis, involving inflammatory and fibrotic changes in the lungs that can severely impair their function. The results obtained in this study confirm some previous findings and also reveal new aspects that allow for a better understanding of the mechanisms underlying this pathological process. According to the study of A. Vats & P. Chaturvedi (2023), bleomycin causes progressive inflammatory and fibrotic changes in the lungs, which coincides with the findings obtained in this research regarding the consolidation of lung tissue. This confirms the universality of this model for studying pulmonary fibrosis and its potential for developing therapeutic strategies. However, according to the study of S. Keshavan *et al.* (2023), the dynamics of fibrosis development can vary significantly depending on the species of laboratory animal. The results of this experiment, obtained in rats, showed a more pronounced reaction to bleomycin, with the formation of dense infiltrates and a decrease in the transparency of the lung fields, which differs from studies on mice, where a less intense reaction was observed.

The methodology of different studies is also a crucial factor. For example, Y. Zhang *et al.* (2023) focused primarily on histological methods, allowing for a detailed study of microscopic changes in lung tissue. This study focused on radiographic methods, which allow for non-invasive assessment of the lung tissue. While this approach has its advantages, a combination of radiographic examination and histology could provide a more complete picture of the changes in the lungs. G. Courtoy *et al.* (2020) applied a quantitative assessment of fibrotic changes, which allowed for a more accurate assessment of the severity of the pathological process. In this study, a qualitative assessment of changes was used, which allowed for a reflection of the disease dynamics, although quantitative methods could have provided more detailed information about changes in the lung tissue.

The duration of the experiment is also an important factor. The results of the 45-day study allowed for the evaluation of both acute and chronic changes in the lungs. The study by I. Savin *et al.* (2022), which focused mainly on the early stages of fibrosis development, helped to understand the mechanisms of the transition from inflammation to fibrosis but did not provide a complete picture of the processes occurring at later stages.

Thanks to the extended observation period, the progression of fibrosis was detected, and the importance of early diagnosis was also evaluated. In this study, radiological methods allowed for the detection of changes in the lungs at various stages of the disease, enabling early identification of the pathology. This is also confirmed by the research of D. Li *et al*. (2022), in which radiography was used as a method for the early diagnosis of pulmonary fibrosis. At the same time, Y. Ishida *et al.* (2023) proved the effectiveness of radiographic examination for monitoring changes in the lungs, in particular, a decrease in transparency and the formation of dense areas on radiographs. This study also confirmed the effectiveness of radiographic examination for assessing the progression of fibrosis. In particular, P. Kolb *et al.* (2020) noted that radiographic methods are very effective for detecting changes in the early stages of fibrosis. Using these methods, it is possible to quickly assess the overall condition of the lungs without resorting to invasive methods such as biopsy. However, it is important to note that radiographic methods cannot always detect all microscopic changes in lung tissue, which is one of the limitations of this approach. D. Zakaria *et al.* (2020) emphasised the importance of combining radiographic and histological methods to obtain more detailed information about pathological changes in tissues. This allowed for a complete picture of the mechanisms of fibrosis development, which is important for developing effective therapeutic strategies.

Another important aspect is the difference in approaches to assessing the severity of fibrosis. In this study, a visual assessment of changes was used, which allowed for a quick assessment of the overall condition of the lungs. However, as shown in studies by F. Ruscitti *et al.* (2020), the use of specialised scales for the quantitative assessment of fibrosis allows for a more accurate measurement of the degree of severity of changes in the lungs. The practical aspect of the study also deserves attention. For example, N. Gupta *et al.* (2024) focused on the study of new therapeutic agents, while the presented research is aimed at improving diagnostic methods. It has been shown that the radiographic method is effective for the early detection of changes in lung tissue, which may be particularly important in veterinary practice.

Overall, the findings of this study confirm the significance of the bleomycin-induced model for studying pulmonary fibrosis, as it allows for the accurate reproduction of the fibrosis development process in the lungs. At the same time, to obtain the most accurate and complete information, it is necessary to combine different research methods, including radiography and histological examination. Moreover, for further research, it is important to standardise the experimental conditions, which will allow for obtaining reliable results in comparison with those already obtained.

Conclusions

Radiographic examination is a leading instrumental method in veterinary medicine, the application of which allows for obtaining high-quality images of rat lung tissue. Therefore, when using radiographic examination to study changes in the lung tissue of rats with bleomycin-induced fibrosis, important conclusions were obtained that significantly expanded the understanding of the development and progression of the studied pathological process. It was established that in clinically healthy rats, the lung tissue appeared homogeneous, indicating a normal physiological state of the organism without signs of pathology. Radiographically, the lungs had a high level of transparency, confirming the absence of any pathological changes. On the 7^{th} day following bleomycin administration, the appearance of initial signs of inflammation was noted, which was especially characterised by an increase in the density of lung tissue and a moderate accumulation of fluid in the pleural cavity. By the 14th day after bleomycin administration, a noticeable increase in the density of lung tissue was established, along with the presence of pleural effusion. Furthermore, by the 30th day, the progression of the pathological process was definitively established: the detection of linear inclusions and a decrease in the transparency of lung tissue, which served as a clear sign of the beginning of structural remodelling, which is a characteristic feature of the intermediate stage of fibrosis. By the 45th day, pulmonary fibrosis had acquired more pronounced features: the lung tissue appeared dull, a typical feature of the late stages of the disease, and was specifically characterised by the formation of fibrotic scar tissue and structural trabeculae, indicating the development of persistent fibrotic changes. Per the results obtained, the study confirmed the progression of fibrosis as a direct consequence of the administration of bleomycin into the pleural cavity of rats, which allowed the outlining of the various stages of pathological changes that occurred in the lung tissue. The results obtained from the radiographic examination are also important for monitoring the progression of lung disease and the ability to choose a therapeutic strategy.

Promising directions for further research include the development of new diagnostic approaches aimed at the early detection of pulmonary fibrosis, as well as the study of potential therapeutic agents designed for the effective treatment of the studied pathological condition.

Acknowledgements

None.

Conflict of Interest

None.

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Рентгенологічне дослідження легеневої тканини в щурів за блеоміциніндукованого фіброзу

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Анотація. Зростання випадків легеневого фіброзу в тварин, спричиненого токсичним впливом, вимагає детального аналізу рентгенологічних змін у легеневій тканині, що важливо для оцінки динаміки фіброзу та розробки ефективних терапевтичних підходів. У цьому контексті мета дослідження полягала у застосуванні рентгенологічного методу для визначення змін у легенях щурів у динаміці часу за моделювання блеоміциніндукованого легеневого фіброзу, що сприятиме вдосконаленню діагностики цього патологічного стану. Штучне відтворення фіброзу в легенях здійснювали на лабораторних щурах завдяки уведенню блеоміцину. Для моніторингу стану легеневої тканини та виявлення прогресуючих змін проводили рентгенологічні дослідження на 7 добу, 14, 30 та 45 добу дослідження. Особливу увагу звертали на виявлення характерних рентгенологічних ознак фіброзу, таких як ущільнення легеневої тканини та зниження її прозорості. Згідно з отриманими результатами дослідження встановили прогресуючий характер інтерстиціальних уражень. З'ясовано, що вже через 7 діб після введення блеоміцину з'являлися ознаки запалення в легенях, що супроводжувалися зниженням прозорості легеневих полів. Через 14 діб спостерігалися початкові ознаки фіброзу, що відобразилися на підвищенні щільності легеневої тканини та появі інфільтратів. На 30 добу розвитку патології відмічали значне прогресування фіброзу, що підтвердилося більш вираженими лінійними включеннями і зниженням прозорості. На 45 добу з'явилися рубцеві зміни, які призвели до значного погіршення структури легень. За отриманими даними підкреслено значення рентгенологічного дослідження як інформативного методу для діагностики та моніторингу перебігу легеневого фіброзу. За описаними рентгенологічними змінами різних стадій розвитку фіброзу можна детальніше охарактеризувати патогенез захворювання, що має важливе значення для оцінки ефективності терапевтичного втручання. Таким чином, результати дослідження можуть бути використані для створення нових діагностичних критеріїв та покращення моніторингу пацієнтів із легеневим фіброзом

Ключові слова: легеневий фіброз; рентгенівські знімки; інтерстиціальні захворювання легень; експериментальна модель; лабораторні щури; діагностика