PEER REVIEWED

Behavior of Cattle Towards Devices to Detect Food-Safety Pathogens in Feedlot Pens

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A contribution of the University of Nebraska Agricultural Research Division, Lincoln, NE 68583. Journal Series No. 13520

Abstract

Our objective was to optimize a protocol using rope devices designed to detect *Escherichia coli* O157:H7 in feedlot pens by 1) determining if the number of devices influenced the percent of cattle sampled, 2) describing when the cattle contacted the devices, and 3) determining if the time of removal or number of devices influenced the recovery of *E. coli* O157:H7. Overall, 2948 cattle were observed in 24 commercial feedlot pens, eight pens each in the autumn, winter and summer. Three or seven devices per pen were placed near the water tank and over the feed bunk approximately one hour prior to sunset. Cattle were observed for a 2-hour period to measure 1) the duration of time until they made contact with the devices, and 2) the type of contact they had with the devices. One tail from each of the devices was collected at the end of the 2-hour observation period (ending approximately one hour after sundown) and the other tail was collected the next morning to test for the presence of *E. coli* O157:H7. Recovery of *E. coli* O157:H7 was not significantly different using three or seven devices ($p>0.10$), or if devices were left available overnight ($p>0.10$). The rate of first contacts did not differ between pens with three or seven devices in any of the 30-minute periods of observation ($p>0.50$). However, regardless of the number of devices in the pen, the first contact rate was highest in the first 30 minutes and decreased significantly with time ($p<0.001$). Over the 2-hour observation period a greater percentage of cattle in pens with seven devices had physical ($p<0.01$), brief oral ($p<0.02$), or prolonged oral contact ($p<0.01$) than cattle in pens with three devices. Therefore, to maximize the number of cattle sampled per pen, the number of devices and length of time the devices are available were important factors of the sampling strategy.

Résumé

Notre objectif était d'optimiser un protocole se servant d'un dispositif de cordes dans le but de détecter la présence de *E. coli* O157:H7 dans des parcs d'engraissement 1) en déterminant si le nombre de dispositifs avait une influence sur le pourcentage d'animaux échantillonnés, 2) en décrivant quand le bétail entrait en contact avec le dispositif et 3) en déterminant si le temps de retrait ou le nombre de dispositifs influençaient le recouvrement de *E. coli* O157:H7. En tout, 2948 animaux ont été observés dans 24 enclos de parcs d'engraissement commerciaux répartis également entre les saisons d'automne, d'hiver et d'été. Soit trois ou soit sept dispositifs par enclos étaient placés près du réservoir d'eau et au-dessus de la mangeoire une heure avant le coucher du soleil. Le bétail était observé pendant une période de 2 heures pour mesurer 1) le temps nécessaire pour approcher les dispositifs une première fois et 2) le type de contact avec les dispositifs. Une portion terminale de chaque

dispositif était ramassée à la fin de la période de 2 heures (finissant approximativement près d'une heure après le coucher du soleil) et l'autre portion était ramassée le lendemain matin pour vérifier la présence de *E. coli* O157:H7. Le recouvrement de *E. coli* O157:H7 n'était pas différent selon que trois ou sept dispositifs étaient utilisés ($p > 0.10$) ou selon que les dispositifs étaient présents durant la nuit ($p > 0.10$). Le taux d'approche initiale n'était pas différent lorsque les enclos étaient munis de trois ou sept dispositifs pour chacune des périodes d'observation de 30 minutes ($p > 0.50$). Toutefois, peu importe le nombre de dispositifs par enclos, le taux initial de contact était plus élevé pendant les premières 30 minutes et diminuait par la suite significativement avec le temps ($p < 0.001$). Pendant la période d'observation de 2 heures, la proportion d'individus qui eurent un contact physique ($p < 0.01$) ou un contact oral bref ($p < 0.02$) ou prolongé ($p < 0.01$) avec les dispositifs était plus élevé dans les enclos avec sept dispositifs que dans ceux munis de trois dispositifs. Donc, pour maximiser le pourcentage d'individus échantillonnés par enclos, le nombre de dispositifs et la durée de leur présence dans les enclos étaient des facteurs importants pour la stratégie d'échantillonnage.

Introduction

An accurate and economical diagnostic strategy is needed so that the presence of *Escherichia coli* O157:H7 can be monitored within feedlot cattle populations. A strategy for pen-level detection of this pathogen has been developed using a device prepared from rope which is placed over feed bunks or water tanks (Smith *et al,* unpublished). When the devices are placed in feedlot pens, the cattle are curious enough to approach the ropes and rub, lick or chew them. The premise of this pen-testing strategy is to gain diagnostic efficiency by culturing a few devices from which numerous cattle could have contributed organisms, so that the probability of recovering *E. coli* O157:H7 from the pen of cattle, if present, is maximized with a minimal number of samples. The devices are relatively simple to use and could reduce the cost of monitoring pens of feedlot cattle compared to testing individual cattle.

Our objective was to optimize the protocol for aggregate detection of *E. coli* O157:H7 in cattle feedlot pens by 1) determining if the number of devices influenced the percent of cattle sampled, 2) describing when the cattle contacted the devices, and 3) determining if the time of removal or number of devices influenced the recovery of *E. coli* O157:H7.

Materials and Methods

The behavior of cattle to the pen-testing devices was measured to understand how the devices might be used as part of an *E. coli* O157:H7 detection protocol. The devices were prepared from 32-inch (81.3 cm) lengths of 1/2-inch (1.27 cm) diameter manilla rope. Devices were attached at the middle to pipe or cable so that each had two tails of approximately 10 inches (25 cm). Devices were placed near the water tank (Figure 1) and over the feed bunk; each device spaced approximately 13.1 ft (4 meters) apart. The devices were made available to the cattle approximately one hour prior to sunset. Twenty-four pens of feedlot cattle were each observed for two hours after placement of the devices: 12 pens with three devices, 12 pens with seven devices. Pens receiving different numbers of devices were matched by the approximate number of cattle, age of cattle and week of observation. Eight pens were observed in each of three seasons: autumn, winter, and summer. The cattle's behavior toward the devices was recorded for a 2-hour period, however the cattle had access to a portion of the devices overnight (Figure 1).

Two observers per pen recorded the animals' activity. Cattle were identified by numbered ear tags, and were observed for the duration of time until they had contact with the device and the type of contact with the devices. The degree of contact was recorded as physical if the animal at least brushed against it, brief-oral if the animal at least made contact to the device with its mouth, or prolonged-oral contact if the animal took the device into its mouth (Figure 2). Therefore, cattle with prolonged oral contact were a subset of those having

Figure 1. Cattle showing interest toward a pen-testing device.

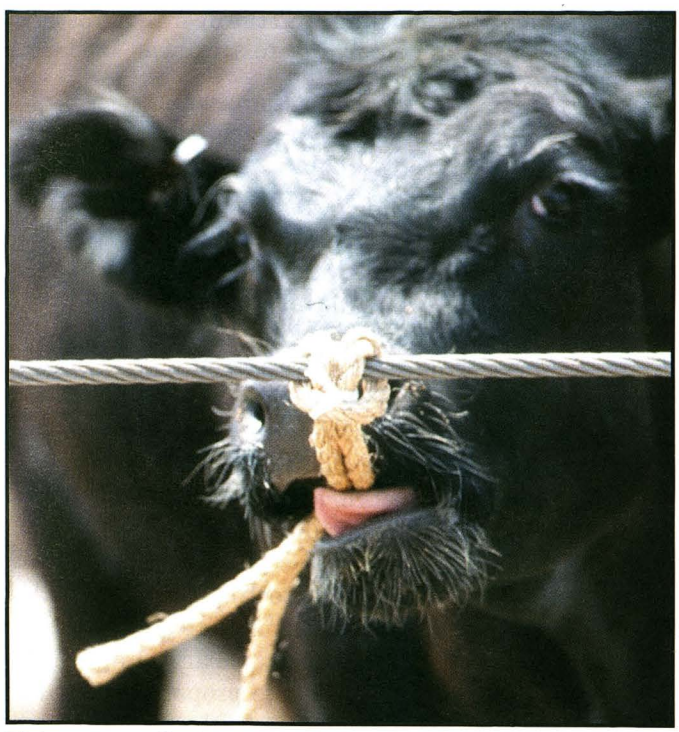

Figure 2. Curious cattle lick and pull the devices into their mouths.

brief oral contact, and those with brief oral contact were a subset of those having physical contact.

Differences between the percentage of cattle within a pen making the various types of contact in the 2-hour observation period were tested for statistical significance considering the matching that occurred by week in the study design. Percentage values were arcsine transformed and tested by analysis of variance considering the week-matched pairs.[a]

Physical contact rates were calculated for each 30-minute interval and differences between time periods and number of devices were tested by analysis of variance considering matching by week. The first contact rate was defined as the number of cattle making their first physical contact within each 30-minute time period divided by the number of cattle at the beginning of the time period that had not yet had physical contact with the devices.

A one-inch tail of each device was collected at the end of the 2-hour observation period (ending approximately one hour after sundown), and the remaining tail was collected the next morning to test for the presence of *E. coli* O157:H7. Culture methods for recovery of the pathogen consisted of pre-enrichment, standard isolation techniques including immunomagnetic separation, and PCR confirmation.[2] Recovery of the pathogen from the devices was considered at the pen-level (recovery of *E. coli* O157:H7 from any device was considered a pen-positive result) and tested by the Fisher exact test. Diagnostic agreement between evening and morning collection of devices for three or seven devices was evaluated by the kappa statistic.[1]

[a]PROC GLM, SAS Institute Inc., Cary, NC.

Results

Overall, 2948 cattle were observed in 24 commercial feedlot pens: 1534 cattle from 12 pens with three devices and 1414 cattle from 12 pens with seven devices. Cattle population in each pen ranged from 55 to 279 (mean 122.8). The number of cattle per pen did not differ between treatment groups pair-matched by the week of study (p=0.25).

Over the 2-hour observation periods the pens with seven devices averaged 55.1% physical, 43.3% brief oral, and 24.9% prolonged oral contact with the devices compared to 42.4%, 32.3%, and 15.4% contacts respectively in pens with three devices (Figure 3). Pens with seven ropes had a significantly greater percentage of physical (p<0.01), brief oral (p<0.02), and prolonged oral (p<0.01) contact than week-matched pens with three ropes.

The rate of first contact did not differ between pens with three or seven ropes in any of the 30 minute time periods (p>0.50); however, regardless of the number of devices in the pen, the first contact rate was highest in the first 30 minutes and decreased with time (p<0.001; Figure 4).

Recovery of *E. coli* O157:H7 within pens did not differ whether devices were cultured after two hours of exposure or left available over night (p>0.1; Figure 5A), nor did recovery differ if three or seven devices were used in the pen (p>0.1; Figure 5B). *E. coli* O157:H7

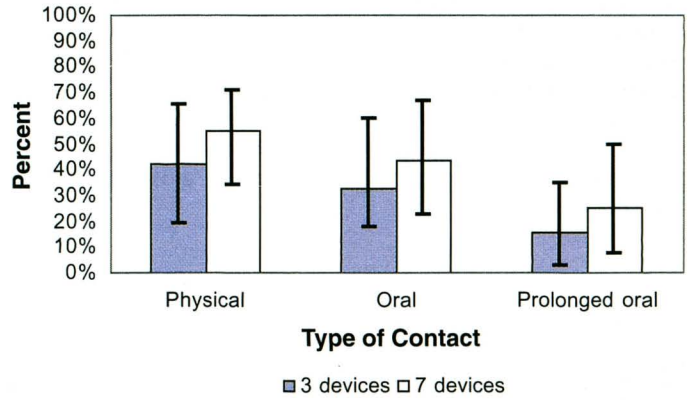

Figure 3. Percentages of cattle in the pen making some form of contact with a device over a 2-hour period when either three or seven devices were available. Bars represent the mean percentage of cattle observed making each type of contact in the 12 pens with each number of devices. Error bars represent the range of percentages observed. For each type of contact a greater percentage of the pen was sampled with seven devices (p<0.05).

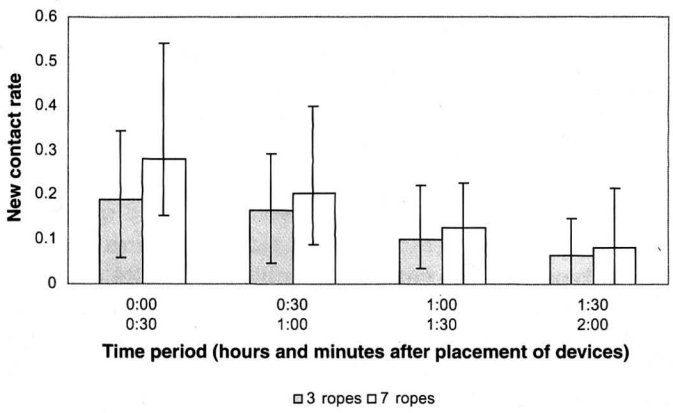

Figure 4. Rate of first contacts occurring in each 30-minute period after placement of the devices. Error bars represent the range of rates observed. There was no difference in the rate of first contact in pens with three or seven devices; however the rates decreased significantly with time ($p<0.001$).

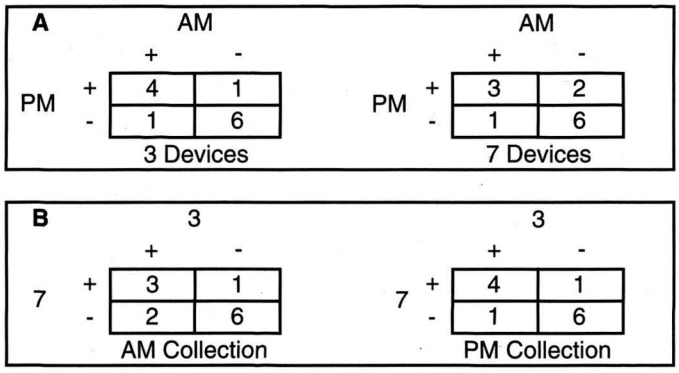

Figure 5. **(A)** Recovery of *Escherichia coli* O157:H7 from 24 pens comparing morning (AM) to evening (PM) collection of the devices in 12 pens with three devices and 12 pens with seven devices. **(B)** Pathogen recovery results from 24 pens comparing three or seven devices available in the pen, twelve pens with AM collection, twelve pens with PM collection. (+) indicates the organism was recovered from at least 1 device in the pen, (-) indicates the pathogen was not recovered from any device in the pen. There were no differences in pathogen recovery comparing use of three devices to seven, or AM to PM collection.

was recovered from at least one device from six of the 12 pens with three devices (50%) and from at least one device from six of the 12 pens with seven devices (50%). The agreement between pen classification from testing devices collected that evening or the following morning was moderate (kappa = 0.66; $p<0.05$) among pens with three devices. The morning to evening agreement between pen classification among pens with seven devices was fair (kappa = 0.47), but not significant beyond the role of chance. Overall from the 24 pens, *E coli* O157:H7 was recovered from 10 pens if the devices were collected in the evening and nine pens if the devices were collected the following morning, and the overall agreement between evening and morning testing was moderate (kappa = 0.57; $p<0.05$).

Discussion

The sampling strategy was based on our empirical observations that cattle are motivated by curiosity to investigate novel items within their environment. The behavior of cattle toward novel items includes smelling, rubbing, licking and chewing the items. In this study we were primarily interested in describing the behavior of cattle to the devices so that the diagnostic protocol could be optimized for efficiency. We have conducted other studies to correlate tests for *E. coli* O157:H7 from the devices with tests of feces from individual cattle within the pen (Smith *et al*, unpublished).

The number of devices placed in a pen may be important to the probability of detecting *E. coli* O157:H7 within a pen of cattle if placement of more devices means that a greater proportion of the cattle are sampled. Regardless of the type of contact the proportion of cattle that contacted the ropes was consistently greater in pens with seven devices compared to three. We observed dominance behavior that might have explained why fewer contacts were observed within the pens with three devices. When three devices were used, not as many animals within the pen necessarily had access to a device even if they appeared to have an interest in it because a few dominant animals could monopolize the devices for long periods of time, thus preventing other animals from making contact.

The rate that cattle made first contact with the devices is analogous to the incidence rate of infectious disease epidemiology. The rate of first contact could define the interest level of the cattle toward the devices. In these terms then the level of interest was similar regardless of whether the cattle were in pens with seven or three devices. Interest was highest immediately after placing the devices and decreased with time even though some cattle never made contact with the devices. This suggests that the time period of exposure needs to be sufficiently long to capture the interest of (and contact with) as many cattle as possible, although there will be diminishing interest with time. Empirically, we observed that by the end of the 2-hour observation period cattle were leaving the bunk areas, romping with each other in the pens, and then beginning to bed down for the evening.

We tested the devices for the presence of *E. coli* O157:H7 after the 2-hour observation period and again the following morning. If any rope tested positive for *E. coli* O157:H7 then the pen was considered positive. Because the pen's status for the presence of the pathogen was determined by whether a rope was positive or

negative, increasing the number of devices could have increased the number of opportunities to find the organism. Also, since a larger proportion of the cattle within a pen was sampled using seven devices, a protocol using seven devices might have had a better chance to detect the organism compared to a protocol using three devices. The proportion of pens classified as positive by the use of three or seven ropes was identical; however, the study was designed to detect large differences in pen classification and may not have had the power to detect smaller differences if they existed.

We also wanted to compare the agreement between testing devices collected in the evening and devices collected the following day. Our interest was in contrasting the concern that the organism might be lost overnight with the convenience of putting the devices out in the evening and returning to collect the devices the following morning (as might be more easily done by commercial feedlot personnel). The agreement between test results by either collection method was moderate, with no strong evidence that either collection time was superior.

Conclusions

To increase the probability of detecting the presence of *E. coli* O157:H7 the number of cattle making contact with the devices should be maximized within practical limits. We concluded from our results that a protocol to maximize the number of cattle making contact must consider the number of devices used and length of time the devices are available for contact.

Acknowledgements

This project was supported in part by funds provided by the Nebraska Beef Council and the University of Nebraska–Lincoln, Agricultural Research Division. We gratefully acknowledge the technical assistance of Mark Blackford, Sharon Clowser, Julie Rau, Chad Rolfes and Spring Younts.

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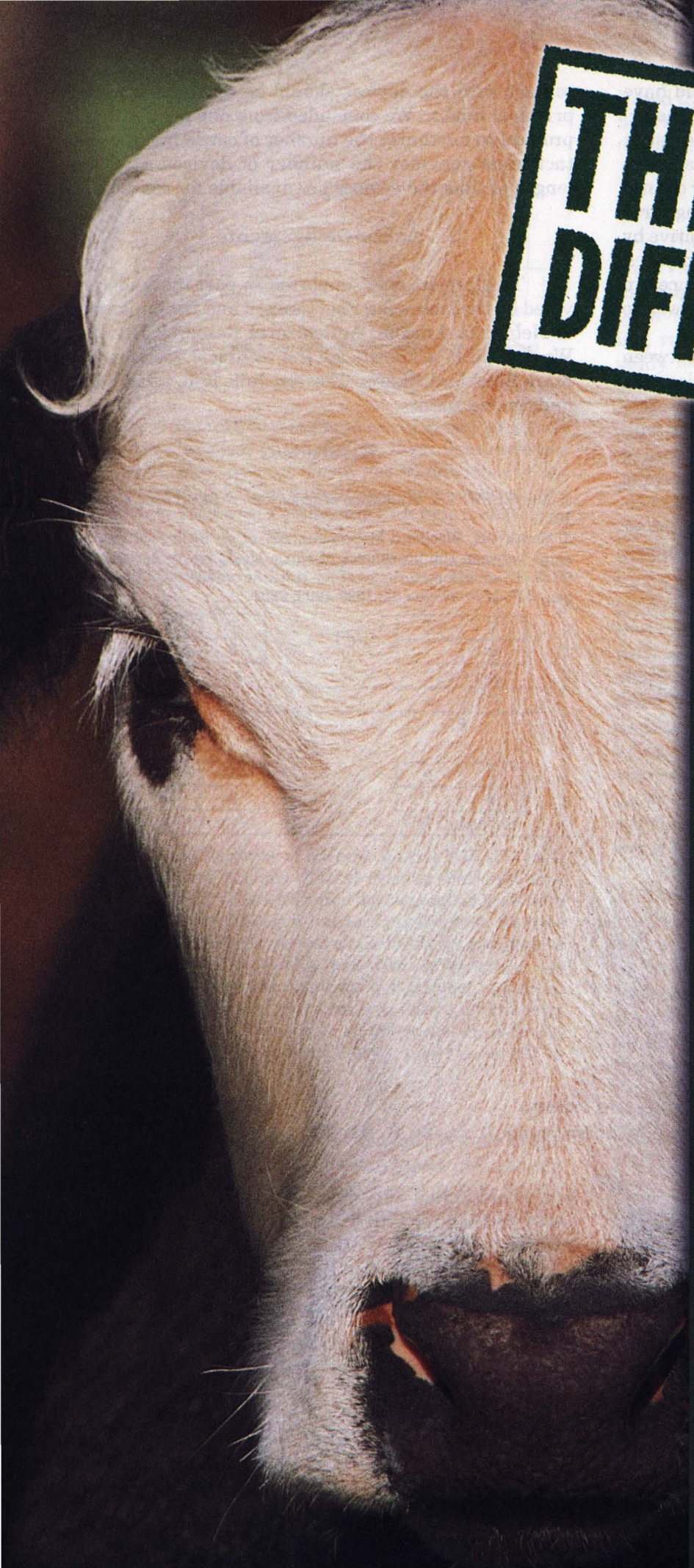
Abstract

Ultrasonography of the Teat Canal in Cows and Sheep

S. Franz, M. Hofmann-Parisot, W. Baumgartner, G. Windischbauer, A. Suchy, B. Bauder

Veterinary Record (2001) 149:109-112

When the isolated teat of a cow was examined with an 8.5 MHz linear array transducer in a vertical plane, the teat canal appeared as a thin, white line, bordered on each side by parallel, thick, grey-black bands. In a horizontal plane a comparable image was obtained. In a sheep, images of comparable quality were obtained with a 12 MHz transducer. Histological studies of the tissues whose removal led to the disappearance of this characteristic ultrasonographic appearance showed that it was associated with the stratified keratinized squamous epithelium with distinct papillae. The content of keratin in the stratum corneum was apparently responsible for the bright zone; the stratum lucidum was not visible, and the surrounding dark, less echoic area was associated with the stratum granulosum. Doppler echography in live animals confirmed this designation. The outer layers of the teat wall were more echogenic.



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