STERNAL ASPIRATION OF BONE MARROW IN ADULT COWS

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ABSTRACT

A method for bone marrow aspiration from the sternal marrow cavity of growing cattle has been optimised for routine application in older animals. By means of this technical adaptation, marrow can easily be sampled from the 3rd or 4th sternebrae of adult cattle.

HISTORY AND SIGNIFICANCE OF BONE MARROW RESEARCH

Bone marrow is a complex biological matrix that is neither routinely examined nor commonly used for research purposes in veterinary medicine. Nevertheless, the potential of bone marrow applications in veterinary medicine may be expected to parallel its human counterpart. The main indication for clinical examination of bovine bone marrow in the early sixties was the presence of contagious diseases affecting calves: leukosis, trypanosomiasis and theileriasis. In adult cattle, bone marrow was rarely investigated. Recently, the hypothesis was put forward that immunomodulation at the bone marrow level might occur around parturition in dairy cows. As haematopoiesis in adult cows is restricted to the bone marrow, alterations in the granulopoiesis could be a causal factor for the observed shift in maturation stage of circulating polymorphonuclear leukocytes (Burvenich *et al.*, 1994), resulting in an increased susceptibility to mastitis and endometritis.

A prerequisite for investigations on bovine bone marrow is a simple and reliable technique to obtain active marrow. Literature describing bone marrow sampling in domestic animals is scarce and dates from a couple decades ago. Sternal puncture was the first technique developed for sampling marrow in domestic animals (Calhoun, 1954).This aspiration site was quickly abandoned, however, because of difficulties both in restraining the cows and in penetrating the sternum. Attempts at marrow sampling were then focused on the iliac bone (Calhoun, 1954). However, the specific bovine anatomy made it impossible to reach the marrow cavity of the iliacal crest, which quickly led to the conclusion that this method was unsatisfactory. Finally, aspiration of bone marrow from the dorsal ends of the ribs seemed to be a suitable alternative. Nevertheless, the technique, as described by Lawrence *et al.* (1962), still had several disadvantages: i) the amount of marrow that could be aspirated was very small, ii) the needle used (Turkel trephine needle; Trephine Instruments Inc., USA) took a plug of bone that could easily block the needle lumen; and iii) if penetration was too deep, there was a risk of puncturing the pleural cavity, resulting in pneumothorax. The initial potential of sternal marrow aspiration was therefore reconsidered. Wilde (1961) succeeded in the aspiration of bone marrow from the sternal marrow cavity, using a human sternal needle - 40 mm in length and 14 gauge. His technique became generally accepted and was routinely used in calves. However, in animals older than 18 months it was nearly impossible to penetrate the cortex of the sternum by simple manual pressure. This limitation was probably mainly due to the material rather than to the procedure itself. This became evident when we tested a stronger and longer needle for penetration of the sternebrae of adult cattle. Following comparative trial of several needle types, we found that the paediatric styletted Janus[å] bone marrow needle, 13g x 90 mm (Figure1; Bignell Surgical Instruments Ltd, England) was best suited for the aspiration of bovine bone marrow. This needle has a butterfly handle to facilitate entry into the bone. The tip of the cannula is tapered to allow the sample to expand as it enters, reducing damage to the sample. The instrument can be completely disassembled for cleaning and sterilisation.

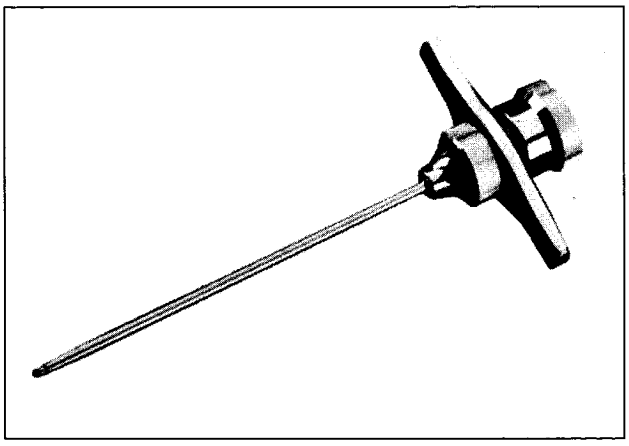

Figure 1. Janus bone® marrow needle, 13 g x 90 mm Bignell Surgical Instruments Ltd, England)

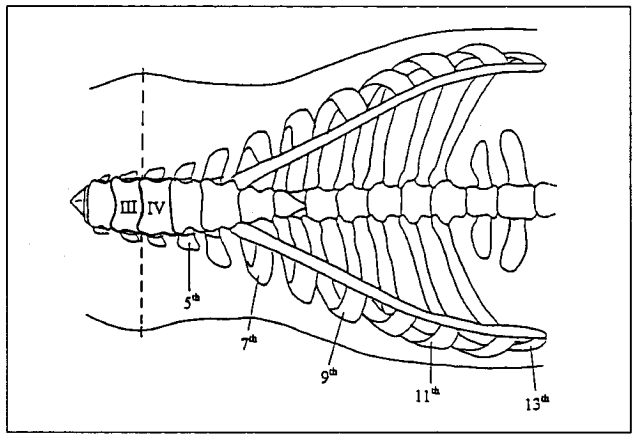

Figure 2. Ventral view of the skeleton of the bovine thorax. The marrow aspiration site, i.e. The 3rd (III) or 4th (IV) sternebrae, is determined by counting of the ribs, starting from the last one. The precise localisation coincides with the intersection of the median line of the sternum and the imaginary line joining the olecranons (dotted line)

STERNAL ASPIRATION OF BONE MARROW IN ADULT COWS

The method for bone marrow aspiration of the sternal marrow cavity of calves described by Wilde (1961) was adapted in order to obtain a suitable technique for adult cows. The procedure was approved by the Ethical Committee of the Faculty of Veterinary Medicine (EC 99/45). Sternal aspirations were performed on six dairy cows of different parities.

The animal was restrained in a head bail; the hind and forelegs were unilaterally secured by a rope, preferably on the right side for a right-handed operator. The cow was sedated with an intravenous injection of xylazine (0.02 mg/kg body weight; Xyl-M 2%; V.M.D., Belgium). The aspiration site (the 3rd or 4th sternebrae) was determined by counting of the ribs, starting from the last one. The precise localisation coincides with the intersection of the median line of the sternum and the imaginary line joining the olecranons (Figure 2). A 10 cm^2 area was shaved and sterilised with 70% ethanol and an iodine surgical scrub (polyvidone-iodine 7.5% solution; iso-Betadine; ASTA Medica, Belgium). All tissue layers in this area - at the deepest part of the pocket between the front limbs – were anaesthetised with infiltration of 7 ml of a 2% lidocaine solution (Xylocaine 2%; Astra Pharmaceuticals, Belgium). A stab incision was then made through the skin and the pectoral muscles, in order to facilitate entrance of the needle. The Janus bone marrow needle (Bignell Surgical Instruments Ltd, England) was introduced on the sternebra under a right angle. Under gentle rotation and with controlled pressure, the needle was pushed upwards until a subtle 'plop' was felt, indicating that the cortex had been pierced and the needle had entered the sternal marrow cavity. The stylet was removed and a 10 ml syringe was fitted onto the cannula. About 2 ml of marrow fluid could thus be aspirated. The cannula was removed with the syringe attached. One catgut suture (Ethicon, Germany) was placed in the skin incision after introducing 5 ml of a benzylpenicilline/dihydrostreptomycine solution (Depomycine 20/20; Mycofarm Belga, Belgium) in the pectoral wound. Three days after bone marrow sampling, the suture was removed. All cows recovered remarkably well and wound healing appeared rapidly.

PRACTICAL CONSIDERATIONS

Up to two days post-operatively, the sternal region was warm and slightly swollen in the six cows. These symptoms were restricted to those observed in the context of an acceptable local tissue reaction. No indication of local infection or systemic illness was observed. We were even able to repeat bone marrow sampling from the sternal marrow cavity of the same animal following a 14 day interval. This finding is in accordance with the observation made by Wilde (1961).

The following critical points should be noted regarding the sternal aspiration method. Firstly, in one cow we did not succeed in the aspiration of marrow fluid. Unsuccessful aspiration can be due to an obstruction of the cannula with trabecular tissue, or to poor needle placement. In the latter case, the cannula should be removed, the stylet replaced and the needle

redirected. Secondly, blood contamination of the marrow sample is inevitable, though it can be minimised by limiting the aspirated volume to 2 ml of marrow liquid. Excessive suction caused damage to blood vessels and sinuses in the marrow, resulting in the mixing of blood into the bone marrow aspirate (Wilde, 1961). For the same reason, it was advised to prepare smears from the very first drops aspirated (Wilde, 1961; Lawrence *et al.*, 1962; Jain, 1986). Thirdly, an anticoagulant was not required when smears were prepared immediately after prelevation. If, for practical reasons, smear preparation was delayed or bone marrow cells had to be isolated subsequently, the sample was transferred into a suitable medium containing an anticoagulant (e.g. 100 U heparin/ml). Cell suspensions were optimally kept cool either on melting ice or at maximally 4°C.

CONCLUSION

Sternal aspiration has already been described for growing cattle up to the age of 18 months (Wilde, 1961). In our hands, bone marrow sampling of adult cows was carried out using an adapted sternal aspiration technique. The most critical factor in overcoming the hardness of the sternum is the quality of the needle. The resulting technique is simple and reliable, and it yields active bone marrow in bovine of all ages. In conclusion, this sampling method can be a valuable tool for studies on bovine bone marrow cell morphology and functionality.

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