Short communication

Dairy herd health, impedance on six acupuncture points and immune response factors in milk: A pilot study

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Abstract

This study aimed to evaluate whether immunological parameters in milk and the impedance at 6 acupuncture points (APs) were related to herd health: good versus poor. The study used a purposively selected sample of 10 herds and in each herd 15 to 18 animals were observed. Statistical models at animal level included a random herd effect, with age and days in milk as covariates. The capacity for immune response was estimated through in vitro proliferation counts in medium, LPS, and ConA. At the right and left bladder meridians we measured impedance at 3 APs related to disease resistance. The energy balance at the onset of lactation and other parameters confirmed the classification in good and poor health herds. The immunological parameters in milk were not significantly related to health status, but LPS values of two herds were consistently lower. The good health herds showed significantly lower impedance at all APs correlated negatively with counts of LPS, indicating that good health animals had consistently higher counts of macrophages, i.e. better innate disease resistance. To confirm prospects of the parameters, we recommend repeating the study with a larger randomly selected sample.

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Keywords: Dairy; Cattle; Health; Electro-acupuncture; Immune response; Impedance

1. Introduction

Health and production of increasing numbers of dairy cows in The Netherlands are declining without a specific cause (De Kruif, 2001). It was suggested that problems relate to herd size, frequent buying-in of animals, and inadequate feed management (Bartels et al., 2001), increased milk production and exacerbated lean dairy characteristic (De Kruif, 2001). Public reports have advised dairy farmers to focus on flexible health management and to breed for robust animals (RLG, 2001). An animal is defined as robust when it recovers homeostasis quickly after an infection or stress, shows clinical signs of diseases only
briefly, and, for a dairy cow, maintains the level of milk production. However, insufficient methods and tools are available to describe robustness and to identify robust animals.

A non-published inventory of complementary parameters for health measurement showed that impedance (or electrical resistance) at acupuncture points (APs) and immunological parameters in milk could be promising. The first, in brief called electro-acupuncture, was initially developed by Voll (1976) and is used in complementary human medicine for comparative diagnostics. APs are classified in meridians (e.g. great governor, bladder) and in association points ('shu') according to the empirical relation with organs; 'ting' APs are end points of the 12 main meridians. The Soviet aerospace program collected large databases of impedance measurements on 24 ‘ting’ APs located at the tips of fingers and toes that allowed imbalances to be discovered before clinical signs of a disease occurred (Sagrijadski et al., 1996). Relatively high impedance values are an indication for acute infections, allergies, poisoning, or stress while extremely low values indicate local disorders or use of sedatives.

The level of immune responses can be quantified in vitro by measuring multiplication of blood cells in milk. The culture medium reflects background cell growth, the stimulation with LPS (lipopolysaccharides) the innate response by macrophages, and the stimulation with ConA (concanavalin A) the adaptive response by T-cells (Boonstra et al., 2000).

This short communication reports on a pilot study that related the mentioned parameters in a purposive sample of good health dairy herds and herds with poor health apparent through recurring health problems and/or unexplained low production.

2. Materials and methods

Herd were selected from amongst about 100 farms participating in farmer learning platforms. A resource person indicated about 20 good health herds and 7 herds with poor health or unexplained low production levels. The resource person, a dairy scientist specialised in nutrition physiology and husbandry practice, based the classification on oral information from the farmers and on visual criteria such as cleanliness of the white coloured part and shininess of the coloured parts of the hair coat. The list of poor health herds was extended by 12 names furnished by a veterinary practice specialised in advising farms with regularly recurring health problems or unexplained low production levels. Ten herds were selected randomly from these 2 limited lists: 4 good health and 6 poor health herds. All farms were visited twice during indoor period, in March and April 2002. Not all herds were housed on slatted floors with cubicles.

For each herd the cost of veterinary interventions, the number of inseminations per calving, the number of cows culled for health reasons and the mortality rate were retrieved for the past year. From each of eight herds, 18 cows not in the first or in last month of lactation were selected randomly; in two herds only 15 were available. We collected individual data on date of birth and last calving, milk yield and fat content (MFC, obtained from the milk recordings), registered specific health problems, if present, and

Fig. 1. Position of the measured APs (small circles; see reference of Table 1 for exact description) and of the area where rigid skin wrinkles were observed (large circle).

Fig. 2. The histological section of an acupuncture point: nerves and capillaries come close to the skin surface; the skin is softer at this point (adapted from Helms, 1996).
Table 1

<table>
<thead>
<tr>
<th>Code</th>
<th>Associated organs</th>
<th>Location relative to APs on the dorsal midline</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL15</td>
<td>BL15</td>
<td>Heart, Lung</td>
</tr>
<tr>
<td>BL49</td>
<td>BL45</td>
<td>Liver, Ovaries</td>
</tr>
<tr>
<td>BL52</td>
<td>BL46-2</td>
<td>Intestines</td>
</tr>
</tbody>
</table>

GV= governor vessel; lat=lateral; L=lumbar vertebra; T=thoracic vertebra.

1.5' lat to GV11, level with T5–T6 space
3' lat to GV06, level with T11–T12 space, 1.5' lat to BL20
3' lat to GV04, level with L2–L3 space, 1.5' lat to BL23

IVAS = International Veterinary Acupuncturist Society.

Kothbauer (1999).


scored body condition. Individual samples of about 500 ml were retrieved from morning milk and kept on ice in brown bottles.

Three APs on the right and three on the left side related to disease resistance were identified (Fig. 1). APs coincide with spots of softer skin where vessels and nerves cross the dense connective tissue, which allows location of the APs (Fig. 2). AP codes according to IVAS were used (Rogers, 2004); an equivalency, description, and location are given (Table 1). Two APs at the bladder (BL) meridian, BL15 and BL52, were identified using a Legger antenna adjusted to a frequency for disease resistance2 (Coquelles, 1993). During the data collection a third AP, BL49, was included after advice from Kothbauer (1999). Impedance was measured in kΩ at all 6 APs in 8 herds and at only 4 APs (excluding BL49) in 2 herds (one good and one poor health herd). Two impedance values were measured at each AP on cows where all 6 AP were included in 2 repetitions. In cows where only 4 AP were included, three measurements were made at each AP. The equipment2 for measuring impedance is a further development of a device used to check the health of Soviet cosmonauts (Treugut et al., 1999). Measurements took place in the morning after local shaving around APs while the cows were fixed.

In 400 ml of milk the mononuclear cells were isolated by Ficoll density centrifugation. Subsequently, cells were counted and stimulated in vitro following methods and materials used by Boonstra et al. (2000). Stimulation index (SI) is calculated as the ratio of counts per minute (cpm) in LPS- or ConA-stimulated culture versus cpm in Medium. From MFC, at the onset of lactation, we estimated the recovery of energy balance by subtracting: MFC(recording 3)–MFC(recording 1)=EB31, in which a positive value indicates a positive energy balance (adapted from de Vries and Veerkamp, 2000).

For the statistical analysis of the results we used SAS (1996). Without considering a herd effect we calculated Pearson correlations and analysed immune response parameters and impedance (impedance values of each AP were averaged for each cow) as dependent variables using PROC MIXED of SAS version 8, specifying the normal distribution. The potential dependency of cows within herds was accounted for by considering herd as random effect and by using an exchangeable correlation structure. The models considered health status as main effect and included age, EB31, BCS and days in milk as covarates.

3. Results

Classical parameters confirmed the experts' classification of herds according to good and poor health (Table 2). The EB31 of the good health herds was significantly higher ($P<0.05$), notwithstanding their higher milk yield ($P<0.05$).

When considering age and days in milk as covariates, no significant difference was shown between the good and poor health herds for any of the immunological parameters in milk (Table 3). When considering age and days in milk as covariates, the impedance
Table 2
General characteristics (mean over last year and standard error of mean) of dairy cattle in 4 herds with good health status and 6 with health or production problems

<table>
<thead>
<tr>
<th>Health status</th>
<th>Average of sample</th>
<th>Characteristics of whole herd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age (years)</td>
<td>DIM</td>
</tr>
<tr>
<td>Good (n=69)</td>
<td>mean</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>S.E.M.</td>
<td>0.3</td>
</tr>
<tr>
<td>Poor (n=105)</td>
<td>mean</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>S.E.M.</td>
<td>0.2</td>
</tr>
</tbody>
</table>

DIM=days in milk; EB31=estimated recovery of energy balance after calving (de Vries and Veerkamp, 2000); BCS=body condition score.

on all 6 APs in the good health herds was significantly lower ($P<0.05$) compared to the poor health herds (Table 4). The Pearson correlation coefficients between LPS and BCS on one hand and the impedance values on the other hand were significantly negative at 5 of the 6 APs: $r^2=-0.21$ ($P<0.03$) and $r^2=-0.22$ ($P<0.04$), respectively. The herd differences were, next to the individual animals' number of days in lactation and the herd health status, the source for most of the variability in the parameters and the values.

4. Discussion and conclusion

The lower veterinary costs for the good health herds (Table 2) contradicts with Bondt and Jansen (2001) stating that low veterinary costs were not an indication of a good health status. This contradiction might be due to our sampling method: we selected herds with a poor health status managed by farmers who had complaints about herd health and/or consulted a special veterinary practice.

On average the good health herds had a low impedance values at the APs. The negative correlation between the values at 5 APs and BCS is in accordance with the significant lower AP values and higher BCS for good health herds, or inversely for poor health herds. The bladder APs are association points for organs or other body functions and higher $\Omega$ values are related to, for example, stress or chronic disturbances. According to Dutch IVAS veterinarians, BL49L located above the spleen is related to lymph circulation and impaired lymph circulation is related to stress or to chronically high activity of the liver. In a herd with high impedance values at BL49L we also

Table 3
Means and S.E.M. of the measured response of immunology parameters for lactating cattle according to the classified health status: reactions on medium, after stimulation with LPS and ConA (all three $\times 1000$), and derived SI-1 and SI-2

<table>
<thead>
<tr>
<th>Status</th>
<th>$n$</th>
<th>Medium</th>
<th>LPS</th>
<th>SI-1</th>
<th>ConA</th>
<th>SI-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>69</td>
<td>1.4 $\pm$ 0.03</td>
<td>8.3 $\pm$ 0.12</td>
<td>6.0 $\pm$ 0.15</td>
<td>28.7 $\pm$ 1.8</td>
<td>19.8 $\pm$ 1.1</td>
</tr>
<tr>
<td>Poor</td>
<td>105</td>
<td>1.6 $\pm$ 0.03</td>
<td>7.1 $\pm$ 0.16</td>
<td>4.9 $\pm$ 0.15</td>
<td>33.8 $\pm$ 1.0</td>
<td>22.5 $\pm$ 0.6</td>
</tr>
</tbody>
</table>

LPS=lipopolysaccharides; ConA=concanavalin A; SI-1=LPS/Medium; SI-2=ConA/Medium.

Table 4
Impedance (m$\Omega$) at 6 acupuncture points (IVAS) of lactating dairy cattle in herds with good ($n=69$) health status or with poor health ($n=100$) ($n$ for BL 49: 51 and 86, respectively)

<table>
<thead>
<tr>
<th>Health status</th>
<th>BL15L</th>
<th>BL15R</th>
<th>BL49L</th>
<th>BL49R</th>
<th>BL52L</th>
<th>BL52R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>4.0 $\pm$ 0.23</td>
<td>3.2 $\pm$ 0.17</td>
<td>3.9 $\pm$ 0.30</td>
<td>3.5 $\pm$ 0.26</td>
<td>3.6 $\pm$ 0.23</td>
<td>3.1 $\pm$ 0.16</td>
</tr>
<tr>
<td>Poor</td>
<td>5.0 $\pm$ 0.19</td>
<td>4.2 $\pm$ 0.15</td>
<td>5.1 $\pm$ 0.21</td>
<td>4.6 $\pm$ 0.20</td>
<td>4.4 $\pm$ 0.18</td>
<td>4.3 $\pm$ 0.17</td>
</tr>
</tbody>
</table>
found a high number of cows with problems in connective tissue, evidenced by parallel rigid skin wrinkles above the heel (Fig. 3). Such wrinkles were more generally observed at the onset of lactation in most dairy cows in the past, when farmers did not use concentrated feed supplements. The veterinary practice specialised in advising farms with regularly recurring health problems used the size of the wrinkles as an indicator. In humans such wrinkles are known as benign lymphedema and related to impaired lymph circulation (Witte and Witte, 1995).

The negative correlation of LPS and the impedance values at 5 APs holds promises for the detection of a functional relationship between the immunological parameters and health characteristics. In vitro stimulation with LPS is widely used to selectively stimulate the multiplication of macrophages reflecting the innate antigen compartment (Boonstra et al., 2000). The count for macrophages in milk is related to their content in the peripheral blood (Park et al., 1992; Saad and Ostersen, 1990), and their low counts in milk thus reflects the low capacity to an innate response by macrophages in the low health herds. The counts after stimulation with LPS were consistently lower for those two poor health herds that the observers ranked as the ones with the most apparent health problems; the main complaint of the other poor health herds was a frequent high cell count in milk. In future experiments sets of cytokines, such as IL-1α, TNF-β, IL-10 and IL-12, will be measured in LPS-stimulated cultures, while IL-4 and IFN-γ will be measured in ConA-stimulated cultures. Collectively, such results would reveal the capacity of immune cells present in the milk to combat infections in the individual animal, and also the transfer of this protective capacity to the offspring.

The herd related variation was high and sample size was too small to confirm that the proposed immunological parameters can discriminate for health and robustness. To confirm prospects of immunological parameters and of impedance at APs for cattle health diagnostics, we recommend repeating the experiment considering factors like housing type and breed in a larger random sample.

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