

Pooled Faecal Samples Compared with Individual Samples for Detection of *Salmonella* in Cattle

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Abstract

The modified ISO 6579:1993 method was used to examine in parallel the presence of *Salmonella* in 4579 individual faecal samples and 210 pooled samples taken from 130 herds of cattle. Twenty-five clinically or subclinically *Salmonella infantis* infected herds were detected. Positive herds were examined once a month for seven months. 112 pooled samples and individual samples, respectively, were negative. 88 pooled samples were *Salmonella* positive. Ten negative pooled samples contained faeces from animals which were found to be *Salmonella* positive in individual sampling. Nine of these false negative pooled samples contained faeces from more than twenty animals and all contained relatively few *Salmonella* positive individuals. Our study indicated that *Salmonella* was reliably detected in pooled faecal samples of a maximum of 20 animals (Kappa test 0.98).

Introduction

Subclinical and clinical *Salmonella infantis* infections increased considerably in cattle in 1994-95, mainly in the provinces of Vaasa and Oulu in Finland. Samples in this survey were collected from all the herds in two adjacent local governmental districts, Teuva and Karijoki located in the province of Vaasa.

This study was performed to estimate the reliability of pooled samples compared with individual sampling to detect *Salmonella* infection in herds. According to Finnish legislation and the national *Salmonella* control programme, faecal samples of individual animals must be collected once a month from *Salmonella* infected herds and examined for *Salmonella*, until two consecutive examinations are negative. This examination is time-consuming and expensive, and so a cheaper but reliable sampling method was needed.

Materials and methods

A practising veterinarian collected 4579 individual faecal samples and 210 pooled samples from 130 herds of cattle. A tablespoonful of each individual sample was pooled at the farm and sent to the laboratory followed by individual samples of the same animals. The pooled samples were mixed thoroughly. All samples were transported after chilling by bus. The transportation time for most of the samples was one day. The number of faecal samples pooled varied from 10 to 48. Twenty-five *Salmonella* positive herds were found. Sampling was repeated in each *Salmonella* infected herds once a month. The examination was carried out during winter months.

Faecal samples were enriched in Rappaport Vassiliadisbroth (Lab M 86) immediately they arrived at the laboratory. The broths were incubated at 42°C for 18 to 24 hours. The selected enrichment broths were streaked onto bromthymol-blue lactose agar (BROLAC, Merck) and brilliant green phenol red agar (Merck) which were incubated at 37 °C for 20 to 24 hours. Typical colonies were identified biochemically (triple sugar, iron agar, urea agar and BROLAC) and serotyped.

Results

Of the pooled samples, 112 yielded negative results with both sampling methods, and 88 were positive for *Salmonella infantis*, the only serovar found. Each positive pooled sample contained faeces from one or more *Salmonella* positive individuals. Ten negative pooled samples were false negative. Nine of them contained samples from more than 20 animals, of which less than 10 % were individually *Salmonella* positive in eight cases. Pooled samples from two *Salmonella* positive farms were false negative in three successive samplings and from one farm twice. The only herd with less than

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21 animals that was false negative in a pooled sample was negative a month later according to both tests.

For statistical analysis the material was divided into two groups, pooled samples containing no more than 20 individual samples (group 1), and 21 to 48 individual samples (group 2). With the Kappa test, the agreement in group 1 was 0.98. In group 2 it was 0.81. *Proteus* overgrowth did not occur in the cultures.

Conclusions

The sampling method is one of the main factors in any test performed. Detection of *Salmonella* in faecal samples can depend on, eg., the amount of specimen, the time between sampling and culturing and the temperature during mailing. This variable was eliminated by pooling the samples at the farm, and sending both individual and pooled samples together to the laboratory. The standard method for isolation and identification of *Salmonella* was used. Pre-incubation

is not included in this procedure, which might have decreased the number of positive findings, especially in pooled samples.

The proportion of *Salmonella* positive animals and the amount of *Salmonella* bacteria in samples must have influenced the result. Animals may variably shed *Salmonella* into faeces. Nine out of the ten false negative pooled samples contained more than 20 individual samples. The negative result may be due to a low number of *Salmonella* bacteria in individual samples. Pooled faecal samples from no more than 20 animals gave reliable results for screening *bovine salmonellosis* on a herd basis.

References

- ISO 6579:1993. Microbiology - General guidance on methods for the detection of *Salmonella*.
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Abstract

Neuropathological and aetiological studies of sporadic non-suppurative meningoencephalomyelitis of cattle

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Sporadically occurring non-suppurative encephalitis appears to be a frequent condition of Swiss Cattle. Fifty-one such cases diagnosed over a period of 10 years were examined retrospectively to investigate whether they constituted one or more distinct diseases, and to search for aetiological agents. Three cases were characterised by periventricular granulomatous encephalitis, and most probably represented a different disease, but the remaining 48 cases had disseminated non-suppurative encephalitis with widespread neuronal changes. Neuronal degeneration was very marked in the hippocampus of 10 cases and in the cerebellar Purkinje cells of 11. It was thought that the latter cases represented morphological variations of the same disease rather than a different disease because of their

overlapping morphological features. The 48 cases had the following features in common: the disease had primarily neurological signs affecting mostly adult cattle, it was a sporadic condition, and there was a clear tendency for it to have a subacute to chronic course. Polymerase chain reaction (PCR) amplification for chlamydial DNA was negative except in one of 32 specimens, and immunohistochemistry did not demonstrate the presence of chlamydial antigens either in the one PCR-positive case or in the other cases examined. Immunohistochemistry for rabies virus, Borna disease virus, and central European tickborne encephalitis virus was negative. In four cases, immunolabelled cells were found in the lesions with antibodies against paramyxovirus antigens.