Determination of maternal pedigree and ewe–lamb spatial relationships by application of Bluetooth technology in extensive farming systems

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ABSTRACT: The objectives of this study were to validate the application of Bluetooth technology to determine maternal pedigree and to determine ewe–lamb spatial relationships in extensive farming systems. A total of 35 first-cross Merino ewes (Merino × Border Leicester and East Friesian) and 23 of their lambs aged 1 to 3 wk were fitted with activity monitors equipped with Bluetooth (BT) technology (ActiGraph wGT3X-BT) by means of halters and collars, respectively. The BT devices on lambs were programmed to receive wireless signals once every minute from nearby BT units on ewes, which were programmed as beacons sending BT signals 4 times every second. Ewes and lambs fitted with sensors were dispatched into the paddocks, and after 10 d, the sensor units were retrieved and the BT signals received by lambs were downloaded using the ActiGraph software. The maternal pedigree of the lambs was determined as the ewe from which the lamb received the most BT signals. The distance between the lamb receiving the signal and the ewe sending the signal was estimated from the strength of BT signal received. The pedigree determined by BT was compared with the pedigree determined by DNA

profiling and verification. The results showed that the accuracy of maternal pedigree determined by BT signals reached 100% within the first 15 min of returning animals to pasture of ewes and lambs fitted with sensors. Maternal signals (counts/d) received by 1-, 2-, and 3-wk-old lambs were 617 ± 102 , 603 ± 54 , and 498 ± 36 , respectively, and the corresponding nonmaternal signals received were 140 ± 27 , 106 ± 30 , and 155 ± 39 , respectively. Maternal signals received during the dark period were significantly higher than the maternal signals received during the light period ($P < 0.05$). Maternal signals received during the light period by 3-wk-old lambs were significantly lower when compared with those received by 1- and 2-wk-old lambs. Over 90% of the BT signals received from within 2 m of the lamb were from its mother. The maternal BT signals expressed as a portion of total BT signals decreased with increasing distance from the lamb. The results show that BT wireless networking is a fast and reliable method for the determination of maternal pedigree of lambs in extensive farming systems. In addition, wireless BT technology is also useful in determining mother–offspring spatial relationships.

Key words: Bluetooth, deoxyribonucleic acid testing, ewe, lamb, maternal pedigree, spatial relationship

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INTRODUCTION

Genetic selection in a sheep flock heavily relies on selection of sires. Further improvements in genetic gain are possible by selecting superior dams

for breeding. However, current methods of determination of maternal pedigree suitable for extensive farming systems, such as genetic testing and electronic tag–based methods, are not widely adopted by farmers due to the high cost and/or poor accuracy. As a result, the majority of breeding flocks in Australia kept in extensive farming systems have little or no record of maternal pedigree. A cheaper and accurate method of determination of maternal parentage is necessary to make large-scale adaptation by farm-

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ers possible. Bluetooth (BT) is a wireless technology standard for exchanging data over short distances, and BT devices previously have been shown to be effective in analyzing proximity and tracking spatiotemporal movement in humans (Versichele et al., 2012). We hypothesized that by determining the proximity of ewes to their lambs using BT technology, it is possible to determine maternal pedigree. In addition, the frequency and strength of the BT signals received by lambs were used to investigate the effects of the age of the lamb and the time of the day on the spatial relationships between ewe and lamb under farm conditions.

MATERIALS AND METHODS

All the procedures and experimental protocols were in accordance with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (NHMRC, 2013) and were approved by the La Trobe University Animal Ethics Committee.

Animal Management

This study was conducted at the Northgate Park commercial sheep farm, Greta, VIC, Australia, in June 2015. A total of 35 twin-bearing ewes were kept in a 9-ha paddock with fairly uniform grazing consisting of predominantly perennial ryegrass and clover and 5% phalaris and 5% other annual grasses. All 35 ewes and 23 of the 70 lambs aged 1 to 3 wk were fitted with sensors for the study.

Sensors

Commercially available sensors equipped with BT technology (ActiGraph wGT3X-BT; ActiGraph, LLC, Pensacola, FL) were used in the study. The sensors were programmed either as a receiver or as a beacon. The receivers were fitted onto a collar (50 cm long and 2.5 cm wide) using cable ties and attached to lambs. The beacons were fitted onto sheep halters using cable ties and attached to ewes. The receivers were programmed to scan for signals from the beacons once a minute, whereas the beacons were programmed to send signals at 4 Hz (4 times a second). The ewes and lambs fitted with sensors were released as one large mob onto the paddocks, and after 10 d, the collars and the halters were retrieved. The BT signals received by the sensors on lambs were downloaded using ActiLife software (ActiGraph, LLC).

Blood Sample Collection, DNA Testing, and Pedigree Verification

Blood samples (10 mL) were collected from a jugular vein of ewes using vacutainer tubes with heparin as an anticoagulant. A small amount (1 to 2 g) of tail tissue was collected from lambs during tail docking and stored at -20°C in sterile plastic tubes until analysis. Blood samples were centrifuged at $1,500 \times g$ for 10 min at 21°C , and the layer containing white blood cells (the buffy coat) was collected and kept at -20°C until analysis for DNA. The samples were sent to Zoetis Australia (Zoetis Inc., Banyo, QLD, Australia) for DNA parentage testing. The DNA was analyzed using Pfizer Animal Genetics (Banyo, QLD, Australia) DNA profiling and analytical software to determine parentage.

Conversion of Signal Strength to Distance

The distance between the beacon and receiver was calculated from the received signal strength indicator (RSSI) values received by the sensors using the following equation: $\text{RSSI (decibel-milliwatts [dBm])} = -65.5817 + 20 \log_{10} \text{distance (m)}$, in which -65.5817 represents the signal gain and loss of hardware estimated from measured RSSI values of known distances in the range of 0.5 to 12 m. A detailed explanation of the derivation of the formula for conversion of RSSI is given in the Appendix.

Statistical Analysis

Data were analyzed by IBM SPSS Statistics 23 software (IBM Corp., Armonk, NY) using the general linear model with age of lambs (1, 2, or 3 wk old) and signal type (maternal or nonmaternal) as fixed factors and BT signals received as a dependent variable. Bluetooth signals were expressed as cumulative counts per hour or as counts per day. For analysis of BT signals at various distances from a lamb, the number of signals received at 0 to 11.99 m, 2 to 3.99 m, 4 to 7.99 m, 8 to 11.99 m, and 12 to 24 m from the lamb were determined and treated as dependent variables for the analysis. Significance was declared at $P < 0.05$.

RESULTS

Figure 1 shows the maternal and nonmaternal signals received from lambs for the first 60 min after dispatching the ewes and lambs into the paddocks. As expected, the cumulative counts received by lambs from both maternal and nonmaternal ewes steadily increased. The maternal signals received by lambs showed a more rapid rise, reaching approximately 30 signals by the end of the first hour. Nonmaternal sig-

Figure 1. Bluetooth signals (cumulative counts) received by lambs from maternal and nonmaternal ewes for the first 60 min after dispatch. Values are mean $\pm$ SE.

Figure 2. Accuracy (%) of determination of maternal pedigree by maternal Bluetooth signals received by lambs. Maternal pedigree was determined as the ewe from which the lamb received the maximum number of signals. Accuracy was determined by comparing the maternal pedigree by Bluetooth with the pedigree determined by DNA analysis and verification. Values are mean $\pm$ SE.

nals received by lambs were much smaller, reaching approximately 7 signals by the end of the first hour.

The accuracy of pedigree by BT was determined by comparing the results with the pedigree determined by DNA testing (Fig. 2). The accuracy of the BT technology to determine pedigree reached 100% within the first 15 min of dispatch of ewes and lambs fitted with sensors onto the paddocks.

The number of daily BT signals received by lambs is shown in Table 1. Signals received by lambs from maternal ewes were more than 3 times higher than the number of signals received from nonmaternal ewes ($P < 0.001$). Maternal signals received by 3-wk-old lambs were approximately 20% lower than the number of maternal signals received by 1- and 2-wk-old lambs ($P < 0.05$). At distances less than 2 m from the lamb, more than 90% of the signals received were from the maternal ewe. As expected, maternal signals received decreased with increasing distance from the lamb: from more than 150 signals per day at distances less than 2 m to less than 30 signals per day at distances ≥ 12 m. Not surprisingly, signals from maternal ewes were signifi-

cantly higher than the signals from nonmaternal ewes at all distances except at distances ≥ 12 m ($P < 0.05$).

Figure 3 shows BT signals received by lambs aged 1 to 3 wk throughout the day. Maternal signals received by lambs showed a clear pattern: a gradual increase from 0000 to 0600 h and a gradual decrease from 0600 to 1700 h followed by a gradual recovery. Maternal signals received by 3-wk-old lambs were significantly fewer than the maternal signals received by 1- and 2-wk-old lambs ($P < 0.05$). No clear pattern of nonmaternal signals received by lambs was observed.

Maternal signals received by lambs during light (0730 to 1700 h) and dark periods (1700 to 0730 h) are shown in Fig. 4. Overall, maternal signals received by lambs during the light period were significantly fewer than corresponding signals received during the dark period. Three-week-old lambs received a significantly lower number of signals when compared with the corresponding signals received by 1- and 2-wk-old lambs ($P < 0.05$).

Table 1. Bluetooth signals (counts/d) received by lambs aged 1 to 3 wk at various distances from lamb. Data are expressed as mean (SD)

Distance from lamb	Signals from maternal ewe, counts/d			Signals from nonmaternal ewe, counts/d		
	1-wk-old lambs	2-wk-old lambs	3-wk-old lambs	1-wk-old lambs	2-wk-old lambs	3-wk-old lambs
0 to 1.99 m	200 (52) ^{a*}	187 (27) ^{a*}	153 (32) ^{b*}	11 (5)	10 (6)	14 (7)
2 to 3.99 m	133 (23) ^{a*}	139 (20) ^{a*}	111 (11) ^{b*}	18 (7)	13 (5)	20 (7)
4 to 7.99 m	157 (24) ^{a*}	157 (17) ^{a*}	133 (14) ^{b*}	37 (8)	28 (8)	41 (9)
8 to 11.99 m	101 (24) ^{a*}	96 (11) ^{a*}	80 (11) ^{b*}	39 (7)	30 (7)	44 (10)
12 to 24 m	27 (9) ^a	24 (6) ^{ab}	21 (5) ^b	35 (13)	25 (6)	36 (12)
Total	617 (102) ^{a*}	603 (54) ^{a*}	498 (36) ^{b*}	140 (27)	106 (30)	155 (39)

^{a,b}Indicate differences between age groups; means with different superscripts differ significantly ($P < 0.05$).

*Indicates significant difference in maternal and corresponding nonmaternal signals received by lambs within the same age group ($P < 0.05$).

Figure 3. Bluetooth signals (counts/h) received by lambs aged 1, 2, and 3 wk from maternal and nonmaternal ewes throughout a 24-h period. Values are expressed as hourly Bluetooth signals (mean $\pm$ SE). Superscripts denote significant differences ($P < 0.05$) in the maternal signals received: ^aSignificant difference between 3-wk-old lambs and 1- and 2-wk-old lambs; ^bsignificant difference between 3-wk-old lambs and 2-wk-old lambs; ^csignificant difference between 1-wk-old lambs and 2- and 3-wk-old lambs

DISCUSSION

To our knowledge, this is the first report of determination of maternal pedigree of lambs in extensive farming systems by the application of BT technology. Knowing the maternal pedigree of lambs is an important part of genetic selection and improvement of productivity of sheep. Current methods used in Australia to determine the maternal pedigree of lambs include DNA profiling and verification, catching and tagging each lamb at birth (mothering up), and Pedigree Matchmaker (PMM; Meridian Agriculture, Yendon, VIC, Australia)—a walk-by system in which animal radio frequency identification data is repeatedly recorded over several weeks to estimate the association between ewes and their lambs (Morris et al., 2012). The first 2 methods are either impractical or too expensive to be used in large commercial herds, and PMM requires several weeks of collecting data and suffers from poor accuracy. The results of the current study show that BT technology provides a rapid and accurate alternative test to determine maternal pedigree. Although the determination of pedigree by PMM and BT are based on the same principle of proximity between maternal ewe and its lamb, the proximity with BT is determined under more natural conditions compared with PMM, where it is necessary to round up ewes and lambs to move them through a walkway. In addition, wide application of BT technology in various platforms including mobile phones has helped in the production of low cost and more reliable sensors (Sørensen et al., 2017). It is noteworthy that whereas DNA profiling and verification is the most accurate method to determine the biological mother, BT technology provides accurate data on moth-

ering of lambs and is less invasive than tissue sampling for DNA testing. We suggest that by combining the results of DNA testing and BT data, it is possible to determine the prevalence of cross-mothering by ewes under extensive farming systems.

In sheep, a strong maternal-offspring bond is formed soon after birth (Smith et al., 1966; Poindron et al., 2007). Maternal selectivity is established in ewes within 4 h after lambing when the mother accepts only her own lambs and rejects alien lambs (Mora-Medina et al., 2016). A strong ewe-lamb bond is essential for lamb survival, and maternal behavior is an important factor affecting lamb survival (Dwyer, 2014). The maternal behavior score, a 5-point scale assessment of maternal response to handling and tagging of her lambs (O'Connor et al., 1985), has been used to estimate lamb survival and performance (Everett-Hincks et al., 2005; de Moraes et al., 2016). Our results show that it is possible to identify ewes that maintain close proximity to their lambs, under field conditions without human interference, by BT technology. Moreover, visual assessment of behavior is possible only during the daytime, whereas our data indicates that the ewe-lamb interactions were more frequent during the dark period. A lower frequency of ewe-lamb interactions in the case of 3-wk-old lambs compared with 1- and 2-wk-old lambs is consistent with previous reports of decreasing mother's interest in her lamb with increasing age of the lamb (Dwyer, 2014; Maldonado et al., 2015).

Previous studies of visual observation of the spatial relationships between Merino ewes and lambs during the daytime reported that the distance between the ewe and its lamb increased with the age of the lamb from 1 to 3 wk (Morgan and Arnold, 1974; Hinch et al., 1987).

Figure 4. Maternal signals (counts) received by lambs during light (0730 to 1700 h) and dark (1700 to 0730 h) periods. Values are mean $\pm$ SE. ^{a,b}Within each period, means with different superscripts significantly differ ($P < 0.05$).

In our study, the strength of the BT signal was used to estimate the distance between ewes and lambs. The estimated distances are very likely overestimated, because interferences under field conditions weaken the signal. However, unlike visual observation and video recordings, BT technology is able to continuously collect proximity data during both day and night times. Our results, in addition to confirming the effect of age of the lamb on ewe–lamb distance, show that ewes spend more time with their lambs during the dark period.

It is interesting to note that the maternal BT signals received by lambs in the first 15 min after returning animals to pasture showed a rapid and linear increase, after which a slower increase was observed. This may indicate a strong need for the maternal ewe to find its offspring when the animals were returned to pasture as a single mob. After the first 15 min, the ewe may allow its lamb to move away under a more relaxed field condition.

A sharp decline in the maternal BT signals received after 0730 h and an abrupt increase at 1700 h correspond with sunrise and sunset, respectively. A likely explanation for the decline at 0730 h is that the ewes are able to keep visual contact with their lambs from a longer distance after sunrise. The sharp increase in the maternal signals received at 1700 h may indicate a strong maternal instinct to keep a closer proximity with the offspring in the dark period. The fewer number of maternal BT signals that were received by lambs coincided with the grazing period between 0900 and 1600 h. Fewer maternal BT signals received by 3-wk-old lambs compared with 1- and 2-wk-old lambs is consistent with a previous report of an increase in the mean distance between ewe and lamb with the age of lamb (Morgan and Arnold, 1974).

Minor problems encountered in our study included detachment of sensors from ewes and lambs (less

than 2%) due to halters and collars slipping off and loss of data due to malfunctioning of sensors (less than 1%). It must be pointed that BT technology must be combined with another form of animal identification such as a radio frequency identification system, because it is not possible to maintain animals' identity once the BT device is removed from the animal.

In conclusion, in addition to a rapid and accurate determination of maternal pedigree, Bluetooth technology is effective in continuously assessing the ewe–lamb spatial relationship without the need for visual observation and video recording.

APPENDIX

For the calculation of distance from the received signal strength indicator, we use a modification of the well-known Friis transmission equation (see, e.g., Sklar, 2001; Shaw, 2013). This modification takes the form

$$P_{RX} = P_{TX} - L_{TX} + G_{TX} - L_{FSPL} + G_{RX} - L_{RX} \quad [1]$$

in which P_{RX} is the received power (dB–milliwatts [dBm]), P_{TX} is the transmitter output power (dBm), L_{TX} is the transmitter losses (dBm), G_{TX} is the transmitter antenna gain (dBm), L_{FSPL} is the free space path loss (decibel–milliwatts [dBm]), G_{RX} is the receiver antenna gain (dBm), and L_{RX} is the receiver losses (dBm).

The expression for the free space path loss (FSPL), can be written as

$$L_{FSPL} = 20 \log_{10}[4\pi(\text{distance}/\text{wavelength})] \quad [2]$$

This equation can further be expressed in decibels as

$$L_{FSPL} = -27.55 + 20 \log_{10}(\text{frequency}) + 20 \log_{10}(\text{distance}), \quad [3]$$

in which L_{FSPL} is expressed in decibels, frequency is expressed in megahertz, and distance is expressed in meters.

Therefore, the hardware is same in all ActiGraph sensors (ActiGraph, LLC) and the signal gain and losses of hardware are constant (P_{TX} , G_{TX} , G_{RX} , L_{TX} , L_{RX} , and frequency). Therefore, combining Eq. [1] and [3] and grouping all the constant values in only one constant C , we have the following simplified expression for the received power P_{RX} :

$$P_{RX} = C + 20 \log_{10}(\text{distance}), \quad [4]$$

in which P_{RX} is expressed in decibels and distance is expressed in meters.

This expression relates the distance between a transmitter and a receiver with the received power (P_{RX}). If we set up an experiment, where the distance is a known priori, and we measure the received power P_{RX} , then it is possible to estimate the value of the constant C by using a statistical approximation, such as least squares estimation (see, e.g., Söderström and Stoica, 1988; Ljung, 1999). Using least squares, we found a value of $C = -65.5817$. Therefore, if we have measurements of the P_{RX} and we know the value of C , then it is possible to find the distance (in meters) by solving Eq. [4].

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