# Evaluation of measurements of the conductivity of quarter milk samples for the early diagnosis of mastitis

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# Evaluation of measurements of the conductivity of quarter milk samples for the early diagnosis of mastitis

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**Measurements of the conductivity of quarter milk samples were made in 31 cows in a 70-cow herd in south-east England, for a period of 15 weeks. Over this period, 42 per cent of cow-weeks and 20 per cent of quarter-weeks had an increase in quarter milk conductivity of 10 per cent of more compared with the mean conductivity of the previous 14 milkings. Fourteen per cent of quarter-weeks had an increase in conductivity of 15 per cent or more. The geometric mean somatic cell count (cell count) was higher in quarter-weeks with a 10 per cent or greater increase in conductivity than in quarter-weeks with a conductivity change of less than 10 per cent. At a conductivity threshold of 10 or 15 per cent and a cell count threshold of 200,000 or 400,000 cells/ml the specificity of this system was estimated to be 85 to 92 per cent, the sensitivity 40 to 54 per cent, the negative predictive value 87 to 93 per cent and the positive predictive value 33 to 55 per cent. The positive predictive value of the individual quarter milk conductivity was insufficiently accurate to be used as the sole criterion for the selection of quarters for early antibiotic treatment.**

WHEN the mammary epithelium is damaged as a result of mastitis, the electrical conductivity of the milk changes because the balance of sodium, potassium and chloride ions is altered. These changes tend to occur before the development of visible clinical signs (Hamann and Zecconi 1998) and could therefore help to detect mastitis early, thus making it possible to improve the efficacy of treatment, improve the bacteriological cure rate, reduce recurrence rates and, potentially, reduce the quantity of antibiotic used per cow. Using dairy cows artificially infected with *Streptococcus uberis*, Milner and others (1997) showed that treatment at the time of the initial rise in conductivity of the milk from an infected quarter was significantly more effective than waiting until the visual clinical signs appeared, and that significantly less antibiotic was required to produce a clinical and bacteriological cure.

This study was designed to determine whether changes in the conductivity of individual quarter milk samples, measured under commercial conditions with a commercially available system, could be used as an early diagnostic test for mastitis, by evaluating the relationship between the conductivity of the samples and their somatic cell count.

## MATERIALS AND METHODS

Data were collected from 31 cows in a 70-cow herd, located in south-east England, for a period of 15 weeks. The cows were milked through a two-box Liberty automatic milking unit which measured and recorded the conductivity of individual quarter milk samples in-line. The conductivity was reported for each quarter in terms of the percentage increase from the mean conductivity during the previous 14 days measurements once it exceeded a 10 per cent trigger threshold. A record was kept of the time of all milkings and the cow's yield at each milking.

### Somatic cell count (cell count)

Individual quarter samples were collected for the determination of the cell count from every cow once a week. They were collected directly from the milking machine below the claw once full flow had been established, preserved with bronopol, and analysed by the Fossmatic technique within 48 hours of collection.

### Data analysis

Data were collected on milk yield, milking interval and days in milk at each milking and analysed for each cow. The cell counts of each quarter sample were analysed. The data were collated to give weekly means, resulting in a maximum of 465 'cow-weeks' of data for whole cow values and up to 1600 'quarter-weeks' of data for individual quarter values. The data were analysed in periods of one week, first by using the data from three days on either side of the routine weekly measurement of cell count, and secondly by examining the data from the seven days before that measurement.

## RESULTS

The 31 cows were mostly in mid-lactation (mean 160 days). They visited the automatic milking unit an average of 2·8 times in each 24-hour period giving an average daily milk yield of 22·1 kg.

### Occurrence of conductivity triggers

Over the recording period 194 cow-weeks (42 per cent) had one or more quarter conductivity triggers (a rise of 10 per cent or more) and 271 did not. Table 1 shows the numbers of cow-weeks in which conductivity triggers occurred in one, two, or three or four quarters of a cow.

Assessed on a quarter-week basis, 310 quarter-weeks (20 per cent) had a conductivity trigger (10 per cent rise or more), and 1229 quarter-weeks (80 per cent) did not. If the conductivity trigger was increased to a 15 per cent increase, less than 14 per cent of the quarter-weeks had a trigger.

### Somatic cell count and occurrence of conductivity triggers

The geometric mean (sd) cell count was significantly higher ($P<0·001$) in quarter-weeks in which there was a conductivity trigger (5·28 [0·09] x $10^5$ cells/ml) than in quarter-weeks with no trigger (3·52 [0·04] x $10^5$ cells/ml).

The geometric mean cell count was significantly higher in quarter-weeks with a greater increase in conductivity ($P<0·001$). It was lowest in quarter-weeks with no conductivity trigger, but increased as the trigger percentage was increased from 10 to 20 per cent (Fig 1). The numbers above the histogram bars indicate the number of quarter-weeks in each category. With the exception of the small number of quarter-weeks with a conductivity trigger above 30 per cent, the quarter-weeks in the trigger bands above 17·5 per cent had a mean cell count above 400,000 cells/ml.

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TABLE 1: Frequency of conductivity triggers of 10 per cent or more during the 15 weeks of the study

| Number of quarters per cow triggered (%) | | | | Total | Total |
|---|---|---|---|---|---|
| 0 | 1 | 2 | 3 or 4 | one or more | cow-weeks |
| 271 (58) | 83 (18) | 68 (15) | 43 (9) | 194 (42) | 465 |

## Estimation of sensitivity and specificity

**Cell count threshold 200,000 cells/ml** Each quarter-week was categorised as uninfected when the cell count was less than 200,000 cells/ml, and infected when it was over 200,000 cells/ml, and these categories were related to the percentage of quarter-weeks which had a conductivity trigger of 10 per cent or more; the results for conductivity triggers recorded three days on either side of the measurements of cell count are summarised in Table 2.

Fewer than 10 per cent of quarter-weeks had no conductivity trigger but had a cell count above 200,000 cells/ml, and were therefore described as false negatives. Slightly more than 10 per cent of quarter-weeks with a conductivity trigger of 10 per cent or more had a cell count of less than 200,000 cells/ml and were described as false positives.

From these data the sensitivity was calculated to be 50 per cent (148/[148+149]) and the specificity 87 per cent (1080/[1080+162]). The positive predictive value was calculated to be 48 per cent (148/[148+162]) and the negative predictive value to be 88 per cent (1080/[149+1080]).

Examining conductivity triggers seven days before the measurement of the cell count changed the distribution very little. There was a slight increase in the percentage of quarter-weeks with a raised cell count that were not identified by a conductivity trigger. The sensitivity was reduced to 46 per cent, the specificity remained unchanged at 87 per cent, the positive predictive value was reduced slightly to 45 per cent and the negative predictive value to 87 per cent.

**Cell count threshold 400,000 cells/ml** The process was repeated at a higher cell count threshold. Each quarter-week was categorised as uninfected when the cell count was 400,000 cells/ml or less, and infected when it was above 400,000 cells/ml, and these categories were related to the percentage of quarter-weeks which had a conductivity trigger of 10 per cent or more; the results for conductivity triggers recorded three days on either side of the measurement of cell count are summarised in Table 3.

Only 5 per cent of quarter-weeks were false negatives, having a cell count of more than 400,000 cells/ml but no conductivity trigger; 13 per cent of quarter-weeks were false positives, having a conductivity trigger of 10 per cent or more but a cell count less than 400,000 cells/ml. From these data the sensitivity was calculated to be 59 per cent and the specificity

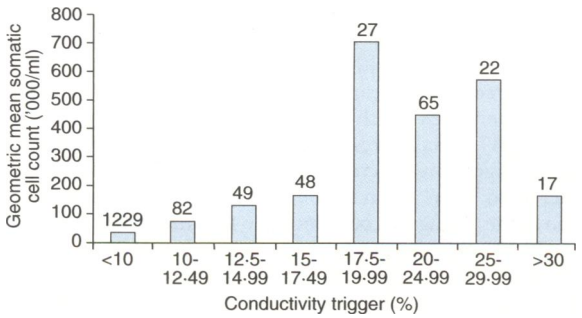


**FIG 1: Geometric mean somatic cell counts in quarter milk samples with increasing conductivity triggers**

TABLE 2: Estimation of false positive and false negative conductivity triggers with the somatic cell count threshold at 200,000 cells/ml and conductivity triggers three days either side of the cell count measurement

| Conductivity trigger | Quarter cell count (cells/ml) (%) ≤200,000 | >200,000 | Total (%) |
|---|---|---|---|
| None | 1080 (70·2) | 149 (9·7) | 1229 (80) |
| 10% or more | 162 (10·5) | 148 (9·6) | 310 (20) |
| Total numbers | 1242 (81) | 297 (19) | 1539 (100) |

85 per cent. The positive predictive value was only 36 per cent but the negative predictive value was 94 per cent.

Examining conductivity triggers seven days before the measurement of cell count changed the distribution very little. The sensitivity was calculated to be 54 per cent, the specificity 85 per cent, the positive predictive value 33 per cent and the negative predictive value 93 per cent.

**Conductivity threshold 15 per cent** The data were re-examined with the conductivity threshold increased from 10 to 15 per cent, and with cell count thresholds of 200,000 cells/ml and 400,000 cells/ml. The data for the 200,000 cells/ml threshold are summarised in Table 4. The analysis was restricted to the data recorded in the seven days before the measurement of the cell count, because at the 10 per cent conductivity threshold there had been little difference between the results obtained by analysing these data and the data recorded three days before and after the measurement of cell count.

With the conductivity trigger at 15 per cent there were 11·4 per cent of quarter-weeks classed as false negatives and 6·1 per cent classed as false positives. The sensitivity was calculated to be 40 per cent and the specificity 92 per cent. The positive predictive value was calculated to be 55 per cent and the negative predictive value to be 87 per cent.

With the conductivity trigger at 15 per cent and the cell count threshold at 400,000 cells/ml there were 5·8 per cent of quarter-weeks classed as false negatives and 7·5 per cent classed as false positives. The sensitivity was calculated to be 51 per cent and the specificity 91 per cent. The positive predictive value was calculated to be 45 per cent and the negative predictive value to be 93 per cent.

Table 5 gives a summary of the calculated sensitivities, specificities, positive predictive values and negative predictive values obtained with the different conductivity and cell count thresholds.

Increasing the cell count threshold increased the sensitivity and negative predictive value, but had little effect on the specificity, and decreased the positive predictive value; increasing the conductivity threshold had no effect on the negative predictive value, increased the specificity and the positive predictive value, and decreased the sensitivity.

## Milk yield and occurrence of conductivity triggers

The mean milk yield was significantly different for cow-weeks with different numbers of triggered quarters ($P<0·001$). The highest milk yield (8·4 [0·16] kg/milking) was recorded in cow-weeks with no conductivity triggers, and it decreased as the number of quarters/cow triggered increased (7·7 [0·27],

TABLE 3: Estimation of false positive and false negative conductivity triggers with the somatic cell count threshold at 400,000 cells/ml and conductivity triggers three days either side of the cell count measurement

| Conductivity trigger | Quarter cell count (cells/ml) (%) ≤400,000 | >400,000 | Total (%) |
|---|---|---|---|
| None | 1151 (74·8) | 78 (5·1) | 1229 (80) |
| 10% or more | 199 (12·9) | 111 (7·2) | 310 (20) |
| Total numbers | 1350 (88) | 189 (12) | 1539 (100) |

**TABLE 4: Estimation of false positive and false negative conductivity triggers with the somatic cell count threshold at 200,000 cells/ml and conductivity triggers seven days before the cell count measurement**

| Conductivity trigger | Quarter cell count (cells/ml) (%) ≤200,000 | >200,000 | Total (%) |
|---|---|---|---|
| None | 1153 (74·9) | 176 (11·4) | 1329 (86) |
| 15% or more | 94 (6·1) | 116 (7·5) | 210 (14) |
| Total numbers | 1247 (81) | 292 (19) | 1539 (100) |

7·4 [0·29] and 6·6 [0·37] kg/milking) for one, two and three or more quarter triggers, respectively.

### Milking interval and occurrence of conductivity triggers

The mean milking interval was significantly different for cow-weeks with different numbers of triggered quarters ($P<0·001$). However, there was no clear pattern, with only cows with two quarters triggered having a significantly longer interval ($P<0·05$) than cows with no conductivity trigger (9·2 v 8·4 hours).

## DISCUSSION

The conductivity of milk can vary substantially in the absence of mastitis owing to factors such as the stage of lactation, milking interval and oestrus (Hamann and Zecconi 1998). These factors complicate the interpretation of changes in conductivity and the accurate selection of cows for early antibiotic therapy. For example, the data from this study show that milk yield and milking interval were both significantly different in cow-weeks with conductivity triggers, in agreement with the trends reported in earlier research (Sheldrake and others 1983, Hillerton and Walton 1991). Changes in conductivity must therefore be assessed on an individual quarter basis, so that the impact of whole udder changes, such as that caused by stage of lactation, are minimised.

The results agree with the findings of Hamann and Zecconi (1998) in that there was a significant relationship between the conductivity of individual quarter milk samples and mastitis, as indicated by increases in cell counts. Cows without a conductivity trigger had a significantly lower mean cell count than cows with a trigger (Table 3), and there was a significant relationship between the level of the conductivity trigger and the mean cell count (Fig 1). However, a significant relationship does not necessarily make a useful diagnostic test; the test must also have predictive value, with a high enough sensitivity and specificity so that useful predictions can be made. Initially, the results were interpreted by using a quarter cell count of 200,000 cells/ml to distinguish between infected and uninfected quarters, as recommended by the International Dairy Federation (Hillerton 1999). However, many commercial herdsmen would consider taking action only on individual cows or quarters with a considerably higher cell count, and the data were therefore also analysed by using a quarter cell count of 400,000 cells/ml as the threshold. The assessments of specificity and sensitivity were also made by using two different conductivity triggers, an increase of 10 per cent or more, or 15 per cent of more, compared with the mean of the previous 14 milkings.

However, neither this change in the thresholds, nor altering the timing of the measurement of cell counts in relation to the conductivity trigger resulted in a sensitivity greater than 54 per cent or a positive predictive value greater than 55 per cent. Thus, the results suggest that the conductivity of individual quarter milk samples is not suitable for use as a reliable early predictor of mastitis, for two reasons. First, the sensitivity is too low, with too high a proportion of cases not being identified early enough, that is, before an increase in cell count. Nevertheless, this poor sensitivity alone would not make the test useless, because the early detection and treatment of 50 per cent of the cows at risk could still have significant benefits. It is the second reason, the proportion of false positives, that precludes the use of individual quarter milk conductivity alone. The positive predictive value ranged from 33 per cent (>400,000 cell count and 10 per cent conductivity trigger) to 55 per cent (>200,000 cell count and 15 per cent conductivity trigger). Thus, if the conductivity of individual quarter milk samples was used to identify cows for early mastitis treatment, it would result in an unacceptable percentage of unjustified treatments, because almost as many cows would be treated unnecessarily as would receive early treatment. The cause of this poor positive predictive value is that, although the number of uninfected quarters with low cell counts which were wrongly identified as infected was a small percentage of all the uninfected quarters, it was a large percentage of all the quarters triggered, because uninfected quarters, with cell counts less than 200,000 cells/ml, were four times more common than infected quarters. This distribution of cell counts is similar to that observed in the general dairy population (Biggadike and others 2000). Thus, even if the sensitivity was much higher, the proportion of false positives would still be a significant proportion of the triggered cows, reaching a minimum of 25 per cent if the sensitivity was 100 per cent.

It is possible that quarters without a high cell count were triggered because the cell counts were measured only weekly. If the cell counts had increased and decreased again within seven days it is likely that more of the conductivity triggers were associated with a high cell count and udder infection than suggested by this data set. However, the mean interval between the trigger and the measurement of cell count was only three days, and it is unlikely that a significant rise and fall in cell count would have occurred during this period. This possibility is supported by the lack of a significant effect on the specificity when the threshold cell count was increased. Furthermore, the predictive value and sensitivity and specificity were similar to those observed by Lansbergen and others (1994) who measured the conductivity and the cell count on the same day.

These data suggest that cows with a raised individual quarter milk conductivity require additional evidence suggesting infection before antibiotic treatment. Possible tests include the N-acetyl-beta-D-glucosaminidase (NAGase) test, which measures the concentration of NAGase, an intracellular, lysosomal enzyme, which is released into the milk compartment as the inflammatory cells become activated or break down, or an ATPase test, which measures the concentration of ATP which increases in proportion to the concentration of cells in the milk. However, neither of these tests is currently readily available commercially. Additionally, the development of an intelligent system which could identify cows that were likely to have an increased conductivity for reasons other than mastitis, would also help in the identification of true mastitis warnings.

**TABLE 5: Sensitivity, specificity and positive and negative predictive values at different thresholds of conductivity trigger and somatic cell count**

| | Conductivity threshold (cells/ml) 10 per cent 200,000 | 10 per cent 400,000 | 15 per cent 200,000 | 15 per cent 400,000 |
|---|---|---|---|---|
| Sensitivity (%) | 46 | 54 | 40 | 51 |
| Specificity (%) | 87 | 85 | 92 | 91 |
| Positive predictive value (%) | 45 | 33 | 55 | 45 |
| Negative predictive value (%) | 87 | 93 | 87 | 93 |

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# Infectious agents associated with respiratory disease in pheasants

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**In a case-control study of the infectious agents associated with natural outbreaks of respiratory disease in pheasants, 28 batches of birds from sites affected by disease and eight batches of birds from unaffected sites were examined by six veterinary laboratories in England, Wales and Scotland, and tested for mycoplasmas, other bacteria and viruses. Sinusitis was the commonest sign of disease and was associated with *Mycoplasma gallisepticum* as detected by PCR in the trachea ($P<0.05$) and conjunctiva ($P<0.01$). Sinusitis was also associated with pasteurella cultured from the sinus ($P<0.05$), antibody to avian pneumovirus (APV) ($P<0.01$) and avian coronaviruses as detected by reverse-transcriptase PCR ($P<0.05$); there was no association between disease and APV as detected by PCR. Avian coronaviruses were the most common infectious agents detected. They were genetically close to infectious bronchitis virus (IBV) but differed in their gene sequence from all the serotypes of IBV previously identified in domestic fowl, and serological tests with six known IBV types showed little cross reactivity. *Mycoplasma* species other than *M gallisepticum* were cultured in 18 batches of pheasants but, with the exception of *Mycoplasma gallinaceum*, were not associated with disease.**

SINUSITIS was first described in pheasants (*Phasianus colchicus*) in the UK by Keymer (1958), who later isolated mycoplasma organisms from affected birds (Keymer 1961). However, he recorded that gamekeepers had encountered respiratory disease in gamebirds, especially pheasants, for many years and it was essential to consider the differential diagnosis, because more than one disease agent might be involved in a single outbreak. 'Gapes' or syngamiosis was reported to be the most prevalent respiratory disease in gamebirds at that time.

Respiratory disease, in particular sinusitis, has continued to be diagnosed in pheasants and is often associated with high morbidity rates, debility and increased mortality (Lister 1989). On one site, Pennycott (2000) found that, despite medication, sinusitis was the most common infectious cause of mortality or culling in adult pheasants in breeding pens. An association has continued to be reported between mycoplasmas (especially *Mycoplasma gallisepticum*) and respiratory disease in pheasants in the UK (Lister 1989, Bradbury and others 2001b), the USA (Cookson and Shivaprasad 1994) and Australia (Reece and others 1986). However, it has become increasingly evident that other infectious agents may be involved in the disease, particularly avian pneumovirus (APV), also referred to as turkey rhinotracheitis (TRT) virus, which has emerged as an important cause of respiratory disease in chickens and turkeys (Cook 2000). The aim of this study was to identify some of the infectious agents currently associated with respiratory disease in pheasants in the UK by means of a case-control study, with particular respect to the roles of mycoplasmas, other bacteria and viruses.

## MATERIALS AND METHODS

### Pheasants

The pheasant 'season' corresponds to the rearing cycle, from the chicks hatched in May through to the breeding adults the following spring. During the 1998/99 and 1999/2000 seasons, live affected pheasants were submitted by gamekeepers via their veterinary surgeons from sites with an outbreak of respiratory disease ('case sites') to one of six veterinary laboratories in England, Wales and Scotland: the Veterinary Laboratories Agency's regional laboratories at Bury St Edmunds, Carmarthen, Luddington, Starcross and Winchester, and the SAC Veterinary Science Division laboratory at Auchincruive; up to eight birds were submitted as a batch from each site. Batches of up to three unaffected live birds were also submitted from sites with no history of respiratory disease in the current or previous year ('control sites'). No stipulation was made as to the age of the birds except that it was not intended to examine cases of septicaemia in young poults or chicks.

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