MEASUREMENT AND ERROR IN HOOF HORN GROWTH RATE IN SHEEP

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RUNNING TITLE: OVINE HOOF HORN GROWTH RATE

SUMMARY

DETERMINING THE RATE OF HOOF HORN GROWTH IN SHEEP IS IMPORTANT TO UNDERSTAND THE PHYSIOLOGY AND PATHOLOGY OF THE FOOT AND THE IMPACT OF THE ENVIRONMENT AND TREATMENT OF DISEASED FEET ON FOOT HEALTH. IT COULD LEAD TO IMPROVED UNDERSTANDING OF THE INTERACTION BETWEEN HOOF HORN AND PASTURE / BARN FLOOR CHARACTERISTICS AND IN METHODS FOR PREVENTION AND TREATMENT OF OVINE FOOT DISEASES. IN THIS STUDY, THE HOOF HORN WAS MEASURED USING A PREVIOUSLY TESTED PROTOCOL ON ALL EIGHT DIGITS OF 21 HEALTHY YEARLING MULE EWES ON A FARM IN NORTH WALES ON FOUR OCCASIONS OVER A PERIOD OF 53 DAYS. THE MEAN HOOF HORN GROWTH RATE WAS 0.11 MM (S.E. 0.02) PER DAY; THE RESIDUAL ERROR VARIANCE WAS 0.024 AND THE R^2 24.5%. THERE WERE NO SIGNIFICANT DIFFERENCES BETWEEN HOOF HORN GROWTH RATES IN FRONT AND HIND FEET OR BETWEEN MEDIAL AND LATERAL CLAWS OR OVER TIME.

Running Title: Ovine Hoof Horn Growth Rate

Summary

Determining the rate of hoof horn growth in sheep is important to understand the physiology and pathology of the foot and the impact of the environment and treatment of diseased feet on foot health. It could lead to improved understanding of the interaction between hoof horn and pasture / barn floor characteristics and in methods for prevention and treatment of ovine foot diseases. In this study, the hoof horn was measured using a previously tested protocol on all eight digits of 21 healthy yearling mule ewes on a farm in North Wales on four occasions over a period of 53 days. The mean hoof horn growth rate was 0.11 mm (s.e. 0.02) per day; the residual error variance was 0.024 and the R^2 24.5%. There were no significant differences between hoof horn growth rates in front and hind feet or between medial and lateral claws or over time.

Introduction

Hoof horn growth rates have been studied in a number of ruminants (Hahn *et al.,* 1986; Sikarskie *et al.,* 1988; Tranter and Morris, 1992; Smith *et al.,* 1999) and in horses (Buffa *et al.,* 1992; Florence and McDonnell ., 2006) and pigs (Quintanilla *et al.,* 2006). Hoof horn growth rate is influenced by the age (Tranter and Morris., 1992; Dekker *et al.,* 2005) and genetics (Quintanilla *et al.,* 2006) of an animal together with its diet; with vitamins such as biotin (Buffa *et al.,* 1992), minerals such as zinc (Smith *et al.,* 1999) and increased energy intake (Butler and Hintz., 1977) enhancing the rate of growth. Environmental factors that influence hoof growth rate include abrasiveness of the floor surface (Hahn *et al.,* 1986) and ambient temperature (Wheeler *et al.,* 1972).

Cattle and horses have different hoof horn growth rates between feet and between the lateral and medial aspects of the hoof. In Holstein cattle, hoof horn growth rate is greater in the rear than in the front feet and greater in the lateral wall than in the medial wall (Hahn *et al.,* 1986). Vermunt and Greenough (1995) summarised hoof horn growth rate in cattle from 9 studies. The rate ranged from 3.9 - 8.5 mm per month across all studies and horn grew faster in cattle on hard surfaces. In horses hoof horn growth rate is greater in the front than the rear feet (Florence and McDonnell, 2006).

The hoof horn growth rate in horses (Florence and McDonnell, 2006), deer (Miller et al., 1986) and cattle (Telezhenko et al., 2009) has been measured using the coronary band as the baseline and in all cases an indent (made with a drill, file and soldering iron respectively) was made on the hoof wall and the distance between the indent and the coronary band measured to determine growth rate. The intra-observer reliability in Florence and McDonnell (2006) was $r>0.93$. Buffa et al.(1992) and Butler and Hintz (1977) also used the coronary band as a marker in horses. An inverted T-shape was heat branded into the hoof from the coronary band and progression distally was measured. The use of a T-shaped marker provides more accuracy than a groove as the meeting point of the two lines making the T allows exact pinpointing for repeat measurements. Livesey and Laven (2007) used tattoo marks just proximal to the coronary band and burn marks (made with a soldering iron) in the hoof as points of reference in cattle. These authors considered that the tattoo was more precise than the coronary band as a reference line. The width of the burn mark (around 2mm) determined the repeatability of measurements in this study.

A greater understanding of ovine (*Ovis aries*) hoof horn growth rate would contribute to our understanding of the normal physiology of the sheep claw and the consequences of overgrowth, extreme wear or trimming hoof horn in diseased and healthy feet (Wassink *et al.,* 2003a; Kaler *et al.,* 2010a). It would also assist in understanding the role of hoof horn in persistence and transmission of infectious foot diseases, such as footrot (Green *et al.,* 2007; Kaler *et al.,* 2010b), contagious ovine digital dermatitis (Wassink *et al.,* 2003b) and of pathogens such as foot and mouth disease virus (Dekker *et al.,* 2005).

To date there have been only two studies estimating hoof horn growth rates in sheep (Wheeler *et al.,* 1972; Dekker *et al.,* 2005). Dekker et al., (2005) measured the growth of hoof horn in five healthy newborn lambs and five healthy ewes. The horn growth was measured from the coronary band to a horizontal groove made in the right lateral claws of the front and hind feet using a hacksaw. Measurements were taken on days 3, 10, 17, 24, 31 and 59, and on each occasion the distance between the groove and the coronary band was measured using a ruler and was rounded to the nearest mm (Dekker *et al.,* 2005). Dekker *et al.* (2005) reported that using the coronary band as a baseline for measurements led to measurement error. The growth rates were 0.44mm per day in the lambs and 0.29mm per day in the ewes. There was no difference between the growth rate in front and hind feet. Wheeler *et al.* (1972) studied hoof horn growth rate under varying temperatures and day length. There was no association between day length and growth rate, but sheep kept in low ambient temperatures had a lower hoof horn growth rate. In their four experiments growth rates ranged widely from 0.024 to 0.2 mm/day. Group sizes ranged from eight to 21 sheep per experiment and they investigated one foot in three experiments, and two feet in one experiment.

Here we present a novel approach to measure hoof horn growth and estimates of precision of this technique in ovine hoof horn in non-lame sheep.

Materials and methods

Sheep and environment

A convenience sample of 21 non lame yearling mule ewes was selected from a flock of 110 yearling ewes in Gwynedd, North Wales. Sheep were on lowland pasture and were not given any food supplement. Data were collected in August and September 2009.

Measuring hoof horn

Pilot studies at the University of Warwick had investigated a variety of methods to measure hoof horn growth. From these it was concluded that the coronary band varied in width by several mm between weekly observations and that this was particularly a concern in sheep with diseased feet (unpublished observation). It was also noted that variation in the angle of measurement from the coronary band to a horizontal line on the hoof horn was possible. As a consequence the final pilot approach was tested where a line in the hair above the coronary band was shaved and the horn was marked with two horizontal grooves and one vertical groove (Figure 1). This was considered to give the most repeatable measurements.

In the current study, the hair above the coronary band of each digit was shaved using wireless clippers (Kim Laube, Lazor clip) and then an ‘X’ shape was shaved deep into the hair above the coronary band on each claw using a beard trimmer (BaByliss for Men Battery Beard Trimmer 7050BU). Two horizontal 0.5mm wide grooves and

one vertical groove were filed into the claw using a file (Nicholson, Three Square Smooth Cut File, 8") (Figure 1).

Measurements were made on days 0, 24, 40 and 53 using digital callipers (Linear, 49-923-150, 150mm/6") in mm rounded to 2 decimal places. The distance from the centre of the shaved 'X' to each of the two horizontal lines at the point at which the vertical groove intercepted each horizontal line (A and C) and the distance (B) between the two horizontal lines were measured (Figure 1).

Data analysis

Claws were excluded when less than two measurements were made, when the marks had faded from the foot between measurements, when a foot was deformed or diseased and when a ewe was lame.

To check the accuracy of the recordings, the variation of A (Figure 1) was assessed for repeated measurements within digit. The sum of A + B was calculated for each measurement and compared with C using a scatter graph to identify outliers and estimate correlation (Figure 2a). Where $A + B \neq C$ the raw data were checked and amended where possible or data were excluded where an error could not be detected. The growth rate was calculated for B for each interval 0 – 24, 24 – 40 and 40 – 53 days. A histogram of the estimated growth rate of B was plotted (Figure 2b) and outliers noted. The growth rate was used as the outcome variable for a three- level hierarchical model with repeated measure within foot within sheep that took the form

$$Y_{ijk} = \alpha + \beta X_{ijk} + \beta X_{jk} + \beta_k X v_k + v_k + u_{jk} + e_{ijk}$$

Where Y_{ijk} = hoof horn growth rate per day for each digit, α = intercept and βX is the vector of fixed effects varying by measurement (ijk), digit (jk) and sheep (k) and v_k is the between sheep residual variance, u_{jk} the between digit residual variance and e_{ijk} is the between measurement residual variance. The model fit was checked and the R^2 calculated.

Results

Missing and excluded data

From a possible 672 digit observations (4 occasions, 8 digits per ewe) there were 372 estimates of growth rate. There were 74 digits from 20 sheep with all four measurements, giving 222 growth rates. There were 108 measurements where sheep were lame and 127 where marks on the skin faded between observations. The mean length of A+B and C were 36.6mm (+/- 0.28) and 36.8mm (+/- 0.28) respectively with a correlation of 0.97. There were four outliers at both ends of the distribution (Figure 2b) that were excluded from further analysis.

Growth rate

The mean (s.e.) hoof horn growth rate based on both 372 and 222 sample estimates was 0.11 (+/- 0.02) mm per day. This equates to 3.6 mm growth per month (30 days). There were no significant differences in the growth rates between front and rear feet (0.0 +/- 0.02 mm) or lateral and medial digits (-0.01 +/- 0.02 mm). The between measurement residual error variance was significant at 0.024 +/- 0.002 (giving a s.d. of 0.15) and R^2 = 24.5%. There was no significant difference in growth rate between sheep or digits. When the growth rates of digits with all three measurements were compared there was no difference between daily growth rates by time points in the study.

Discussion

Precision and repeatability were important concerns in this study. Overall, after excluding outliers, the measurement error variance was 0.024 across all 372 positive measurements. In earlier pilot studies we had observed that the coronary band varied in depth within a digit over time (data not shown) and this, together with the fact that it is a horizontal line, made it difficult to identify a consistent point from which to measure vertically down to hoof horn. Consequently, we placed a cross in the hair above the coronary band to give a point to measure from. This cross faded rapidly and one error in the current study was that on 127 occasions the baseline cross had disappeared by the subsequent measurement. Ideally, a permanent mark, such as a tattoo, would have been used (Livesey and Laven 2007). The ethics justifying the possible pain involved in applying the tattoo are debatable but once applied a sheep could be handled less frequently, so reducing stress, because the mark above the coronary band would be permanent. It might make longer term studies, such as impact of season, more feasible especially since the current study does suggest that not many sheep would need to be studied within one age group because hoof horn growth rate did not vary significantly between sheep.

Measurement errors also arose from the width of the marks on the skin and grooves in the horn (for example, the filed grooves were approximately 0.5mm wide), which would have reduced precision in the results and also explain the apparent negative growth rates. Significant unexplained variability was only present in repeated measures within digit and was probably mostly measurement error. It was not reduced when we included only digits with three repeated measurements in the analysis, indicating that this was not an issue of insufficient sample size.

Taking measurements A, B and C and using C = A + B to check for data recording errors and plotting a histogram helped to identify large outliers in the analysis. One individual measuring the sheep throughout the study prevented between observer bias.

This study was conducted during August and September 2009. Previous studies (Tranter and Morris, 1992; Wheeler *et al.*, 1972) have reported that hoof horn growth rate is less at colder temperatures; in dairy cattle in New Zealand the rates of hoof

horn growth and wear were lowest in winter and greatest in summer. Wheeler *et al.,* (1972) hypothesised that this was because there was a decreased blood supply to the distal limb. If this is also the case in sheep then the growth rate calculated in this study is likely to be greater than growth rates during winter.

There were no significant differences in growth rates between front and rear feet in our study, this was also reported by Dekker *et al.,* (2005), suggesting that the fact that 60% of the weight is taken by the front feet of sheep (Kim and Breur, 2008) is not sufficient to cause greater wear of front feet that would stimulate a faster growth rate as reported in cattle (Vokey *et al.,* 2001).

The growth rate of 0.11 mm per day or 3.6 mm per month in our study was in the same range as results from Wheeler *et al.,* (1972) and for cattle on pasture. However, it is considerably less than the 0.29 mm per day (8.7 mm per month) calculated by Dekker et al., (2005) in five sheep. The difference between the two estimates may be due to differences in environmental factors such as ambient temperature or nutritional status, age or genetics or because the floor surface in the Dekker *et al.,* (2005) study was hard (the sheep were housed) whilst the sheep in the current study were at pasture. Differences in experimental techniques may also account for some of the differences in growth rate. In our study digital callipers were used to take measurements and distance was measured from a distinct point in the centre of the shaved 'X' to the intersections of the grooves, whereas Dekker et al (2005) used a ruler and the distance was measured from two lines, which may have reduced the accuracy of their measurements but it is unlikely to account for all of the difference.

A traditional technique to treat footrot in sheep has been to trim away horn to expose the lesion in the foot (Wassink *et al.,* 2003; Kaler *et al.,* 2009). This often requires trimming horn from the wall of the foot. Recent research has indicated that trimming hoof horn of sheep with footrot delays healing compared with treatment with systemic antibiotics and topical spray (Kaler *et al.,* 2010a). From our study we estimate that hoof horn trimmed by 1cm will take approximately 3 months to re-grow. If sensitive tissue is exposed during trimming (and this is the aim of therapeutic trimming) then in this 3 month period a sheep might be more likely to damage the exposed sensitive tissue and more likely to develop footrot again (Kaler *et al.,* (2010b).

Hoof horn growth rate varies by age (Dekker *et al.,* 2005), nutritional status (Buffa *et al.,* 1992; Smith *et al.,* 1999; Butler and Hintz., 1977) and hardness of the walking surface (Vokey *et al.,* 2001) and so these were kept consistent in the current study. In addition, breed was consistent, although the mule breed is a cross of two pedigrees. Consequently, the results give an estimate of hoof horn growth rate for one year old, mule sheep on pasture. We would expect this to vary by age, breed and environment.

The current study has been an important step in establishing a procedure to measure hoof horn growth rate in sheep and has highlighted improvements that can be made

in future studies. Future studies can use this technique to investigate whether the rate of hoof horn growth varies by breed of sheep and hardness of walking / standing surface. Importantly how infectious foot disease (footrot) and therapeutic and corrective foot trimming affect hoof horn growth rate would be of great interest.

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Figure 1: Markings in hair and horn and distances A, B and C used to measure hoof horn growth.

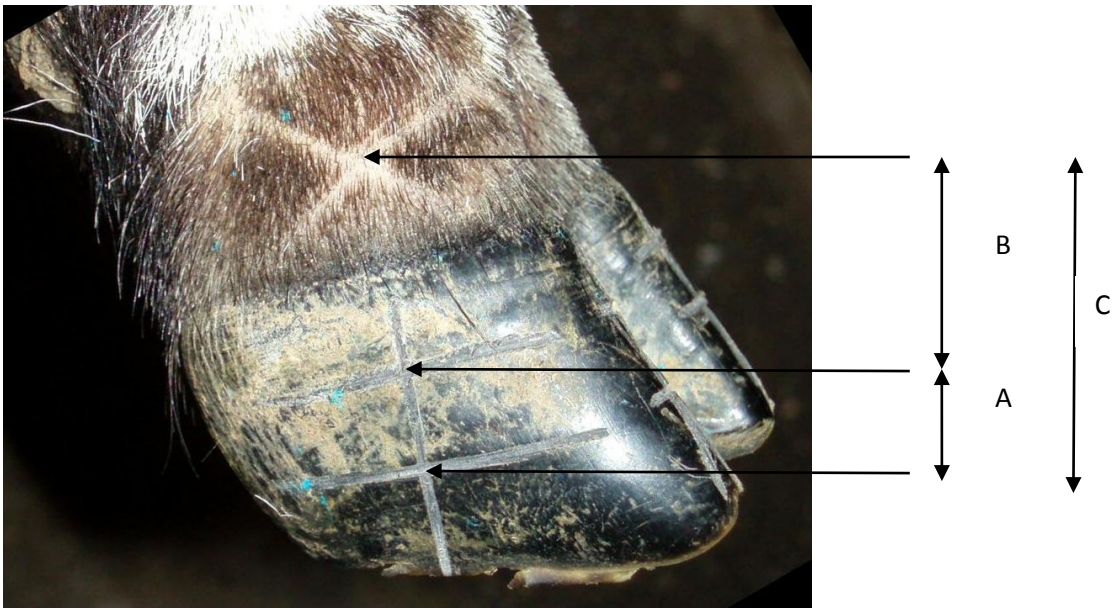

Figure 2a Scatter plot of (A+B) against C in mm in 373 observations

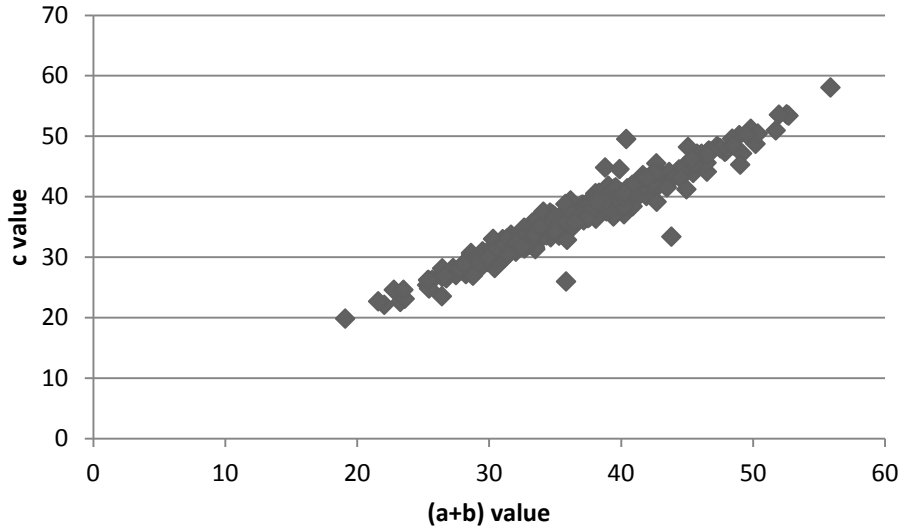

Figure 2b Histogram of hoof horn growth rate per day (four clear outliers at either end of the distribution)