1. Introduction
Since the introduction of scintigraphy as a diagnostic imaging tool in the 1960's [1], nuclear medicine has provided a wide range of sophisticated methodologies to study the physiology and disorders of the human lung. In the last few years, several of these investigative procedures have been adapted to the equine species and up to now, the areas covered are:

1. the study of regional lung function through ventilation and perfusion scanning;
2. the detection of subclinical inflammatory processes using alveolar clearance measurement;
3. the determination of inflammatory cells involvement in lung diseases;
4. imaging of pulmonary infection and/or inflammation;
5. the assessment of mucociliary clearance and
6. the study of aerosol deposition within the equine lung.

Following a brief introduction to the scintigraphic technique, this chapter will survey these nuclear procedures with an emphasis on their application to the practise of clinical respiratory medicine in the equine species.

2. Nuclear Scintigraphy
2.1. Basic Principles of Scintigraphy
The basic concept of scintigraphy is the administration to the patient of a gamma emitting substance to allow imaging and functional study of an organ, tissue or system. An external scintillation detector interfaced to a counting device detects the gamma rays emitted by the radionuclide distributed within its target. Two types of detectors are available in the veterinary field: the hand-held counting system and the gamma camera. Hand-held device records the whole counts (i.e., radioactive disintegrations) reaching a small detection area. In bone scintigraphy, this system is used to compare the number of counts received in a region versus the contralateral one (e.g., contralateral limb) and/or a reference site (e.g., the lateral wing of the atlas). Gamma camera, consisting of an array of photon detectors, is more appropriate for lung scintigraphy than hand-held counter. Indeed, owing to a data-processing system connected on-line to the camera, regional counts can be displayed by images in which chromatic variations are proportional to the regional radioactivity (Fig. 1).

![Figure 1. Obtaining a scintigraphical image. Gamma rays emitted by the radiopharmaceutical distributed within the target organ (A) are recorded by a gamma camera (B) linked to a computer (C). The local concentration of the radiopharmaceutical is displayed by showing an image in which the colour scale varies proportionally to the regional radioactivity [i.e., in this illustration, the colour scale gradually varies from black (no radioactive disintegration recorded), blue, green, yellow to red (highest number of gamma rays recorded by the camera)].](image)

2.2. Radiopharmaceuticals
Radionuclides are rarely administered to the subject in the pure state. Usually, they label a chemical compound formulated to focus the target to be imaged. Most routinely used radiopharmaceuticals are technetium-99m (99mTc) labelled compounds that are prepared by adding sodium pertechnetate [Na+(99mTcO4-)] to "cold" (i.e., non-radioactive) lyophilised ingredients supplied in a "kit" form suitable for administration to humans. Sodium pertechnetate can be obtained on-site for several days by eluting with saline a 99mTc-generator made of its product parent, molybdenum-99 (99Mo). (Fig. 2).
Technetium-99m has ideal physical characteristics for scintigraphy: its 6-hours half-life limits the radiation dose to the patient and its 140-keV gamma ray emission is in the range of gamma camera detection. Because of these qualities, a wide variety of chemicals to be labelled with 99mTc have been developed and are commercially available. The biodistribution of radiopharmaceuticals depends on their physical and chemical characteristics. For labelled compounds, the simplistic approach tends to ascribe the same behaviour to the radiopharmaceutical as to the carrier. The choice of the radiopharmaceutical will be determined by the function to be imaged. Absorption, distribution, metabolism and/or excretion of the radiopharmaceutical can be followed by scintigraphy. Depending upon their indicated use, radiopharmaceuticals may be administered orally, by injection, instillation or even by inhalation. The radiopharmaceuticals of interest for equine lung scintigraphy and their administration route(s) are summarized in Table 1.

### Table 1. Radiopharmaceuticals of interest for equine lung scintigraphy and their indication(s)

<table>
<thead>
<tr>
<th>Radiopharmaceutical</th>
<th>Study</th>
<th>Administration Route(s)</th>
<th>Indication(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>81m Kr</td>
<td>Ventilation</td>
<td>Inhalation</td>
<td>- Assessment of regional ventilation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- On-line monitoring of functional changes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Study of the ventilation/perfusion relationship</td>
</tr>
<tr>
<td>99mTc-Nanocolloid</td>
<td>Ventilation</td>
<td>Intravenous</td>
<td>- Perfusion study</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- On-line monitoring of functional changes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Study of the ventilation/perfusion relationship</td>
</tr>
<tr>
<td>Technegas</td>
<td>Ventilation</td>
<td>Inhalation</td>
<td>- Assessment of degree of airflow obstruction</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Study of the ventilation/perfusion relationship</td>
</tr>
<tr>
<td>99mTc-DTPA</td>
<td>(Ventilation)</td>
<td>Inhalation</td>
<td>- See 99mTc-Nanocolloid</td>
</tr>
<tr>
<td>99mTc-albumine aggregates or 99mTc-microspheres</td>
<td>Perfusion</td>
<td>Intravenous</td>
<td>- See 99mTc-Nanocolloid (the ventilation image may be of poor value under lung disorders)</td>
</tr>
<tr>
<td>99mTc-white blood cells or 111-In-white blood cells</td>
<td>Intavenous</td>
<td></td>
<td>- Cell kinetic studies</td>
</tr>
<tr>
<td>99mTc-RBC</td>
<td>Perfusion</td>
<td>Intravenous</td>
<td>- Detection of intra-thoracic infection</td>
</tr>
</tbody>
</table>

3. Lung Imaging Procedure

According to the function to be studied, the radiopharmaceutical may be administered apart of the imaging procedure or during acquisition of images with the horse’s thorax positioned close to the face of the detector. The large area of the equine lungs necessitates acquisition of one cranial and one caudal view for each lung to be entirely imaged. Radioactive reference marks placed on the chest wall, outside of the lung field, enable computed reconstruction of lateral views of each lung.
Images can be acquired in static or dynamic mode. In the case of static imaging, acquisition of the image is stopped after a defined time or a certain number of counts has been attained (Fig. 3). In dynamic mode, a series of images are acquired in a movie-like fashion, i.e., sequential images of a defined duration are recorded for a defined period. The composite image obtained comprises a sequence of frames demonstrating the distribution of the radiopharmaceutical over the total period of recording (Fig. 4). These frames may be viewed in “cine mode” or used to produce time-activity curves.

Figure 3. Image acquired in static mode. Image (thirty seconds of acquisition time) acquired twenty minutes following administration of in vitro labelled autologous red blood cells. The spleen as a red blood cell reservoir and a site of cell destruction is well visualised. - To view this image in full size go to the IVIS website at www.ivis.org . -

Figure 4. Images acquired in dynamic mode. Sequential images (two seconds of acquisition time per frame) demonstrating the distribution of in vitro labelled autologous red blood cells following their intravenous administration; image 1: arrival in the pulmonary vessels; images 2 & 3: perfusion of the lung; images 4 & 5: tracer circulation in the caudal aorta; images 6 to 8: distribution of the radioactive cells in the whole circulation (the spleen is progressively visualised from the 5th image). - To view this image in full size go to the IVIS website at www.ivis.org . -

Processing of Lung Images - Planar scintigraphy produces images which are a two dimensional projection of the three dimensional distribution of a radiopharmaceutical within the lungs; pixels of a lateral view represent a column of tissue and the counts recorded are issued from both lungs with a larger contribution of the lung closer to the camera. Consequently, counts recorded in a specific region have no direct anatomical significance but give an indication of what is happening in the lungs area corresponding to the pixel; appropriate image processing enables major information to be extracted from lung scan.

The majority of clinical software packages installed in nuclear medicine computers dedicated to planar scintigraphy consists of several programs that can be grouped into four main functions: Image Display, Image Processing, Curve Analysis and Data Management & Storage. As opposed to equine bone scanning, direct interpretation of equine lung images is of limited value; therefore, the Image Display function that includes image enhancement programs (to highlight specific features in the original image) is mainly utilized for presentation and communication of the results. Conversely, programs included in the Image Processing and Curve Analysis functions are extensively used to treat the rough images for qualitative and quantitative evaluation of the lung function; briefly, the main operations performed on pulmonary images are:

- the smoothing of data to reduce statistical noise;
- the drawing of region of interests (ROIs). The purpose of drawing a ROI may be the delineation of the lung edge for comparative studies (e.g., pre vs. post treatment images, healthy vs. diseased horses, and ventilation vs. perfusion images)
- the calculation of the counts within ROIs. In dynamic mode acquisition, the number of counts within ROIs as a function of time may be used to plot a curve from which functional parameters may be derived;
- the subtraction, division or multiplication of one image by another one or by a numeric factor (e.g., to create a ventilation-perfusion ratio image, to correct for background or decay, to normalise frames)
- the alignment of sequential images using a motion correction program.

Methods of lung image processing for specific physiologic parameters to be obtained are detailed elsewhere [2-6].

4. Main Applications of Pulmonary Scintigraphy

4.1. Study of Regional Lung Function

Air distribution from conducting airways to parenchyma called lung ventilation and blood distribution through the pulmonary artery ramifications called lung perfusion may be studied with scintigraphy. In contrast to other pulmonary function tests that measure the function globally, lung scintigraphy has the unique capability to provide topographical analysis of the lung function. Furthermore, the ventilation-perfusion relationship may be determined.

4.1.1. Ventilation

Ventilation in horses may be visualised by using either radioactive aerosols such as 99mTc-labelled nanocolloid of human
albumin either the radioactive krypton-81m (81mKr) gas.

4.1.1.1. Radioactive Aerosols as Ventilation-imaging Agents - The technique requires a lead-shielded aerosol producing system, an airtight facemask and a collection system to be designed. In the collection system, a filter traps undeposited radioactive aerosol particles contained in the exhaled air (Fig. 5).

**Figure 5.** Radioactive aerosol administration. The radioactive aerosol produced by a generating system (A) reaches the horse through a flexible tube (B) connected to an airtight facemask (C). A one way valve (D) on the mask assures air complementation during inspiration. Exhaled air is collected by another tube (E) to be vented owing an extractor fan (F) to a filter (G) were undeposited radioactive aerosol particles are trapped. The delivery and collection systems are enclosed in a lead-shielded box (H). - To view this image in full size go to the IVIS website at www.ivis.org. -

Aerosol deposition images are acquired following administration of the ventilation-imaging agent. Imaging must start as soon as possible after the administration procedure to avoid significant changes in the distribution pattern due to mucociliary clearance mechanism. Alveolar clearance may also change the ventilation image over time. Most of the ventilation studies involving horses have used 99mTc-diethylene triamine penta acetic acid (99mTc-DTPA). However, 99mTc-DTPA clears out of the lungs by passive diffusion through the intercellular junctions of the alveolar-capillary barrier. The rate of 99mTc-DTPA alveolar clearance (see "Alveolar clearance measurement") considerably increases in lung disorders thus competing with the time required to acquire lung images (around 20 to 30 minutes). A radiopharmaceutical that does not permeate the lungs, and therefore whose distribution remains virtually static, should be preferred. Satisfactory images of horse’s lung have been obtained with a nanocolloid of human albumin (Venticoll-Solmed, Birsfelden, Switzerland) [3,7]. This nondiffusible compound may be labelled with 99mTc.

The extent of aerosol penetration and deposition within both airways and ventilated alveoli depends on the aerodynamic properties of the aerosol, breathing pattern and airways geometry. To minimise impactation of aerosol droplets in conducting airways, the system generating the radioactive aerosol should produce an aerosol suspension with droplets’ size ranging from 0.5 to 2 µm, i.e., droplets that may potentially reach the alveoli and settle. Pathological conditions that modify the breathing strategy and/or the geometry of airways disrupt the ability of radioactive aerosols to depict lung ventilation. In human lung disorders, ventilation is better visualised using Technegas (Tetley Medical Ltd, Sydney, Australia) rather than 99mTc-"wet" aerosols inhalation. Technegas is a dry aerosol produced by heating (at 2,500 ºC) Na+(99mTcO4) in a graphite crucible under an argon atmosphere. The 99mTc-labelled carbon particles obtained measure between 0.005 and 0.2 µm (depending on the source) [8-11]. The small particle size enables Technegas to go deep into the lungs similarly to a gas and to adhere to alveolar walls similarly to conventional aerosols. Technegas does not diffuse through the alveolar-capillary barrier and the only limiting factor for image acquisition is the 99mTc half-life [12]. To the author knowledge, no publication reports the use of Technegas in the equine species but this ventilation-imaging agent has been tested in the bovine species with good results [13,14].

4.1.1.2. Krypton-81m Ventilation Imaging - The 81mKr is obtained by eluting with air a 81mKr-generator made of its cyclotron product parent, Rubidium-81 (81mRb). The 81mKr gas is a short-lived noble gas (13 seconds) consequently, images of ventilation must be acquired during the continuous tidal breathing of the gas. This short physical half-life confers to 81mKr several advantages over 99mTc-labelled aerosols:

1. there is no risk of environmental contamination;
2. a system to trap exhaled air is not required;
3. there is no need for horse’s isolation after the procedure and;
4. ventilatory modification (e.g., in response to bronchodilator administration) can be monitored on-line. As 99mTc, the 81mKr gamma ray emission (190 keV) can be transmitted out of the horse’ body and fulfils condition of gamma camera detection.

Unfortunately, the 81mKr presents two major disadvantages that preclude its use in clinical routine: the expensiveness of the generator in addition to the short half-live of 81mRb (4.58 hours) that supplies 81mKr gas for only a single day. The others noble radioactive gases that may be used in human medicine (Xenon-133, Xenon-127, Oxygen-15, Nitrogen-13, etc.) are not suitable for ventilation studies in the equine species because of inappropriate physical properties (half-life and/or gamma energy emission).
4.1.1.3. Clinical Studies and Research Applications - In healthy horses, images acquired following inhalation of small-sized 99mTc-aerosol show uniform distribution of the radiopharmaceutical throughout the lung field. Lung disorders induce patchy and more central distribution of the radioactive aerosol. This altered distribution results from variation in breathing strategy (e.g., changes in the rate of airflow, tidal volume, breathing frequency) and/or modifications of airway geometry (e.g., obstruction to airflow due to bronchoconstriction, inflammation, secretions in the lumen) that usually accompany respiratory tract diseases. Although non pathognomonic, this modified aerosol distribution pattern may be considered as a means to discriminate normal from abnormal lung function (Fig. 6). In addition, measurement of aerosol dispersal and/or penetration within the lung can be used to determine the severity of functional disturbance as well as to monitor the efficiency of a treatment to restore a normal function.

Figure 6. Aerosol deposition image of the right lung in a heaves-affected horse before (A) and after (B) treatment. The functional modifications present during symptoms of heaves result in patchy dispersal and poor alveolar deposition of the radioactive aerosol (A). Therapy by inhalation of aerosolised drugs improves the lung function and restores a more homogeneous distribution of the radiopharmaceutical (B). - To view this image in full size go to the IVIS website at www.ivis.org . -

The 81mKr gas allows topographical distribution of true ventilation (V) to be obtained. Using this technique, Amis and collaborators [2] demonstrated the existence of a vertical gradient of pulmonary ventilation: the dorsal lung regions are less ventilated than the lower ones. Up to now, there are no reports the use of 81mKr for the study of modification induced by respiratory disorders. Another potential use of 81mKr is for the study of the sequential filling and emptying of the equine lung. Knowledge of topographical distribution of air over the inspiration phase of the respiratory cycle would help to determine adequate moment for puff inhalation of drug to target specific site(s) within the lungs.

4.1.2. Perfusion
The two possible methods for perfusion imaging are the entrapped particle technique and the infusion of radioactive gas.

4.1.2.1. Perfusion Imaging with Radioactive Entrapped Particles - The standard method for lung perfusion imaging uses a suspension of radioactive particles (i.e., microspheres or albumin aggregates available as lyophilised kits ready to label with 99mTc) that are entrapped in the pulmonary vascular bed following their intravenous administration. The particle size of the injectate (> 10 µm) exceeds that of precapillary diameter therefore, the particles are completely trapped in the pulmonary vasculature in a distribution proportional to regional blood flow [15]. Because the number of vessels blocked is very small compared to the total number of pulmonary vessels, this procedure has no detectable effect on the pulmonary function [16]. The radiopharmaceutical is progressively cleared from the blood by the reticulo-endothelial system. Because pulmonary blood flow is influenced by changes in alveolar pressure associated with breathing, the radiopharmaceutical must be injected over several breaths [17]. Images are taken immediately afterwards. The resulting images average distribution of blood flow during inspiration and expiration and represent the perfusion at the moment of administration.

4.1.2.2. Radioactive Gases as Perfusion Imaging Agents - Among the different radioactive gases, the 81mKr gas is the most adapted for perfusion imaging in the horse. Images of lung perfusion are acquired while 81mKr, dissolved with dextrose in water, is infused intravenously.

4.1.2.3. Clinical Studies and Research Applications - Pulmonary perfusion distribution in the equine species has been studied using both 81mKr [2] and the entrapped particles technique [18-20]. With infused 81mKr, only perfusion of the caudal lungs can be studied because of radioactivity circulating in the heart that is visualised on the lateral view of the cranial lungs. As for ventilation studies, the short half-life of 81mKr enables changes in perfusion distribution induced by specific conditions (e.g., position change in anaesthetised horse) to be monitored on-line. With entrapped particles, the whole lung area can be considered. Because the thickness of the lungs varies in all directions, the perfusion image does not permit direct visualisation of the blood flow distribution; in each pixel of the image, the colour is representative of the total amount of perfusion in the corresponding lung sample. Therefore, analysis of pulmonary blood flow distribution requires either relative comparison (e.g., perfusion versus ventilation or perfusion under different conditions) or slaughtering of the horse in order to count, with a gamma well counter, radioactivity in lung sections of the same size. Entrapped fluorescent microspheres have also contributed to the understanding of mechanisms determining the perfusion distribution. Different colours of fluorescent microspheres are used to discriminate different injection times (e.g., administration at rest and at different levels of exercise). The major drawback of this technique is the necessity of...
slaughtering the horse to measure the fluorescence emitted by small lung pieces. On the other hand, spatial resolution of regional blood flow measurement is highly improved. In the conscious standing horse, scintigraphical studies demonstrated a vertical gradient of pulmonary perfusion in which the pulmonary blood flow increases from dorsal to ventral lung zones [2,19]. This gradient was thought to mainly result from gravity and the balance between pulmonary arterial, pulmonary venous and alveolar pressures. However, anaesthesia redistributes the blood flow to caudo-dorsal region of the lungs whatever the position of the horse (e.g., lateral, dorsal or ventral recumbency) [17,19]. This finding and the demonstration that perfusion heterogeneity exists within isogravitational planes [17,21] suggest that some factors other than gravity might significantly influence blood flow distribution. The fixed structure of the pulmonary arterial tree (i.e., its fractal geometry) would play a major role in perfusion distribution [21] and would limit the perfusion redistribution under exercise despite significant increases in pulmonary artery pressure and cardiac output [22]. However, perfusion redistribution with exercise exists with flow increasing to the dorsal region of the lung [20,22]. To summarise the current opinion, the regional perfusion would be primarily determined by the intrinsic structure of the pulmonary vascular but neither influence of the gravity [23] nor contribution of local factors such as vasoactive mediators can be neglected [24,25]. The observed perfusion redistribution to the upper part of the lungs with exercise (Fig. 7) may conceivably contribute to the location of exercise-induced pulmonary haemorrhage (EIPH) lesions in the dorso-caudal region of the lung [26].

Figure 7. Perfusion redistribution induced by exercise (image of the left lung in a healthy horse). Computed image obtained by dividing, on a pixel by pixel basis, the exercising perfusion image by the resting perfusion image. The areas more perfused during exercise compared to rest are displayed in red. Exercise induces perfusion redistribution to the dorsal region of the lung. - To view this image in full size go to the IVIS website at www.ivis.org . -

4.1.3. Ventilation-Perfusion Relationship

Study of the ventilation-perfusion relationship provides insight into basic lung function and contributes to the comprehension of pathological mechanisms involved in pulmonary diseases that disturb the balance between ventilation and perfusion distributions.

4.1.3.1. Dual Imaging Using Two Different Isotopes - Ventilation and perfusion images can be acquired simultaneously using radiopharmaceuticals labelled by isotopes with different gamma emission. For example, 81mKr ventilation and 99mTc perfusion images may be recorded concurrently. The major benefit of dual isotope acquisition is the perfect concordance between pixels of both images.

4.1.3.2. Ventilation-perfusion Imaging Using the Same Isotope for Ventilation and Perfusion Studies - Using 81mKr, perfusion and ventilation can alternately be imaged without interference from one study to the other. In addition, as mentioned earlier, the course of functional modification induced by different conditions may be followed on-line. Using radiopharmaceuticals labelled with 99mTc, ventilation and perfusion images are subsequently acquired. Therefore, the radioactivity deposited following nebulisation of the ventilation-imaging agent "contaminates" the perfusion image. This undesirable activity must be removed from the perfusion image by computed subtraction. This subtraction requires (1) the images to be perfectly matched in the acquisition matrices and (2) decay correction for the time elapsed between both images acquisition [3]. Radioactive reference marks taped on the thoracic wall may be used to align ventilation and perfusion images. Ventilation-perfusion study using 99mTc labelled radiopharmaceuticals is the less expensive option in veterinary nuclear medicine department because of 99mTc-generator availability.

4.1.3.3. Clinical Studies and Research Applications - In awake standing horses, the vertical gradient of ventilation is matched by a similar gradient of perfusion [2,3]. This matching is thought to be necessary to ensure efficient blood oxygenation and it is commonly accepted that unbalanced ventilation-perfusion distributions is the major cause of hypoxaemia in lung diseases [27]. Scintigraphy performed on heaves-affected horses demonstrated that the disease induces mismatches between the ventilation and perfusion distributions. The ventilation-perfusion pattern disruption was still present after a medical treatment despite clinical recovery. This study suggested that functional impairment requires a longer period to resolve than do clinical signs [7]. Scintigraphy performed with horses suffering from EIPH demonstrated significant reduction in ventilation and perfusion to the dorso-caudal lung fields, i.e., at the bleeding sites. The perfusion deficit was more pronounced than the ventilatory change and the net result was an abnormally high ventilation-perfusion ratio in those areas of the lung [28]. Scintigraphical analysis of ventilation-perfusion relationship enables the degree of inadequacy between both functional parameters to be quantified. Therefore, scintigraphy may repeatedly be performed in horses with diagnosed abnormalities to
monitor response to therapy.

4.2. Alveolar Clearance Measurement
The integrity of the alveolar epithelium membrane can be assessed by measuring its permeability to 99mTc-DTPA. The 99mTc-DTPA is a small hydrophilic compound that passively permeates the lungs following its administration to alveoli by nebulisation. The 99mTc-DTPA molecules slowly diffuse through the alveolar-capillary barrier at the site of cell junctions. Because the pulmonary endothelial junctions are much more permeable than the alveolar ones, the epithelial junctions are the limiting factor of the alveolar-capillary barrier to the speed of 99mTc-DTPA passage. Inflammatory processes involving the lung damage the alveolar epithelium and cause the junctions to become more porous to solute permeation, thus resulting in an accelerated clearance of the 99mTc-DTPA from alveoli.

Images of 99mTc-DTPA aerosol deposition are acquired in dynamic mode or repeatedly at time intervals. From all images acquired, the computer can create dynamic curves that enable the rate of 99mTc-DTPA removal to be extrapolated. Result can be expressed either as a half-time clearance from lung to blood (T<sub>1/2</sub>) or as a rate constant (k).

Clinical Studies and Research Applications - A significant difference in 99mTc-DTPA lung clearance rate was found when healthy horses were compared to horses with symptoms of heaves [4]. Following remission of the disease obtained by putting horses at pasture for two months, there was a complete recovery with restoration of normal alveolar permeability and normal pulmonary function tests (i.e., mechanics of breathing and blood gas analysis). Stabled in a controlled environment (i.e., poor in allergens responsible of the symptoms’ exacerbation), heaves-affected horses showed a 99mTc-DTPA clearance rate intermediate between healthy and clinically-affected horses. The alveolar clearance measurement was a more sensitive indicator of lung damage than pulmonary function tests that were not significantly modified by the ongoing subclinical inflammatory process [29].

These studies demonstrated that measurement of alveolar clearance has a role to play in the early detection of alveolar epithelium damage. This early recognition is critical for the prevention of clinical symptoms and/or irreversible structural deterioration as well as in monitoring of therapies (medical and/or environmental management) used to treat inflammatory lung diseases.

4.3. Determination of the Involvement of Inflammatory Cells in Lung Diseases
Sub-populations of white blood cells can be separated by centrifugation on a density gradient and subsequently, these blood cells can selectively be labelled in vitro to be re-injected to the donor. In the equine species, neutrophils, eosinophils and platelets have successfully been labelled with 111-In or 99mTc [30-34] (Fig. 8a and Fig. 8b).

Figure 8a. A simple method for separation of pure neutrophils from equine whole blood. - To view this image in full size go to the IVIS website at www.ivis.org . -

I. Blood is withdrawn from the horse’s jugular vein into syringes containing acid citrate dextrose (ACD) anticoagulant;
II. a part of the blood is dispensed into tubes containing hydroxyethyl starch (Hespan) that favours red blood cell (RBC) aggregation and let to stand for RBC sedimentation;
III. the remaining blood is centrifuged to obtain cell-free plasma (CFP);
IV. following sedimentation, the leukocyte-rich-platelet-rich plasma (LRPRP) obtained is layered onto a double density polysucrose solution gradient and centrifuged;
V. after centrifugation, the neutrophil layer is located at the bottom of the tube;
VI. the cells, collected in a single tube, are washed with CFP by centrifugation and finally,
VII. the neutrophils, suspended with a small amount of CFP, are ready to be labelled.

In human medicine, a 99mTc-anti-granulocyte monoclonal antibody Fab' fragment (LeukoScan ®, Immunomedics Europe, Amsterdam, The Netherlands) has been introduced to tag granulocytes in vivo. LeukoScan ® recognises an antigenic structure (a surface glycoprotein called "non-specific cross reacting antigen", NCA-90) of granulocytes. The fragment binding to granulocytes does not affect the function of the cells as it may be by in vitro leukocytes labelling procedure [35]. In human, LeukoScan ® has proved to be of value for determining the location and extent of soft-tissue and bony infections [36]. A preliminary study [37] suggests that LeukoScan ® could be valuable in investigating neutrophil recruitment into the equine lung.
Clinical Studies and Research Applications - Radiolabelled leukocyte scanning exploits the natural migratory behaviour of white blood cells in order to determine inflammatory cell involvement in lung diseases and/or to image inflammatory site (see "Imaging of pulmonary infection and/or inflammation"). In heaves-affected horses submitted to antigen challenge, scanning with 111-In-labelled leukocytes enabled the investigators to follow the time course of white blood cell accumulation into the lung as well as to correlate this lung invasion to the appearance and further worsening of pulmonary dysfunction [32]. In addition, the active migration of neutrophils from the blood compartment to the air space of the lung can be demonstrated (Fig. 9) [37].

Scanning with labelled leukocytes is particularly useful to determine the role of specific inflammatory cells in the pathogenesis of respiratory disorders [32] and for investigating effect of pro or anti-inflammatory drugs on cells recruitment into the lung [38-41].

4.4. Imaging of Pulmonary Infection and/or Inflammation
The most common scintigraphic methods to detect pulmonary infection and/or inflammation are Gallium-67 (67-Ga) citrate and labelled leukocytes scanning. Following its intravenous administration, 67-Ga firmly binds to the iron transport protein, transferrin. Circulating tracer is subsequently redistributed into inflamed and infected tissues to iron-binding proteins produced by macrophages and bacteria. Scintigraphy with 67-Ga enables chronic and diffuse inflammation to be imaged [42].

An acute infection and/or inflammation will be better visualised with labelled leukocytes. To this aim, a mixed population of leukocytes is isolated from a sample of autologous blood, labelled and given back to the patient. The mixed population predominantly contains granulocytes that are the cells primarily involved in acute inflammation. In spite of the labelling procedure, granulocytes keep the property to actively migrate into infectious and/or inflammatory sites. Site of focal infection and active inflammation appears as areas of abnormal radioactivity concentration on the image of the thoracic wall.

Clinical Studies and Research Applications - To the author knowledge, no study performed with horses reports neither the use of 67-Ga for pulmonary scanning nor the use of labelled leukocytes for detection of focal inflammatory sites in the
thorax. However, this last technique has successfully image an abdominal abscess in a horse [43]. Imaging pulmonary infection might be of interest in the investigation of intra-thoracic infection especially in the foal affected with *Rhodococcus equi*. Accurate detection of Rhodococcus pneumonia infection at its beginning might permit early therapy with antibiotics before the occurrence of extensive damage which may occur prior to the development of clear clinical signs.

4.5. The Assessment of Mucociliary Clearance

Mucociliary clearance is a non-specific mechanism that aims at keeping the lung free from environmental contaminants (e.g., bacteria, aeroallergen, dust). This defence mechanism may be studied by scintigraphy. The technique for assessing the mucociliary clearance efficiency in the tracheal portion of the respiratory tract has been described in the horse [44]. The mucociliary clearance is evaluated by measuring the tracheal mucous transport (TMT) rate: the migration of a minute suspension of radioactive insoluble particles deposited in the trachea is followed by sequential imaging of the trachea; the TMT rate is then calculated by dividing the distance moved by the radioactive tracer by the time taken. Studies should be performed in unsedated horse because sedation is reported to decrease TMT rate [45]. The mucociliary clearance in the portion of airway down to the trachea (i.e., large airways) has not been studied in the horse lung. Efficiency of mucociliary clearance in large airways may be assessed by determining the rate of removal of an inhaled radioactive aerosol deposited in conducting airways by nebulisation.

Clinical Studies and Research Applications - Within horses, measures of TMT rates are repeatable but largely vary among horses [46]. Therefore, routine clinical study of TMT in individual horse has no obvious clinical application.

Pulmonary diseases may disrupt ciliary activity and/or induce biochemical and rheological changes of the bronchial secretions. Scintigraphy demonstrated impairment of tracheal mucociliary clearance in horses with respiratory diseases [47,48]. Several pharmaceutical agents are used in the therapy of equine respiratory diseases with the aim of improving mucociliary clearance. However, their beneficial mucokinetic effects are postulated from human medicine studies and have rarely been assessed in clinical studies involving horses. Scintigraphy might potentially answer key questions regarding the correlation between the viscoelastic properties of mucus and clearance efficiency.

4.6. The Study of Aerosol Deposition Within the Equine Lung

The inhaled route for respiratory drugs has gained a major interest for treating equine respiratory disorders [49]. Clinical trials have demonstrated the validity of this route of administration. Surprisingly, the deposition of therapeutic aerosols in the equine respiratory tract has not often been studied. Nevertheless, the knowledge of the fraction of inhaled drug reaching the lungs and the drug distribution pattern in the whole lung or even in specific parts of the lungs might provide unique information to improve inhalation therapy. Scintigraphy allows to visualise and quantify the distribution of radioactive aerosol within the different parts of the respiratory tract. Therefore, it might significantly advance the technique of aerosolised drugs.

4.6.1. Radioactive Tracers

The labelling of drug complexes with gamma ray emitters is the best technique to correlate pulmonary function changes with the dose and pattern of drug deposition. For drugs that may not be labelled with a radionuclide, scintigraphy may be performed with the drug formulation containing free 99mTc. However, the distribution of the radioactivity may not represent the drug’s distribution since dissociation of components may occur during both aerosolization and passage within conducting airways. A most acceptable possibility is the use of ventilation-imaging agents (with the exception of DTPA because of lung clearance) with physico-chemical characteristics similar to the drug of interest.

4.6.1.1. Clinical Studies and Research Applications - By means of suitable calibration and/or calculation procedures [5,6] scintigraphy allows:

1. the percentage of activity released from an inhalation device
2. the percentage of the dose released reaching the lungs and/or a specific area
3. the deepness of aerosol penetration and/or the amount of aerosol restrained to large airways
4. the evenness of aerosol distribution within the different lung regions and
5. the aerosol distribution outside of the lung field (e.g., trachea, oro-pharyngeal region, nostrils) to be determined.
These information are of great value to define:

1. the physicochemical characteristics required for drug particles to attain a specific lung location,
2. requirements of delivery systems,
3. administration techniques,
4. the location of drug receptor sites,
5. the correlation between the degree of airflow obstruction and accessibility of the aerosolised drug to its target
6. and finally, to recommend dosages for a particular delivery system.

Up-to-now, only a few aerosol deposition studies have been performed in the equine species. For instance, scintigraphy demonstrated the feasibility of nebulising aerosols to horses with two different operating systems namely, an ultrasonic nebuliser (UN; Ultra-Neb 200HI, DeVilbiss, Somerset, USA; 1.63 MHz) and a jet nebuliser (JN; Nebul ®, Agritronix, Meux, Belgium; 600 kPa) [50]. With both systems, the percentage of the dose released reaching the lung was lower than the average 10% achieved with similar devices in the human lung (i.e., 5.09 ± 0.66% for the UN vs. 7.35 ± 1.96% for the JN). A pressurised metered dose inhaler especially designed for the equine species (3M Equine Aerosol Delivery System, 3M Animal Care Products, Saint Paul, Minnesota, USA) would enable 23.3 ± 2.3% of the total dose released to reach the mid-thoracic region [51].

Aerosol deposition studies may also investigate the efficacy of bronchodilators to "open" airways in order to facilitate access of a drug further administered by nebulisation. For example, there was a greater penetration of the radiopharmaceutical after effective bronchodilation induced by puff inhalation of albuterol sulphate in a group of heaves-affected horses than in untreated horses [52].

4.7. Further Advances in Lung Scintigraphy

Scintigraphy is currently experiencing major developments in radioactive tracers. In the field of immunoscintigraphy, radiolabelled antibodies to endothelial adhesion molecules activated in inflammatory process start to be available. Such a radiopharmaceutical might be of interest for the study of drugs that would aim at controlling neutrophil emigration in heaves-affected horses. Labelled cytokines (e.g., interleukin-8) that bind with high affinity on specific cell-surface receptors might contribute to the understanding of mechanisms involved in neutrophil recruitment. Chemotactic peptides, which target inflammation, should also be available in a next future [53].

Benefit of scintigraphy in EIPH detection and quantification has still to be proven. However, the labelling of equine red blood cells is possible [54] and in human, scintigraphy is successfully performed to detect low-grade gastrointestinal bleeding using labelled red blood cells [55].

5. Nuclear Imaging Facility and Radiation Protection

Over the last few years, many universities and large veterinary centres have purchased second-hand gamma camera to mainly investigate equine orthopaedic conditions. There is no additional requirement for the nuclear imaging facility to perform pulmonary scintigraphy [56-58]. The use of radioactive materials must be authorised by the national Radiation Safety Control organisation and the procedures must follow the recommendations of the International Commission on Radiological Protection [59] as well as specific local regulations.

During scintigraphy, any faeces and urine voided in the examination room must be collected to be stored for decay of contamination and then, flushed into normal waste system. After completion of scintigraphy, horses have to be kept in a radiation isolation box until sufficient radioactive decay has occurred (in Belgium, at Liege University, horses imaged with 99mTc-labelled products must stay in quarantine for forty-eight hours).

6. Conclusions

Despite the high initial cost of nuclear imaging equipment, several equine clinics and hospitals have established a nuclear imaging unit. However, only of few publications report the use of scintigraphy in the investigation of equine lung function. The time-consuming nuclear procedures and necessary image analyses have precluded its wide application. Nevertheless, the potential of equine lung scintigraphy is enormous and extends from functional and/or fundamental studies to clinical applications. Scintigraphy quantifies functional disturbance, even subclinical, induced by respiratory diseases. Area(s) to be targeted by medical treatment can be identified and the ability for a drug to reach its target can be demonstrated. In addition, scintigraphy may be performed repeatedly in horses with diagnosed abnormalities to follow the course of a disease process and/or to monitor response to therapy.
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