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LUNG BIOPSY: TECHNIQUES AND INDICATIONS

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Summary: Trans-thoracic lung biopsy has been used in horses to confirm pulmonary fibrosis, lung tumor, chronic bronchiolitis, interstitial pneumonia and in particular lung silicosis.

The technique of lung biopsy has improved with development of fast biopsy devices. The aim of the present study was to compare complications of lung biopsy in horses and the quality of the lung specimens after biopsy with the manual Tru-Cut biopsy needle (TC) and with an automated biopsy needle (ABN).

Lung biopsies were performed in 50 horses with one instrument on one side of the thorax and with the other instrument on the other side. Postmortem examination was performed in 20 of the 50 horses. Coughing was detected in 10 of 50 horses and epistaxis in 6 of 50 horses. Endoscopy revealed bleeding into the airways in 16 of 49 horses and in 5 of 49 horses after biopsy with the TC or, respectively the ABN. The ABN induced a significantly smaller amount of bleeding. Pneumothorax was detected by radiographic examination in one of 50 horses. The size of hematoma diameter determined in 7 of 20 horses at postmortem examination, was significantly smaller after biopsy with the TC than with the ABN. The quality of the lung specimen was good.

Trans-thoracic lung biopsy has been used in horses to confirm pulmonary fibrosis (Viel, 1983), lung tumor (Godber et al., 1993), chronic bronchiolitis (Nyman et al., 1991; Viel, 1983), interstitial pneumonia (Detlev et al., 1985; Donaldson et al., 1998; Jacobs, 1985; Savage et al., 1998) and in particular lung silicosis (Berry et al., 1991).

Lung biopsy was first described in the horse in 1970. At that time, biopsy was performed with a trephine activated by a high-speed electric hand drill. The procedure was undertaken on two horses under general anesthesia that abolished spontaneous movements. One of the horses showed a transient dyspnea, and both recovered without further clinical symptoms. There were no clinically manifest complications noted using the same technique, mainly on horses under general anesthesia (Schatzmann et al. 1974). Nine horses were examined at necropsy between one day and four months after biopsy. There were signs of local bleeding and pleural reaction around the biopsy site. The quality of the biopsy specimens was considered adequate and free of artefacts.

Compared to the first biopsy attempts performed under general anaesthesia (Dungworth et al., 1970), the technique using the Vim manual Tru-Cut biopsy needle in the standing horse without sedation is far less invasive and expensive (Raphel et al., 1981). The biopsy specimens are of good quality and permit a proper histological examination and precise evaluation of the bronchiolar and peribronchiolar structures (Naylor et al., 1992). The complications of this procedure are coughing and hemoptysis in two of the 20 horses of one study (Raphel et al., 1981) and hemoptysis in one of the 16 equine patients suffering from different degrees of chronic airway disease (Naylor et al., 1992). Postmortem examination performed on 20 horses one day to five months after the biopsy showed no more than a local hemorrhage around the biopsy site (Raphel et al., 1981). A survey performed showed that 23% of 44 veterinarians performing lung biopsies in horses had not noticed any complications after that procedure (Savage et al., 1998).

The purpose of the present study was to compare the complications occurring during and after lung biopsy performed with the Tru-Cut biopsy needle and with a biopsy instrument involving a spring-loaded biopsy needle. The manual Tru-Cut biopsy needle has been described to provide lung biopsies of good quality while inducing epistaxis in three from 46 horses (Naylor et al., 1992; Raphel et al., 1981). The spring-loaded biopsy needle was described before for the biopsy of testicles and the flexor tendons of horses (Wagels et al., 2001). Furthermore the quality of the biopsies collected with both instruments was to be also compared in the present study.

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MATERIALS AND METHODS

Horses
Study 1 was performed on 50 mature horses. It was performed in compliance with the German Animal Welfare Law (registration number: 99/254). The horses were 22 mares, 28 geldings, aged 4 to 18 years and weighing 420 kg ± 55 kg. A clinical examination including endoscopy of the upper airways and trachea was performed on all horses one day before biopsy. The horses were euthanized one day after biopsy for anatomical studies and in 20 horses a post mortem examination was performed 4 hours after euthanasia. In the mean time the horses had been lying in lateral recumbency.

Instruments
A 14-gauge, 15.2-cm long manual Tru-Cut biopsy needle (Fig. 1) was used for comparison with an automated biopsy needle (Fig. 2) in which a disposable 14-gauge, 10-cm long core biopsy needle was inserted. Both needles have a similar device for cutting a lung specimen (Raphael et al., 1981). The needle of the automated biopsy needle fits into a metal box containing two small mobile carriages that glide on a common rail. The inner obturator-specimen rod and the outer cannula fit into one of the carriages. These are pulled back one after the other when the instrument is loaded. When retracted, the carriages compress a spring that will thrust them forward when the release is activated. Both inner and outer cannulae are propelled forward with a delay of one-tenth of a second, so that the biopsy is performed at a high velocity.

Technique
The biopsies were performed under radiological monitoring of the chosen biopsy site to avoid areas close to the diaphragm (Fig. 3).

In each horse one biopsy was performed on one side of the thorax with the manual Tru-Cut biopsy needle, and a second one on the other side of the thorax to allow comparison of complications between the two instruments. The sides of the thorax on which the manual Tru-Cut biopsy needle was used was alternate between horses. The horses were sedated (xyloclane: 0.5 mg/kg BW, i.v.) and standing. A roentgen-opaque marker was taped to each side of the thorax in the eighth intercostal space at mid-thorax. The markers were of different sizes to allow distinction between the right and left sides of the thorax. A laterolateral chest radiograph was taken at end of inspiration with the technique previously described (Venner et al., 1995). The site of biopsy was selected according to the roentgen-opaque markers or adjusted to the horse’s individual anatomy as revealed by radiography (Fig. 3). Care was taken to avoid areas close to the diaphragm. As a biopsy of the lung periphery was taken, no particular attention was paid to the localisation of the pulmonary arteries.
pleural space as a signal of hemothorax were registered. To quantify the amount of fluid gathered in the pleural space was evaluated in relation to the number of intercostal spaces in which fluid was noted in the inspiratory phase. In horses presenting a pneumothorax on the chest radiographs, sonography was performed on both sides of the thorax in the caudodorsal area with the purpose to identify the side of the thorax on which pneumothorax was induced. The findings were paper-printed.

Radiography of the lung was performed two hours after biopsy in order to detect a pneumothorax. To enhance the chance to visualize one or both collapsed lungs, a laterolateral image of the thorax was taken at end of expiration. The side of pneumothorax was determined subsequently by sonography.

Postmortem macroscopic examination of 20 horses was performed to verify the sonographical findings. These horses were euthanized 24 hours after biopsy, remained recumbent on one side for four hours and were then processed for presentation to students during anatomy lectures. Four hours after euthanasia, an evaluation of local hemorrhage around the biopsy area, the diameter of the hematoma, was measured on the lung surface and the pleural space was examined for signs of hemothorax.

**Statistical analysis**
Coughing and epistaxis were only registered but not statistically evaluated, because it was not possible to define the origin of these phenomena or which instrument was the cause of bleeding. It was possible to determine which instrument was responsible for more trauma of the lung by endoscopy of the airways. As a biopsy was performed with both instruments on every horse, a McNemar test for dichotomous repeated values was used to evaluate which instrument was more likely to induce bleeding.

The quality of the biopsy specimens was defined according to the quantity of erythrocytes present in the alveoli. The signed rank test was used for repeated non-parametric values to compare induced bleeding into alveoli with both biopsy instruments and to compare induced hematoma in the lung tissue evaluated postmortem and with a non-Gaussian distribution (Steel et al., 1960).

**RESULTS**
No pain reaction and no flinching of the horse were noted during the biopsy procedure.

Coughing was observed in 10 of the 50 horses after biopsy. Six horses developed epistaxis within 30 minutes after biopsy. Life-threatening bleeding occurred in one horse immediately after the biopsy using the manual Tru-Cut biopsy needle so that no biopsy could be performed with the other biopsy device. This horse could not be considered for further evaluation of the biopsy instruments.

One horse developed a small unilateral pneumothorax without clinical signs. Sonography revealed that the pneumothorax developed on the side where the biopsy had been performed with the manual Tru-Cut biopsy needle.

Bleeding was compared in each horse after biopsy with both instruments. Because of strong coughing right after the biopsy with the manual Tru-Cut biopsy needle in one horse only 49 horses were available for comparison of both instruments. In 16 of 49 horses endoscopy revealed bleeding after biopsy with the manual Tru-Cut biopsy needle. Bleeding occurred in only five horses after biopsy with the biopsy device. There was a significant difference in the amount of bleeding into the airways induced by the different biopsy instruments (p = 0.005).

A hematoma at the site of biopsy was revealed by sonography in 7 horses on the side of the manual Tru-Cut biopsy needle and only in 3 horses on the side of the automated biopsy needle. This difference was significant (p = 0.046).

The hematomas measured on the surface of the pleura were 1.4 ± 1.5 cm with the Tru-Cut and 0.5 ± 0.7 cm with the automated biopsy needle (p = 0.015).

Histological examination of the 99 biopsy specimens taken showed that 97 were lung tissue and 2 skeletal muscle. In 12 cases one block of lung specimen was either muscle or cartilage, but the second block of the same biopsy specimen was lung tissue.

The median value of bleeding into the lung parenchyma in specimens collected with the manual Tru-Cut biopsy needle and with the automated biopsy needle was 1 (scale: 0 to 3). No significant difference was observed in the number of erythrocytes in the alveoli.

**DISCUSSION**
The results of this study showed that 6 of the 50 horses submitted to a double lung biopsy developed epistaxis. Further, lung biopsy performed with the manual Tru-Cut biopsy needle induced bleeding into the airways in 16 of 50 horses, whereas bleeding in the main bronchus was present in only 5 of 49 horses on the side where the automated biopsy needle was used. Considering experience on endoscopy of race horses screened to evaluate the incidence of exercise induced pulmonary hemorrhage, a delay of 60 to 90 minutes after the race is accepted as the proper period to identify EIPH (Pascoe et al., 1981). The time of endoscopy of 30 minutes after the biopsy in the present study might appear short. But as the horses developing hemoptysis showed this in the 10 minutes following biopsy, this delay chosen seemed adequate to the authors to evaluate bleeding into the airways after lung biopsy.
Sonography allowed the detection of a comet-tail artefact at the surface of the lung in the area of the biopsy in 7 of 20 examined horses on the side of the thorax where biopsy had been performed with the manual Tru-Cut biopsy needle and in 3 of 20 horses after use of the biopsy device. This difference was also significant. This result correlates with the postmortem evaluation of the extent of the hematoma at the biopsy site. The hematoma measured on the surface of the visceral pleura was significantly smaller after biopsy with the device compared with the manual Tru-Cut biopsy needle.

This study shows that the biopsies obtained with the manual Tru-Cut biopsy needle and with the automated biopsy needle are of similar and good quality. Subjective evaluation of a few investigators describe samples of “good quality, permitting a proper histological examination” (Raphel et al. 1981) and “free of artifacts” (Schatzmann et al., 1974). The quality of biopsies of the present study was evaluated on histological examination of semi-thin probes. As the collapse of alveoli depends on the manipulation of the biopsy specimen and on the direction of the cut of the probe fragment, it was decided not to define a quantitative evaluation scale for this parameter. Further, it was possible to evaluate systematically the bleeding into alveoli induced by the biopsy. There was no significant difference in the amount of erythrocytes found in the alveoli of lung specimen collected with the manual Tru-Cut biopsy needle or with the biopsy device. However, there are more complications such as hematoma at the site of biopsy and bleeding into the airways following biopsy with the manual Tru-Cut biopsy needle compared with the biopsy device.

Major complications after biopsy of the lung appeared to be rare. They consisted of profuse epistaxis in one out of 50 non dyspneic horses and puncture of abdominal organs. Accidental puncture of the liver, the stomach or other abdominal organs can be avoided if the site of the intended biopsy is confirmed with radiography and if care is taken to perform the biopsy at the end of inspiration.

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* Dispomed Witt oHG, Gelnhausen, Germany
* Pro-Mag 2.2, US Biopsy engineered medical products, Franklin, IN, US
* Diasonic Sonotron, Garching, Germany
Fig. 1: Manual Tru-Cut biopsy needle

Fig. 2: Automated biopsy needle

Fig. 3: Chest radiograph of a horse thorax with markers on area of intended biopsy.