

# A longitudinal survey of anti-*Ostertagia ostertagi* antibody levels in individual and bulk tank milk in two dairy herds in Normandy

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## Abstract

The *Ostertagia*-specific antibody levels in milk were monitored in 2 dairy herds to investigate seasonal variations and the relationship between individual and bulk tank milk antibody levels. Bulk tank and individual milk samples from all lactating animals were collected over a 1-year period at weekly and monthly intervals, respectively. The *Ostertagia*-specific antibody levels were measured with an indirect ELISA and the test results were expressed as optical density ratios (ODR). A clear seasonal pattern that followed the expected intake of infectious larvae was observed in the individual and bulk tank milk antibody levels of both herds. Within each herd, there was a large variation in the individual ODRs. This variation remained large when the distribution of individual ODRs was plotted according to high and low bulk tank milk ODR categories. The results suggest that the effect of seasonal variations on cut-off levels that predict production responses after anthelmintic control, needs to be assessed.

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## 1. Introduction

Gastrointestinal nematode infections in adult cows remain usually at the subclinical level. However, in some cows and/or herds, subclinical infections can negatively affect milk yield (Gross et al., 1999; Sanchez et al., 2004a). On the individual level, the anti-*Ostertagia ostertagi* antibody level in milk samples taken in late lactation has shown promise to predict the milk production response after anthelmintic treatment at calving (Sanchez et al., 2002, 2005). Sanchez et al. (2005) found some evidence of a positive treatment response in cows with a test result higher than 0.4 optical density ratio (ODR). However, it is not known if this cut-off value can be used in each sea-

son. An obvious seasonal pattern in the *Ostertagia*-specific serum antibody level has been described (e.g. Agneesssens et al., 2000; Borgsteede et al., 2000). Seasonal differences could also occur in the *Ostertagia*-specific milk antibody level and affect the interpretation of the test results.

In comparison with individual diagnosis, herd diagnosis based on the *Ostertagia*-specific bulk tank milk antibody level would have benefits such as ease of collection and reduced laboratory costs. In addition, significant negative relationships between the anti-*Ostertagia* antibody level in bulk tank milk and the herd average annual milk yield have been demonstrated (Charlier et al., 2005a).

In the present study the *Ostertagia*-specific antibody levels in individual and bulk tank milk were monitored monthly during one year in 2 dairy herds to investigate possible seasonal trends and the relationships between the individual and the bulk tank milk antibody level.

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## 2. Materials and methods

### 2.1. Herds and sample collection

The study was conducted on 2 dairy herds (A and B) in Normandy (France) over a 1-year period. The cows in both herds were turned out to pasture in the first half of April and were housed in the beginning of November. There was no previous history of *Fasciola hepatica* infections, and no anthelmintic treatments were administered to the dairy cows for at least 2 years prior to the start of the survey. Herds A and B consisted of on average 47 Normande or Normande crossbred and 59 Holstein Friesian cows, respectively.

Bulk tank and individual milk samples from all lactating animals were collected at weekly and monthly intervals, respectively from April 2003 until March 2004. However, in herd B, no bulk tank milk samples were collected in April. After collection at the farms, the samples were stored at  $-20^{\circ}\text{C}$  until further processing.

### 2.2. Laboratory methods

The milk samples were thawed and centrifuged ( $16,000 \times g$ , 5 min) before analysis. The supernatant was collected and centrifuged a second time to remove all the fat. Next, the samples were tested with an ELISA that detects specific IgG levels against *O. ostertagi* (Charlier et al., 2005b). The test results were expressed as ODRs.

### 2.3. Data analysis

Two bulk tank milk ODR categories were created based on a hypothetical cut-off level of 0.9. This value was chosen because it represents the overall mean bulk tank milk ODR in this study and in a previous large field survey (Charlier et al., 2005a). Based on the mean monthly bulk tank milk ODR, a category was assigned to each herd for that particular month. Finally, the frequency distribution of individual ODRs was plotted within each bulk tank ODR category. A  $\chi^2$  test was performed to investigate if the num-

ber of cows with a high individual ODR differed significantly between the high and low bulk tank milk ODR category.

## 3. Results

### 3.1. Seasonal variations

The mean ODR values of the individual and bulk tank milk antibody levels over the period of follow-up are shown in Fig. 1. The individual milk antibody levels followed a seasonal pattern in both herds with higher values from June until October. In herd A, the increase between April and October was small (0.073 ODR), but a peak was observed in July. In herd B, the increase between April and October was 0.316 ODR. From October onwards, the antibody levels decreased in both herds and reached their minimal levels between December 2003 and March 2004. A similar seasonal pattern was observed in both herds for the bulk tank milk antibody levels (Fig. 1).

### 3.2. Relationship between individual and bulk tank milk antibody levels

Within each herd, there was a large variation in the individual ODRs. This is demonstrated in Fig. 2 where the distribution of individual ODRs in the months April and September 2003 are given for both herds. The range of individual ODR values that was observed within the two bulk tank ODR categories (higher or lower than 0.9) is represented in Fig. 3. In both categories, a large variation in individual ODRs was observed, but there were significantly more cows with high ODR values in the high bulk tank milk category ( $P < 0.001$ ).

The bulk tank milk ODR was on average 0.301 units (53%) higher than the mean of the individual ODRs with a range of 0.021–0.627 units. The standard deviation of bulk tank milk samples collected in the same month was low ( $\leq 0.112$ ) except in the months July/August in herd A and July/August/September in herd B where a larger variation was observed (Fig. 1).

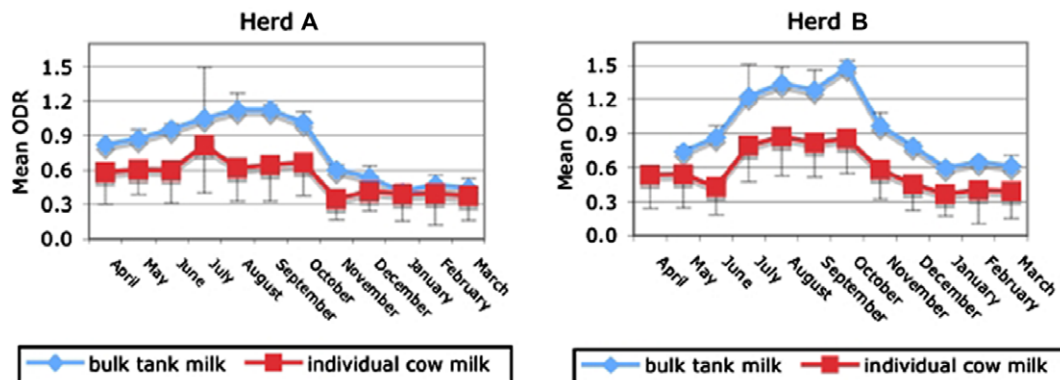


Fig. 1. Mean monthly ODR values of individual and bulk tank milk samples on herds A and B during the months April 2003 until March 2004. Bars represent standard deviations.

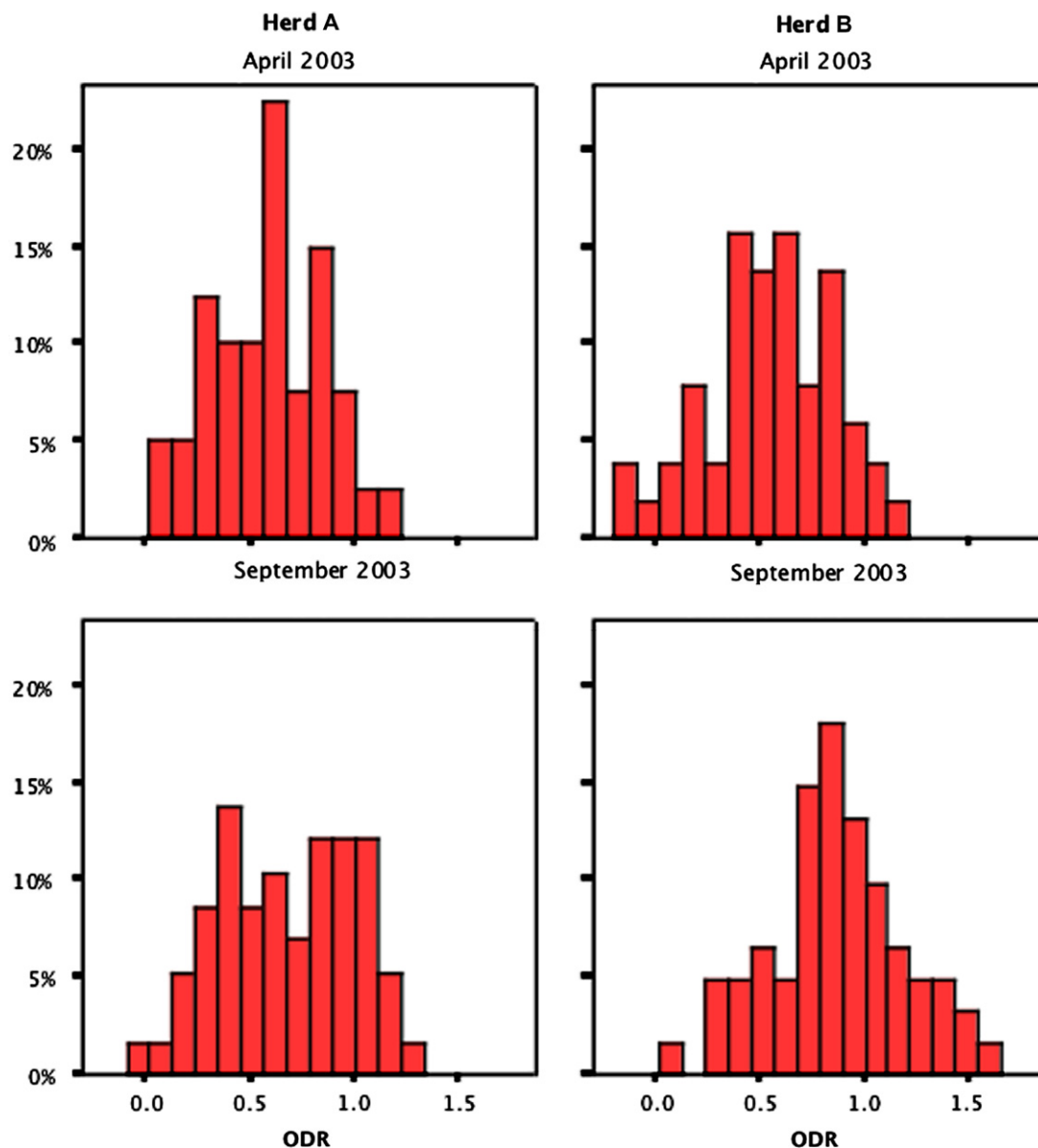


Fig. 2. Frequency distribution of individual ODR values in April and September in herds A and B.

#### 4. Discussion

A clear seasonal pattern was observed for both the individual and the bulk tank milk ODRs. Such a pattern is known to occur in the *Ostertagia*-specific serum antibody level of pastured dairy cows (Agneessens et al., 2000; Borgsteede et al., 2000) and follows the expected intake of infectious larvae. The antibody levels increase during the pasture season and decrease during the housing period, when most larvae are present in the form of inhibited L4 stages. Seasonal trends in *Ostertagia*-specific milk antibody levels have been previously described in field surveys, but the effects were rather small (Sanchez et al., 2002; Charlier et al., 2005a). The present results indicate that the seasonal variation within a herd can be considerable and that threshold levels that predict a positive milk production response after anthelmintic treatment might differ per season.

There was a large variation in individual ODRs within the 2 categories of bulk tank milk ODR. It has been shown that cow factors such as milk yield, age and days in milk do not greatly influence ODR values and that most of the observed variation is a reflection of the parasite contact in the digestive tract (Sanchez et al., 2004b). This suggests that in both categories there are animals that would benefit from anthelmintic control and that it is useful to test the individual animals. On the other hand, testing and giving anthelmintic treatment to individual animals is less practical than testing one bulk tank milk sample per herd and performing a whole-herd treatment.

It was observed that the bulk tank milk ODR was higher than the mean of the individual ODRs (on average 53%). Previously, a similar but smaller difference (22%) has been described (Sanchez et al., 2002). This is probably explained by high ODR cows contributing relatively more antibodies

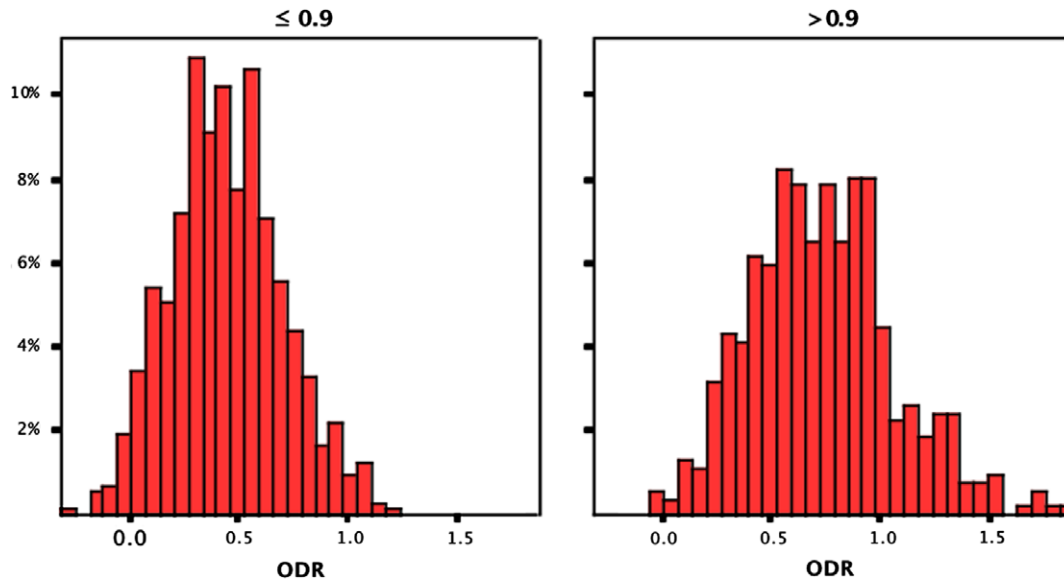


Fig. 3. Distribution of individual cow milk ODR by bulk tank milk category.

to the bulk milk than low ODR cows due to a saturation of the antibody binding reaction of the ELISA at high antibody concentrations in the milk (Charlier et al., 2006).

In general, there was a small variation between the test results of bulk tank milk samples collected in the same month, suggesting that testing one bulk milk sample each month is enough for monitoring a herd in time. However, a considerably higher variation was observed during the summer months. Different explanations could be hypothesized for this observation such as a higher proportion of cows at the end of lactation or a higher bacterial contamination of the milk in summer months.

From this study, we can conclude that large seasonal variations in *Ostertagia*-specific milk antibody levels can be observed within herds and animals. Subsequently, their effect on possible cut-off levels that predict production responses after anthelmintic control/treatment needs to be assessed. A possible way to do so is to compare the predictive value of the *Ostertagia*-specific antibody level on the production response after anthelmintic treatment, separately in different seasons (e.g. spring vs. autumn). The large variation in individual milk ODRs within the low and high bulk tank milk category suggests that in both categories of herds it could be useful to test individual animals. Therefore, the *O. ostertagi* milk ELISA is a promising tool to detect herds or the animals within a herd that require anthelmintic treatment.

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