

From the National Veterinary Institute, Oslo, Norway.

## POST-MORTEM EXAMINATION IN THE DIAGNOSIS OF JOHNE'S DISEASE IN GOATS

By

*Finn H. Fodstad and Eggert Gunnarsson*

FODSTAD, F. H. and E. GUNNARSSON: *Post-mortem examination in the diagnosis of Johne's disease in goats.* Acta vet. scand. 1979, 20, 157—167. — Post-mortem examinations play an important role in Johne's disease programmes in Norway. The results of such examinations of samples of viscera from 2997 goats carried out during the 5-year period 1972—1976 are given. The investigations show that the demonstration of macroscopical changes in mesenteric lymph nodes and small intestine has only limited value as a guideline in the post-mortem diagnosis of Johne's disease in goats. Often macroscopical changes were not seen or they were non-specific. Caseous and/or calcified foci in mesenteric lymph nodes in infected animals were demonstrated quite often whilst observed intestinal changes were strikingly few. Corrugation of the mucosa was rare. However, in sections of macroscopically unchanged intestine marked epithelioid cell infiltrations and abundant acid-fast bacilli were not uncommon. In sporadic cases productive inflammation with tubercle formation was seen in lymph nodes in infected animals.

Bacteriological culture was by far the most reliable post-mortem diagnostic method. By this method 92 % of the infected goats were detected. The corresponding figures for histological examination and microscopy were 54 % and 47 %, respectively.

macroscopy; histology; bacteriology; Johne's disease in goat.

The *in vivo* diagnosis of Johne's disease in the goat is very uncertain. In the early stages of the disease the animal shows no symptoms and in the later stages symptoms are more or less non-specific. Most commonly, progressive emaciation is observed. The animal becomes dehydrated and anaemic, and milk yield gradually declines. In contrast to observations of diseased cattle, faeces usually remain normal though may have a somewhat pasty consistency. Only very occasionally is diarrhoea a problem.

Hypersensitivity reactions and serological tests are subject to considerable margins of error. The complement fixation test has been used successfully in Norway in mapping the disease, but has only limited value as a diagnostic aid in the individual animal. This is due to, among other factors, the occurrence of anergic phases in infected animals during the course of the illness, to non-specific reactions of unknown cause and to cross-reactions because of other mycobacterial infections (*Ringdal* 1960, *Rankin* 1961, *Pearson & McClelland* 1962, *Merkal et al.* 1968).

Because of the unreliability of in vivo diagnostic methods, post-mortem examinations have played an important role in Johne's disease control programmes in Norway. They have been useful for checking clinical and serological findings and for assessing the effect of vaccination.

This paper describes post-mortem examinations performed at the National Veterinary Institute from 1972—1976, of samples of viscera from goats.

## MATERIALS AND METHODS

### *Samples of viscera*

The dispatch of suitable material to the National Veterinary Institute was based on agreements with veterinarians in general practice, abattoirs and herd owners. In districts in which vaccination against Johne's disease was carried out, material consisted of samples from as many dead or slaughtered goats as possible. In other districts, samples were mainly limited to suspicious patho-anatomical findings, together with samples from goats which showed a positive antibody titre against Johne's disease. In most cases material comprised parts of jejunum and ileum with associated mesenteric lymph nodes. During the investigational period, the National Veterinary Institute received samples from 2997 goats for further examination with respect to Johne's disease.

### *Macroscopic examination*

The aim was to observe the extent to which macroscopic changes are present in the small intestine and mesenteric lymph nodes in Johne's disease in goats, as well as to ascertain which patho-anatomical findings are most important in making the

diagnoses. Patho-anatomical findings were recorded and described. As regards the mesenteric lymph nodes, special attention was paid to size, consistency, colour, nodule formation and the presence of caseous or calcified foci. Any thickening or corrugation of the mucosa of the intestinal samples examined was registered.

#### *Histological examination*

Tissue samples from the intestinal sections and mesenteric lymph nodes were fixed in 10 % buffered formalin. Paraffin sections were stained both with haematoxylin-eosin and according to Ziehl-Neelsen's method.

#### *Bacteriological examination*

Dubos' medium containing 2 % mycobactin was used for the routine culture and isolation of *Mycobacterium paratuberculosis* (Smith 1953, Stuart 1965). After macroscopic examination pieces of tissue exhibiting changes were finely divided and ground in a mortar together with sterile sand. Five % oxalic acid was used as decontaminating agent, 40 ml of this being gradually added to the material during grinding. After being allowed to stand for 5 min, the suspension was filtered through 4 layers of sterile gauze. The filtrate was transferred to sterile tubes and incubated at 37°C for 30 min. After centrifuging for 20 min at 1200 × g, the supernatant was removed and the sediment suspended in about 2 ml saline. Smears were stained for acid-fast bacilli according to Ziehl-Neelsen. Approx. 0.2 ml of the suspension was inoculated onto the surface of Dubos' slant serum-agar containing 2 % mycobactin. Three culture tubes were used for each sample. Inoculated tubes were incubated, with the surface of the medium in the horizontal plane, for 48—72 h at 37°C and then for up to 3 months with weekly readings being taken after the 5th week. The prolonged reading period was due to great variation in the speed and intensity of growth of the different strains of *M. paratuberculosis*. The diagnosis was verified by microscopy of smears prepared from cultures showing possible or certain growth of *M. paratuberculosis*. In doubtful cases, subculture was made on Dubos' medium both with and without the addition of mycobactin.

## RESULTS

Johne's disease was demonstrated in 274 (9 %) of the goats examined. As regards 262 of these, examination of material comprised macroscopic description, microscopy of direct smears, bacteriological culture and histological examination. Diagnosis was made on the basis of 1 or more of the last 3 methods.

*Macroscopic findings*

Mesenteric lymph nodes from 2745 goats were examined. Of these 255 showed infection. Table 1 shows that macroscopic changes which could be indicative of Johne's disease were found in 65 % of the goats which were ascertained to be infected. Most

Table 1. Macroscopic examination of mesenteric lymph nodes from 2745 goats.

| Patho-anatomical findings       | 255 goats,<br>infection<br>demonstrated |      | 2490 goats,<br>infection not<br>demonstrated |      |
|---------------------------------|---|------|--|------|
|                                 | number                                  | %    | number                                       | %    |
| Abscesses                       | 5                                       | 2.0  | 2  | 0.1  |
| Miliary grain-sized nodules     | 18                                      | 7.0  | 23   | 0.9  |
| Caseous/calcified foci          | 53                                      | 20.8 | 37   | 1.5  |
| Swollen, oedematous lymph nodes | 88                                      | 34.5 | 294  | 11.8 |
| No changes                      | 91                                      | 35.7 | 2134   | 85.7 |

common were swollen oedematous lymph nodes. Caseous and often partially calcified processes, mainly confined to the cortical region, were found in 20 % of the infected goats, while macroscopic changes were not demonstrated at all in 36 % of the infected animals. Some degree of enlargement of the lymph nodes was found in 12 % of the non-infected goats, while miliary to pea-sized nodules or caseous foci were demonstrated in 2—3 %. Macroscopical changes were not observed in 86 %.

Parts of the jejunum and ileum from 2947 goats were examined. Of these, 273 showed infection (Table 2). Macroscopic changes were found in almost half the samples from animals in which infection was demonstrated. Most common were various degrees of thickening of the mucosa. In about 7 % of the infected animals marked corrugation of the mucosa which could not be stretched out was found. Partial thickening of the mucosa was

**Table 2.** Macroscopic examination of sections of small intestine from 2947 goats.

| Patho-anatomical findings | 273 goats, infection demonstrated |      | 2674 goats, infection not demonstrated |      |
|---------------------------|-----------------------------------|------|--|------|
|                           | number                            | %    | number                                 | %    |
| Marked corrugation        | 20                                | 7.3  | 0                                      | 0    |
| Thickened mucosa          | 109                               | 39.9 | 315                                    | 11.8 |
| No changes                | 144                               | 52.8 | 2359                                   | 88.2 |

found in 12 % of the non-infected animals. The intestine samples from the remaining non-infected animals showed no macroscopic changes.

Table 3 shows the various macroscopic findings correlated with infection. As in cattle, corrugation of the intestinal mucosa seems to be a relatively specific finding (*Jubb & Kennedy 1970*). All goats showing this macroscopic change proved to be infected.

**Table 3.** Correlation between macroscopic findings and infection.

| Organ                  | Patho-anatomical findings   | Number of samples | Demonstrated infected samples |       |
|------------------------|-----------------------------|-------------------|-------------------------------|-------|
|                        |                             |                   | number                        | %     |
| Mesenteric lymph nodes | Abscesses                   | 7                 | 5                             | 71.4  |
|                        | Miliary grain-sized nodules | 41                | 18                            | 43.9  |
|                        | Caseous/calcified foci      | 90                | 53                            | 58.9  |
|                        | Swollen, oedematous         | 382               | 88                            | 23.0  |
|                        | No changes                  | 2225              | 91                            | 4.0   |
| Small intestine        | Marked corrugation          | 20                | 20                            | 100.0 |
|                        | Thickened mucosa            | 424               | 109                           | 25.7  |
|                        | No changes                  | 2503              | 144                           | 5.8   |

*Microscopy of direct smears*

Short acid-fast bacilli were demonstrated in smears from 123 goats. Results from bacteriological and histological examinations were negative for 2 of these animals. As a rule, large numbers of bacilli were present in the smears. Only in a few cases was the presence of bacilli more sporadic.

### *Bacteriological culture*

After 3 months of incubation growth of *M. paratuberculosis* had occurred from samples from 241 goats. Growth characteristics of the different strains varied greatly. Some strains showed abundant growth after only 6 weeks of incubation. Others showed growth of only a few colonies after 3 months. Some cultures were contaminated such that any mycobacteria present would have been completely overgrown by saprophytic bacteria.

### *Histological findings*

Histological changes characteristic of Johne's disease were demonstrated in 142 goats. Bacteriological examination of 19 of these animals was negative. Histological changes were usually present both in the mesenteric lymph nodes and the small intestine. In most animals there seemed to be a clear correlation between macroscopic and histological findings in the mesenteric lymph nodes. The most common finding in large, swollen mesenteric lymph nodes was more or less confluent areas of epithelioid cells, mostly confined to the cortical region. These cells usually contained abundant acid-fast bacilli. Giant cells of Langhans' type were rarely seen in this early stage of the disease. In later stages with obvious nodule formation or caseous and/or calcified foci in the lymph nodes, far fewer acid-fast bacilli were found in the histological sections. Nodules showing a typical tubercle structure were demonstrated in the cortical region in sporadic cases. Acid-fast bacilli were then difficult, if not impossible, to find. The most common histological finding in the mucosa of the small intestine in the infected goats was the occurrence of variously sized accumulations of epithelioid cells, containing large numbers of acid-fast bacilli. These cell infiltrations occasionally penetrated into the submucosa and exceptionally down into the connective tissue between the 2 layers of stratum muscularis. A striking finding was the marked changes and abundant acid-fast bacilli in sections from macroscopically normal small intestine.

### *Diagnosis*

Figure 1 shows the basis on which Johne's disease was diagnosed in those goats examined bacteriologically, histologically

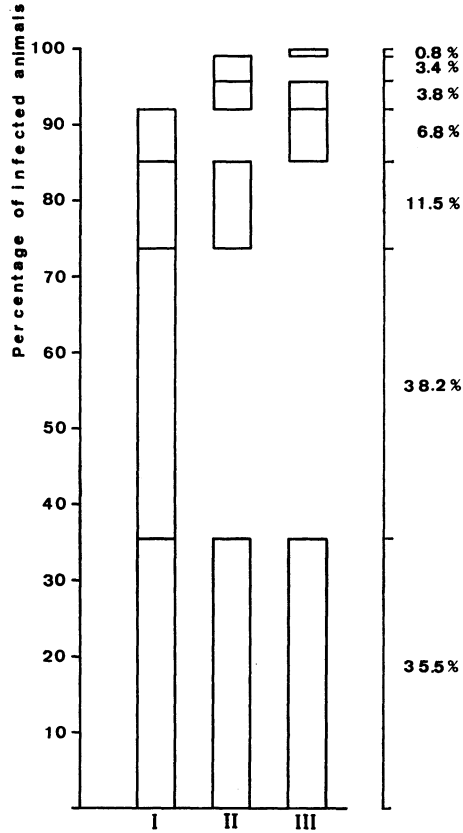


Figure 1. Basis for the diagnosis of Johne's disease in 262 goats. The columns numbered I, II and III indicate the percentage of infected goats diagnosed by bacteriological culture, histological examination and direct microscopy, respectively. The figures to the right indicate the distribution of the goats according to the basis for the diagnosis.

and by microscopy of direct smears. The 3 diagnostic methods gave the same result in 35 % of cases, while the diagnosis was based on 2 of them in 22 %. Bacteriological culture detected 92 % of the infected goats. Corresponding figures for histological examination and microscopy were 54 % and 47 %, respectively. The most striking result is that bacteriological culture was alone responsible for 38 % of the positive diagnoses.

## DISCUSSION

The investigations show that the demonstration of macroscopical changes in mesenteric lymph nodes and small intestine has only limited value as a guideline in the post-mortem diagnosis of Johne's disease in the goat. Similar changes may be associated with several other conditions. Post-mortem findings may often be quite negative. In these cases the disease is probably at an early stage.

Macroscopic findings deviate in several respects from those usually found associated with Johne's disease in cattle. Thus caseous and/or calcified foci in mesenteric lymph nodes were demonstrated in approximately only 20 % of the infected goats. This is in accordance with earlier observations (*Holmboe & Slagsvold* 1934). According to *Goudswaard* (1971) such changes indicate a chronic course. Corresponding changes have also been described in Johne's disease in sheep (*Holmboe & Slagsvold, Stamp & Watt* 1954). In the literature there had always been general agreement that caseation of the lymph nodes did not occur in Johne's disease in cattle, until a case was recorded a few years ago (*Richards & Muhm* 1972). The figures in Table 3 seem to indicate a strong correlation between abscess formation in mesenteric lymph nodes and infection in goats. However, the material with abscesses is very small. No real significance can therefore be attached to this relationship. In infected cattle a common finding is pronounced macroscopical change in various sections of the intestine, most often in the form of marked thickening and corrugation of the mucosa. In contrast, observed intestinal changes in the goats examined were strikingly few. Moderate thickening of the mucosa was seen relatively often, whilst corrugation was rare. Most commonly, intestinal sections were macroscopically normal. The present investigation showed little correlation between macroscopic and histological findings in the small intestine. This is in accordance with earlier observations (*Jubb & Kennedy* 1970). Thus, marked epithelioid cell infiltrations and abundant acid-fast bacilli were not uncommon findings in sections of macroscopically unchanged intestine.

Productive inflammation in the form of tubercle formation occurs very rarely in Johne's disease. In sporadic cases, however, such inflammatory changes have been demonstrated in mesenteric lymph nodes in infected goats (*Jubb & Kennedy*). This was also the case in the present material, as typical tubercles were



found in the cortical zone of mesenteric lymph nodes in a few animals. In such cases acid-fast bacilli were either present in very small numbers or not demonstrable. It is possible that such histological changes are an expression of a recovery from infection.

The investigation shows that bacteriological culture is by far the most certain post-mortem diagnostic method. There may be several reasons for this. Infection may have taken place so shortly before examination that histological changes had not yet become manifest in the tissues. Dispatched material may have been in such a state that histological examination was almost worthless. Moreover, the histological picture may vary quite considerably within a limited area of the same organ.

As regards the reliability of bacteriological culture in Johne's disease, results are in agreement with the reported situation in cattle (Pearson 1962, Ringdal 1963) and sheep (Kluge *et al.* 1968). However, the method is both laborious and time-consuming. Moreover, results show that approx. 8 % of infected animals were not detected by this method. Thus it is necessary to supplement with histological examination and microscopy of direct smears. Most positive diagnoses can then be made much more quickly.

Recent investigations in the fields of cellular and humoral immunology have opened up new perspectives as regards the rapid and safe diagnosis of Johne's disease in animals in vivo (Bendixen 1977, Gunnarsson & Fodstad 1979). However, until appropriate techniques in these fields are further developed, post-mortem examination as described in the present paper is considered to be the most safe method of diagnosis of Johne's disease in the goat.

#### REFERENCES

- Bendixen, P. H.: Application of the direct leukocyte-migration agarose test in cattle naturally infected with *Mycobacterium paratuberculosis*. Amer. J. vet. Res. 1977, 38, 1161—1162.
- Goudsvaard, J.: Studies on the incidence of *Mycobacterium johnei* in the organs of experimentally infected goats. Neth. J. vet. Sci. 1971, 4, 65—75.
- Gunnarsson, E. & F. H. Fodstad: Analysis of antigens in *Mycobacterium paratuberculosis*. Acta vet. scand. 1979, 20, 200—215.
- Holmboe, F. D. & L. Slagsvold: Paratuberkulose hos sau og geit. (Paratuberculosis in sheep and goats). Skand. Vet.-T. 1934, 24, 573—584.

- Jubb, K. V. F. & P. C. Kennedy*: Pathology of Domestic Animals. Chapter 2. Acad. Press, New York and London 1970.
- Kluge, J. P., R. S. Merkal, W. S. Monlux, A. B. Larsen, K. E. Kobecky, F. K. Ramsey & R. P. Lehmann*: Experimental paratuberculosis in sheep after oral, intratracheal, or intravenous inoculation: Lesions and demonstration of etiologic agent. *Amer. J. vet. Res.* 1968, 29, 953—962.
- Merkal, R. S., A. B. Larsen, K. E. Kobecky & R. D. Ness*: Comparison of examination and test methods for early detection of paratuberculous cattle. *Amer. J. vet. Res.* 1968, 29, 1533—1538.
- Pearson, J. K. L.*: Studies on Johne's disease in cattle in Northern Ireland. III. Diagnosis of non-clinical infections — Review of literature. *Brit. vet. J.* 1962, 118, 86—96.
- Pearson, J. K. L. & T. G. McClelland*: Studies on Johne's disease in cattle in Northern Ireland. IV. Diagnosis of non-clinical infections. Serological and allergic tests in Northern Ireland. *Brit. vet. J.* 1962, 118, 97—106.
- Rankin, J. D.*: The non-specificity of a complement-fixation test used in the diagnosis of Johne's disease in cattle. *Res. vet. Sci.* 1961, 2, 89—95.
- Richards, W. D. & R. L. Muhm*: Isolation of *M. paratuberculosis* from a cow at routine slaughter. *Proc. 75th Ann. Meet. U.S. Anim. Health Ass.* 1972, 518—522.
- Ringdal, G.*: Diagnosis of Johne's disease in cattle. *Nord. Vet.-Med.* 1960, 12, 513—531.
- Ringdal, G.*: Culture of *Mycobacterium johnei*. *Acta vet. scand.* 1963, 4, 85—91.
- Smith, H. W.*: Modifications of Dubos's media for the cultivation of *Mycobacterium johnei*. *J. Path. Bact.* 1953, 66, 375—381.
- Stamp, J. T. & J. A. Watt*: Johne's Disease in sheep. *J. comp. Path.* 1954, 64, 26—40.
- Stuart, P.*: Vaccination against Johne's disease in cattle exposed to experimental infection. *Brit. vet. J.* 1965, 121, 289—318.

#### SAMMENDRAG

##### *Post mortem diagnostikk ved paratuberkulose hos geit.*

Post mortem undersøkelser spiller en betydelig rolle i bekjempelsesprogrammet mot paratuberkulose hos geit i Norge. I femårsperioden 1972—1976 ble organprøver fra 2997 geiter undersøkt. Disse undersøkelsene viste at makroskopiske forandringer i krøslymfeknuter og tynntarm-avsnitt bare har begrenset verdi som rettesnor ved post mortem diagnostikk av paratuberkulose hos geiter.

Mange infiserte dyr viste ingen makroskopiske forandringer i krøslymfeknuter eller tarmavsnitt. Hos andre var de registrerte forandringer overveiende uspesifikke. Av typiske forandringer hos infiserte dyr kan nevnes mer eller mindre svulne, ødematøse krøslymfeknuter. Ofte fantes forostede og/eller forkalkede foci i disse. Tarmforandringer var få og oftest lite uttalte. Bare i enkelte tilfeller fantes fortykket

slimhinne med foldedannelser. I histologiske snitt fra makroskopisk normale tarmavsnitt fantes imidlertid ofte betydelige epitheloide celleinfiltrasjoner og rikelig med syrefaste staver. Hos ganske få infiserte dyr påvistes produktiv betennelse med begynnende tuberkeldannelser i krøslymfeknuter.

Av de påviste infiserte dyr ble 92 % funnet ved bakteriologisk dyrking. De tilsvarende tall for histologisk undersøkelse og direkte mikroskopi var henholdsvis 54 % og 47 %.

*(Received September 13, 1978).*

Reprints may be requested from: Finn H. Fodstad, the National Veterinary Institute, P.O.Box 8156, Oslo Dep., Oslo 1, Norway.