Paratuberculosis in dairy and pygmy goats: an underestimated problem?

Paratuberculose bij melk- en dwerggeiten: een onderschat probleem?

[1,2]A. Tuerlinckx, [3]J. Vicca, [2]P. Deprez, [2]B. Pardon

[1]DAP Aan De Watergang, Kemphoekstraat 47, 9170 Sint-Gillis-Waas
[2]Department of Large Animal Internal Medicine, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke
[3]Department of Agro- and Biotechnology, Odisee, University College KULeuven, Hospitaalstraat 23, 9100 St.-Niklaas

Bart.Pardon@UGent.be

ABSTRACT

Paratuberculosis is a chronic intestinal disease affecting goats and other ruminants worldwide. The objective of this review was to summarize current knowledge on the prevalence, diagnostic possibilities and possible prevention and control measures for paratuberculosis in dairy and pygmy goats in Europe. The herd level prevalence of *Mycobacterium avium subspecies paratuberculosis* (MAP) in dairy goat farms is very high, namely 71%, 63% and 86% in Germany, France and the Netherlands, respectively. The prevalence in pygmy goats is undocumented. Antibody ELISA, fecal culture and PCR testing are readily available for diagnosis. A highly suggestive finding for paratuberculosis is the presence of enlarged intestinal lymph nodes on abdominal ultrasonography. To control the disease a combination of 'test and cull' and environmental hygienic measures are recommended. In contrast to cattle, vaccination is available and might be an additional tool in the control program for goats. Vaccine antibodies are no longer detectable after one year, and therefore serological monitoring can be continued on vaccinating herds. The worrisome prevalence data from neighboring countries indicate the need for a prevalence study on dairy goat farms in Belgium.

SAMENVATTING

Paratuberculose is een chronische, intestinale aandoening bij geiten en andere herkauwers. Het doel van dit overzichtsartikel is om de huidige kennis omtrent prevalentie, diagnostische mogelijkheden, preventie en mogelijke controlemaatregelen tegen paratuberculose bij melk- en dwerggeiten in Europa samen te vatten. De bedrijfsprevalentie van *Mycobacterium avium subspecies paratuberculosis* (MAP) is in de ons omringende landen zorgwekkend hoog, namelijk 71%, 63% en 86% in Duitsland, Frankrijk en Nederland, respectievelijk. De prevalentie bij dwerggeiten is onbekend. Antistof ELISA, cultuur en PCR van mest zijn vlot beschikbaar om de diagnose te bevestigen. Een sterk suggestief letsel voor paratuberculose is de aanwezigheid van vergrote intestinale lymfeknopen op abdominale echografie. Om deze aandoening onder controle te krijgen, is een combinatie van "test and cull" en hygiënische maatregelen sterk aangeraden. In tegenstelling tot bij rundvee is vaccineren mogelijk en zou het een bijdrage kunnen leveren tot controleprogramma's voor paratuberculose bij geiten. Vaccinantistoffen zijn niet meer detecteerbaar één jaar na vaccinatie, waardoor serologische monitoring mogelijk blijft op gevaccineerde bedrijven. De verontrustende prevalentiecijfers in aangrenzende landen tonen de dringende noodzaak van een prevalentiestudie op melkgeitenbedrijven in België aan.

INTRODUCTION

Paratuberculosis is a chronic contagious disease caused by *Mycobacterium avium subspecies paratuber-culosis* (MAP) (Verdugo et al., 2014)*,* affecting several animal species: predominantly ruminants (cattle, sheep, goat, deer, etc.), but also camelids, rabbits, foxes, pigs, horses, llamas, alpacas, deer and weasels are

susceptible (Windsor, 2015). Animals get infected at a very young age, but due to the long incubation time, clinical signs appear much later; on average at the age of two (Mercier et al., 2010).

Paratuberculosis has been less studied in goats than in cattle (Nielsen et al., 2008). There are important differences between both species regarding their susceptibility and clinical presentations of paratuberculosis. Goats have a less efficient humoral immunity reaction to MAP (Kostoulas et al., 2006) and clinical illness develops at an earlier stage in the pathogenesis. In contrast to cattle, chronic diarrhea is not a prominent clinical sign in goats (Robbe-Austerman et al., 2011), and MAP bacteria are almost never found in goat's colostrum (0%) or milk (4%), even when antibodies against MAP are detected (Lievaart-Peterson, 2017). Another important difference with cattle in the control of paratuberculosis is that vaccines against MAP are available for goats and sheep. These inactivated vaccines induce antibodies, which might interfere with the use of serology as a diagnostic tool in control programs (Sevilla et al., 2008).

The aim of this review is to provide an overview of the current knowledge on the prevalence of paratuberculosis in goats, the different diagnostic possibilities and difficulties, possible prevention and control strategies and the influence of vaccination on prevalence studies and control programs.

MATERIALS AND METHODS

A search was conducted in different databases (PubMed, Web Of Science and Google Scholar) with the following key words: goat, paratuberculosis, diagnosis, prevalence, MAP, prevention and vaccination.

PREVALENCE OF PARATUBERCULOSIS IN EUROPE

In Europe, peer-reviewed prevalence studies have been published in Germany, France and the Netherlands. In Germany, 1609 unvaccinated animals (1473 sheep and 136 goats) (10 animals with the lowest body condition score in each farm) were tested using serum enzyme linked immunosorbent assay (ELISA) (sensitivity (SE) 90% and specificity (SP) 99%, as determined in cows. Of the sheep and goats, 14% and 21% tested positive, respectively. The herd-level prevalence on 167 farms, i.e. 150 sheep and 17 goat farms, was 65% and 71%, respectively. The within-herd prevalence was 21% and 32%, in sheep and goats, respectively (Stau et al., 2012). In France, in 2010, 105 dairy goat farms were screened with an antibody ELISA (SE= 53% and SP= 100%), covering 11,847 unvaccinated goats older than six months old. Of the farms, 62.9% (95% confidence interval (CI)= 41.4 – 84.4) tested positive and the within-herd prevalence was 5.5% (95% CI= 2.62 – 3.24) (Mercier

et al., 2010). In the Netherlands, 36 dairy goat farms were tested using antibody ELISA; 86% (95% CI= 72% - 94%) of the farms tested positive. In contrast to France and Germany, 26 farms of this Dutch population vaccinated their goats against paratuberculosis. All vaccinating farms tested positive (95% CI= 87% - 100%). Of the ten non-vaccinating herds, only 50% (95% CI= 19% - 81%) tested positive. Depending on the cut-off, the within-herd prevalence varied between 4% (0% - 7%) and 8% (0% - 16%) (Lievaert-Peterson, 2013). It is important to bear in mind that these studies report apparent prevalence, and given the low SE of some ELISAs, true prevalence might be different. For Belgium, no peer-reviewed prevalence studies are available. In an unpublished study, nine commercial dairy goat farms of the approximately 60 specialized dairy goat farms in Belgium were tested by sampling 10% of the animals aged between two and five years old. All tested positive and the within-herd prevalence ranged between 11% and 39% (Vicca, unpublished results). The only identified herd-level risk factor for paratuberculosis is increasing herd size (Stau et al., 2012). This suggests that the prevalence of paratuberculosis in the European countries studied is much higher in goats than in cattle (3-27% true prevalence and 7-55% apparent prevalence on antibody ELISA) (Garcia and Shalloo, 2015). Data on the prevalence in pygmy goats are currently lacking, but case reports are available (Tuerlinckx, 2017). The close contact between pygmy goats and humans and the fact that goat's cheese has been more frequently consumed in recent years, warrant a closer estimate of the possible zoonotic risks (Statistics Belgium, 2016).
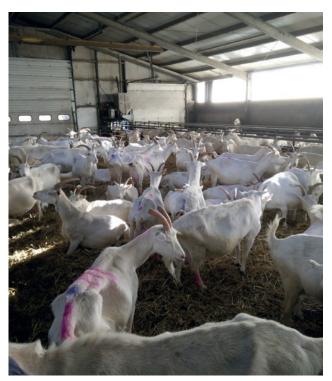

Figure 1. Marked weight loss in a dairy goat with paratuberculosis (front, marked with pink).

DIAGNOSTIC POSSIBILITIES AND DIFFICULTIES

The incubation time of paratuberculosis is long and highly variable (Robbe-Austerman, 2011). In goats, the main clinical signs are cachexia, production loss, a dull and crustaceous hair coat, exercise intolerance (Boelaert et al., 2000; Windsor, 2016), selective food intake (leaving the concentrate) and sometimes intermittent diarrhea (Maynard-James et al., 1997). In a study on 54 goats with paratuberculosis, 54% had normal feces, 24% had pasty feces, 15% had intermittent diarrhea and only 7% suffered from chronic diarrhea. Dairy goats generally don't reach the 'diarrhea stage', possibly because they are removed earlier due to declining production. In contrast, pygmy goats do get to the diarrhea stage (GD, 2014). The only systematic clinical sign is cachexia without anorexia (Fernandez-Silva et al., 2014) (Figures 1 and 2). Therefore, it is impossible to diagnose paratuberculosis only by clinical observation and examination (Bastida et al., 2011).

Several diagnostic tests are available: on the one hand culture, polymerase chain reaction (PCR) analysis and Ziehl-Neelsen staining of feces or intestinal or lymphoid tissue, and on the other hand, detection of antibodies in serum or milk by ELISA. The long and variable incubation time (one to four months at 37°C) (Eriks et al., 1996) makes isolation of MAP from feces difficult. Fecal culture has a low SE (8% (2% - 17%)) because of the difficult in vitro incubation, the intermittent shedding of MAP and the unequal division of bacteria in the feces (Kostoulas et al., 2006). Ziehl-Neelsen solution stains mycol-acid in the bacterial membrane. *Mycobacteria* are able to hold the reddish color despite decoloring attempts with alcohol. Therefore, *Mycobacteria* will turn red on a blue background. The Ziehl-Neelsen procedure is not specific because all *Mycobacterium spp.* color red (Navarro et al., 1991). Moreover, the SE is low (36.4% in cows) (Zimmer et al., 1999).

Next to culture and Ziehl-Neelsen staining, antibody ELISA can be performed on milk or serum. The SE and SP for milk and serum are comparable, and ELISA on milk has been suggested as a good screening tool for dairy goat farms (Windsor, 2015). Since 2007, the Flemish Animal Health Service (Diergezondheidszorg Vlaanderen) offers an antibody ELISA on milk with a SE of 86.7% and a SP of 99.7%, in Belgium. The problem with ELISA is that antibodies are detected, which are only present during the humoral T_H2-immunity stage, after the cell-mediated T_H1-immunity stage. The T_H2-immunity is suppressed by the cell-mediated T_H1-immunity, which starts shortly after infection. Therefore, there is a high risk of false negative results when testing serologically with ELISA before the humoral T_H2-immunity stage (Kostoulas, 2005). ELISA is not suitable to quantify the number of MAP bacteria and to detect paratuberculosis before the humoral stage. The most recently available

Figure 2. Detail of the lumbar region in a dairy goat with cachexia as a consequence of paratuberculosis.

test is PCR, which has the highest SE (94.1%) and SP (100.0%) to detect an infection. The test can be applied on feces, intestinal and lymphoid tissue or milk. It allows to differentiate low and high shedders. This method has only recently been implemented in the dairy goat industry, since the PCR-test has become cheaper and more accurate (Bastida et al., 2011).

The gold standard for diagnosing clinical paratuberculosis in goats remains post-mortem examination (Robbe-Austerman, 2011). The following pathological findings can be seen: depletion of fat depots, muscle atrophy, thickening of the intestinal wall and enlargement of the mesenteric lymph nodes (Windsor, 2015). Mesenteric lymph nodes enlarge up to 60 – 70 mm x 20 – 30 mm. Several white-yellow spots of necrosis may be visible on the cutting surface. Sometimes goats suffering from clinical paratuberculosis show mineralized nodules in the cortex of the lymph node (Lybeck et al., 2015). To confirm the diagnosis of paratuberculosis at necropsy, histology with hematoxylin-eosin staining can be used. In some cases, a chronic granulomatous enterocolitis with infiltration of macrophages, lymphocytes and plasma cells in the lamina propria can be noticed (Fernandez-Silva et al., 2014).

In goats, there are roughly two kinds of histological findings, namely diffuse and local lesions (Windsor, 2015). Diffuse lesions show a granulomatous infiltration of the mesenteric lymph nodes and the intestinal wall, with transmural enteritis. The intestinal villi are shortened, enlarged and erosion and ulceration of the intestinal wall are sometimes remarked. In contrast, in goats with local lesions, multiple granulomas and a high number of lymphocytes are present in the intestinal wall and mesenteric lymph nodes. The intestinal wall is not as enlarged as it is in goats with diffuse lesions, and often the epithelia of the intestine are unaffected (Lybeck et al., 2013). Further detail on the histopathological findings is available elsewhere (Windsor, 2015). A final diagnosis can only be made by testing feces or tissue samples by PCR or culture (Robbe-Austerman, 2011). Furthermore, Ziehl-

Neelsen staining can be applied on ileal or mesenteric lymph node samples.

An alternative method to detect goats infected with paratuberculosis is ultrasonography. This method has become more accessible with pygmy goats being kept as pets. Their owners are more willing to pay for a more individual and specialized diagnostic approach. With a 7.5 MHz transducer penetrating up to 12 centimeters, a standing goat can be examined. Shaving is often needed as only making contact with alcohol and/or gel on the goat coat often provides insufficient image quality. Three aspects of the small intestine need to be visualized to suspect paratuberculosis: (1) the thickness and the (2) presence of folds in the mucosa of the intestinal wall and the size of mesenteric lymph nodes. Tharwat (2012) found that only 44% of 54 seropositive goats had a thickened intestinal wall (2 mm up to > 5.1 mm), the other 56% showed no thickening of the intestinal wall (< 2mm). Transversal folding of the mucosa of the intestinal wall was not a very reliable criterion, since only 13% of the seropositive goats showed this on ultrasound. The most sensitive ultrasonographic finding was the enlargement of the mesenteric lymph nodes. Of goats with paratuberculosis, 91% (n=49) showed this image on echography (confirmed by necropsy) (Figure 3). Of these goats, 73% showed a hypo-echogenic cortex and hyper-echogenic medulla. None of the mesenteric lymph nodes of the control population could be visualized by ultrasound (Tharwat et al., 2012).
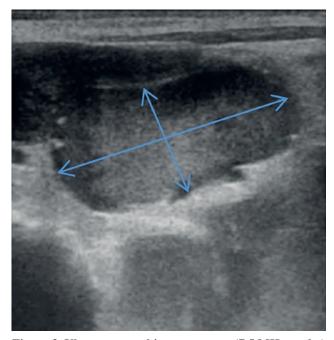

Figure 3. Ultrasonographic appearance (7.5 MHz probe) of a typical enlarged mesenteric lymph node (3.85 cm x 1.71 cm) in a 2.5-year-old pygmy goat suffering from paratuberculosis.

PREVENTION AND CONTROL

To the authors' knowledge, in Europe, paratuberculosis programs in goats are only available in the Netherlands (voluntary) and in Norway (obligatory for herds delivering to certain dairy companies); this is in contrast to the situation in cattle. Most cattle paratuberculosis programs are based on the principles of 'test and cull' and decreasing pathogen transmission by improved management (hygiene and in extenso translated into external and internal biosecurity) (Bastida et al., 2011). The same two principles can be applied to goats, but also vaccination can be applied in replacement stock to increase their resistance to infection (Windsor, 2015). The oro-fecal route is the most common route of infection in goats. An infected goat sheds up to 10^8 MAP per gram of feces (Windsor, 2015). Also colostrum and milk are considered infectious; however, in contrast to cattle, colostrum apparently plays a minor role in goats (Leonor Mundo et al., 2013). In a recent Dutch survey, MAP could not be detected in any of 121 colostrum samples, whereas 95% contained MAP antibodies (Lievaart-Peterson, 2017). In addition, transplacental infection is possible in goats (Manning et al., 2003). MAP survives for over one year in the environment and can be retrieved from airborne dust particles. Not only the farm itself

but also neighboring fields where cows, sheep or other goats are grazing are a reservoir of MAP (Robbe-Austerman, 2011). Moreover, wildlife might contaminate pasture or drinking ponds; however, up till now, only wild ruminants (deer, etc.) and lagomorphs have been evidenced to shed MAP in their feces of fecal shedding, thereby spreading MAP by contaminating the environment (Stevenson et al., 2009).

To prevent farms from being infected, purchased animals need to be tested. However, given the low sensitivity, only relying on a purchase test is insufficient. In addition, it is strongly advised to only purchase animals from a herd of origin with a known negative paratuberculosis status. Also male goats should be tested (Robbe-Austerman, 2011). Given the currently estimated high prevalence in Belgium, purchase from negative herds will be difficult.

In positive herds, control measures need to be implemented to gradually reduce the prevalence and eventually become negative. Three main pillars to control paratuberculosis in goats have been mentioned: management measures to decrease pathogen spread, 'test and cull' and vaccination. The most important management measure to avoid spread is motherless raising. Goat milk is a potential source (in 4% of the cases (Lievaart-Peterson, 2017)) of MAP. Separation of the lambs immediately after birth and subsequent prolonged individual housing are still recommended (Robbe-Austerman, 2011). Even though the presence of MAP in colostrum has not been demonstrated in Dutch dairy goats (Lievaart-Peterson, 2017), replacement colostrum from other MAP free goat or cattle

farms is recommended. Alternatively, colostrum of positive farms might be pasteurized. Low-temperature long-time pasteurization (60°C during 60 minutes) is the only possibility, because colostrum antibodies are destroyed at higher temperatures. In a small-scale study, in each of four replicate batches, no viable MAP could be retrieved after such a pasteurization protocol (Godden et al., 2006). However, there is no consensus on the 100% efficacy of this procedure. Moreover, pasteurization of small amounts of colostrum is practically difficult: most devices work with 1-4 liter bags. It should be noted that in cattle, this pasteurization protocol has been shown to be ineffective in the long term, most likely because animals become infected through the environment later in life (Godden et al., 2015). In contrast to cattle, the effects of supplemental hygienic and combined external and internal biosecurity measures on the incidence of new MAP infections in goat herds have not been studied yet.

The traditionally most important pillar of a paratuberculosis control program is a test-and-cull policy. In the dairy goat industry, test-and-cull is controversial, since dairy goats have a high economic value. Another issue is the large number of false negatives due to the low sensitivity of the different diagnostic tests (Windsor, 2015). In the Netherlands, a voluntary paratuberculosis control program has recently been initiated for dairy goats. The program is based on serial (annual) antibody ELISAs on milk or serum and differentiates three statuses: status A= no infection, status B= infected animals and animals with antibodies are culled; status C= infected animals and animals with antibodies are not culled. A difference with the Dutch paratuberculosis program for cattle is that no fecal culture confirmation is required for goats. Unfortunately, to date, only 5% of the Dutch dairy goat farms participates (personal communication, M. Holzhauer, GD, the Netherlands). The program does not include vaccination. In Norway, all goat farmers delivering milk to the dairy industry are obliged to check for antibodies against MAP on bulk tank milk five times a year. In Australia, a national, voluntary Johne's Disease Control Program is in action and depends on the widespread use of vaccination (Windsor, 2015).

THE SIGNIFICANCE OF VACCINATION FOR MONITORING AND CONTROL PROGRAMS

An important difference with cattle is that a goat vaccine against MAP is available for control programs (Gudair®, CZ Veterinaria, Porriño, Spain). This vaccine with heat inactivated MAP bacteria is registered in Spain and the Netherlands. The vaccination scheme includes a once-in-a-lifetime vaccination at the age of 2-3 weeks up to 6 months of all animals (including adults at initiation of the herd vaccination). A meta-

analysis has shown that vaccination reduces microbial contamination, and reduces or delays production losses and pathology (Batista and Juste, 2011). However, vaccination can not fully prevent infection and large variations between the available vaccines have been shown. In Australia, the national paratuberculosis control program uses the Gudair® vaccine. Since the introduction of this vaccine in 2002, in Australia it is believed to be the most economical way of controlling paratuberculosis (Windsor, 2015). The results are twenty times more satisfying than the test-and-cull strategy using serum ELISA (Bastida et al., 2011). An average decline of damage and infection of 94.8% (measured in effect at tissue level), 79.3% of the epidemiology (microbiological infection risks, measured in number of isolations of MAP in feces or tissue) and 45.1% less production losses (losses measured as number of clinical cases or deaths) have been shown in the Netherlands (GD, 2014). A study of 1998 revealed that, when vaccinating with the Gudair® vaccine, mortality and the prevalence of MAP shedders declined with 90%, and shedding started twelve months later (Dhand et al., 2016). However, shedding could not be prevented. Different studies have shown very good results; however, the results were not as satisfying in populations with a low or medium prevalence. Therefore, the high expectations of the Gudair® vaccine can only be met when vaccinating a population with a high prevalence, as currently in Belgium (Windsor, 2013). Vaccination should be continued, even after the whole population is immunized. Shedding of MAP may continue up to six years, meaning that even buying vaccinated goats poses a risk of introducing MAP (Windsor, 2013). Remarkably, many of the available vaccination studies do not include a control group (Bastida et al., 2011). In conclusion, based on the available data, the effects of vaccination should not be overestimated; moreover, it appears to be impossible for a farm to become negative only by vaccination (Windsor et al., 2014). Nevertheless, in Australia, the control program with vaccination has been of enormous economic benefit (Windsor, 2015).

An important disadvantage of paratuberculosis vaccination is the cross reactions with the intradermal tests for *Mycobacterium tuberculosis*, hampering national tuberculosis control programs (Windsor, 2015). Because the Gudair® vaccine is not a marker-vaccine, interference with the serological diagnosis and monitoring of goat farms might also occur. A series of Dutch studies have shown that antibody levels start to reduce 16 weeks after vaccination and can no longer be detected one year after vaccination (Lievaert-Peterson, 2013). Therefore, monitoring of vaccinated herds remains possible, but needs to be done in a representative number of animals to avoid false positives. Finally, attention should be paid to avoid self-injection when administering the vaccine as extensive local necrosis can be effected (Windsor, 2015).

CONCLUSION

Available studies show a worrisome high prevalence of paratuberculosis in dairy goats in the neighboring countries of Belgium. The prevalence in companion goats is currently undocumented. Based on this information, a prevalence study on Belgian dairy goat farms is urgently warranted. Identical diagnostic tools as in cattle are available and identical issues with the long incubation period exist. Enlargement of the mesenteric lymph nodes is a highly suggestive finding on abdominal ultrasonography. Prevention and control programs should include a combination of test-and-cull and management measures to reduce pathogen spread. In contrast to cattle, vaccination offers perspective as an additional control tool. As vaccine antibodies can only be detected until one year after vaccination, serological monitoring in vaccinated herds remains possible.

REFERENCES

Bastida F., Juste R.A. (2011). Paratuberculosis control: a review with a focus on vaccination. *Journal of Immune Based Therapies and Vaccines 9*, 1-8.

Boelaert F., Walravens K., Biront P., Vermeersch J.P., Berkvens D., Godfroid J. (2000). Prevalence of paratuberculosis (Johne's disease) in the Belgian cattle population. *Veterinary Microbiology 77*, 269-281.

Dhand N.K., Eppleston J., Whittington R.J., Windsor P.A. (2016). Changes in prevalence of ovine paratuberculosis following vaccination with Gudair®: Results of a longitudinal study conducted over a decade. *Vaccine 34*, 5107-5111.

Eriks I.S., Munck K.T., Besser T.E., Cantor G.H., Kapur V. (1996). Rapid differentiation of Mycobacterium avium and M. paratuberculosis by PCR and restriction enzyme analysis. *Journal of Clinical Microbiology 34*, 734-737.

Fernández-Silva J.A., Correa-Valencia N.M., Ramírez N.F. (2014). Systematic review of the prevalence of paratuberculosis in cattle, sheep, and goats in Latin America and the Caribbean. *Tropical Animal Health and Production 46*, 1321-1340.

Garcia A.B., Shallo L. (2015). Invited review: The economic impact and control of paratuberculosis in cattle. *Journal of Dairy Science 98*, 5019-5039.

GD (2014) ParaTBCwijzer. http://www.gddiergezondheid. nl/~/media/siteactiva/paginas/paratbc/paratbc-wijzer-pdf.ashx.

Godden S., McMartin S., Feirtag J., Stabel J., Bey R., Goyal S., Metzger L., Fetrow J., Wells S., Chester-Jones H. (2006). Heat-treatment of bovine colostrum II: Effects of heating duration on pathogen viability and immunoglobulin G. *Journal of Dairy Science 89*, 3476-3483.

Godden S.M., Wells S., Donahue M., Stabel J., Oakes J.M., Sreevatsan S., Fetrow J. (2015). Effect of feeding heat-treated colostrum on risk for infection with *Mycobacterium avium ssp. Paratuberculosis,* milk production, and longevity in Holstein dairy cows. *Journal of Dairy Science 98* (8), 5630-5641.

Kostoulas P., Leontides L., Billinis C., Amiridis G.S, Florou M. (2005). The association of sub-clinical paratuberculosis with the fertility of Greek dairy ewes and goats varies with parity. *Preventive Veterinary Medicine 74*, 226-238.

Kostoulas P., Leontides L., Enøe C., Billinis C., Florou M., Sofia M. (2006). Bayesian estimation of sensitivity and specificity of serum ELISA and faecal culture for diagnosis of paratuberculosis in Greek dairy sheep and goats. *Preventive Veterinary Medicine 76*, 56-73.

Leonor Mundo S., Gilardoni L., Hoffman F., Lopez O. (2013). Rapid and sensitive method to identify *Mycobacterium avium* subsp. *paratuberculosis* in cow's milk by DNA methylase genotyping. *Applied and Environmental Microbiology 79*, 1612–1618.

Lievaart-Peterson K. (2013). Paratuberculose in de melkgeitenhouderij. https://www.verantwoordeveehouderij. nl/show/Paratuberculose-in-de-melkveehouderij.htm

Lievaart-Peterson K. (2017). Eindverslag: Publiek private samenwerking kleine herkauwers (PPS-KH) Paratuberculose in de melkgeitenhouderij. http://www.agripress. be/_STUDIOEMMA_UPLOADS/downloads/Paratuberculose_in_de_melkgeitenhouderij_pdf.pdf

Lybeck K.R., Storset A.K., Djønne B., Valheim M., Olsen I. (2010). Faecal shedding detected earlier than immune responses in goats naturally infected with Mycobacterium avium subsp. paratuberculosis. *Research in Veterinary Science 91*, 32-39.

Mercier P., Baudry C., Beaudeau F., Seegers H., Malher X. (2010). Estimated prevalence of Mycobacterium avium subspecies paratuberculosis infection in herds of dairy goats in France. *Veterinary Record 167*, 412-415.

Nielsen S.S., Toft N. (2008). A review of prevalence of paratuberculosis in farmed animals in Europe. *Preventive Veterinary Medicine 88*, 1-14.

Robbe-Austerman S. (2011). Control of paratuberculosis in small ruminants. *Veterinary Clinics of North America: Food Animals 27*, 609-620.

Sevilla I., Li L., Amonsin A., Garrido J.M., Geijo M.V., Kapur V., Juste R.A. (2008). Comparative analysis of *Mycobacterium avium subsp. paratuberculosis* isolates from cattle, sheep and goats by short sequence repeat and pulsed-field gel electrophoresis typing. *BMC Microbiology 8*, 1-8.

Stau A., Seelig B., Walter D., Schroeder C., Ganter M. (2012). Seroprevalence of Mycobacterium avium subsp. paratuberculosis in small ruminants in Germany. *Small Ruminant Research 105*, 361-365.

Statistics Belgium (Algemene Directie Statistiek). http:// statbel.fgov.be/nl/statistieken/organisatie/statbel/informatie/statbel/in_de_kijker_archief/In_de_kijker_2017/ belgische_productie_verse_zuivel.jsp

Stevenson K., Alvarez J., Bakker D., Biet F., de Juan L., Denham S., Dimareli Z., Dohmann K., Gerlach G.F., Heran I., Kopecna M., May L., Pavlik I., Sharp J.M., Thibault V.C., Willemsen P., Zadoks R.M., Greig A. (2009). Occurrence of Mycobacterium avium subspecies paratuberculosis across host species and European countries with evidence for transmission between wildlife and domestic ruminants. *BMC Microbiology 2009*, 9-212.

Tharwat M., Al-Sobayil F., Hashad M., Buczinski S. (2012). Transabdominal ultrasonographic findings in goats with paratuberculosis. *Canadian Veterinary Journal 53*, 1063-1070.

Tuerlinckx A. (2017). Paratuberculose bij dwerg- en melkgeiten: een onderschat probleem? Master's thesis, Ghent University.

Verdugo C., Jones G., Johnson W., Wilson P., Stringer L., Heuer C. (2014). Estimation of flock/herd-level true Mycobacterium avium subspecies paratuberculosis prevalence on sheep, beef cattle and deer farms in New Zealand using a novel Bayesian model. *Preventive Veterinary Medicine 117*, 447-455.

Windsor P.A. (2013). Understanding the efficacy of vaccination in controlling ovine paratuberculosis. *Small Ruminant Research 110*, 161-164.

Windsor P.A., Eppleston J., Dhand N.K., Whittington R.J. (2014). Effectiveness of Gudair™ vaccine for the control of ovine Johne's disease in flocks vaccinating for at least 5 years. *Australian Veterinary Journal 92*, 263-268.

Windsor P.A. (2015). Paratuberculosis in sheep and goats. *Veterinary Microbiology 18*, 161-169.

Zimmer K., Dräger KG., Klawonn W., Hess RG. (1999). Contribution to the diagnosis of Johne's disease in cattle. Comparative studies on the validity of Ziehl-Neelsen staining, faecal culture and a commercially available DNA-Probe test in detecting *Mycobacterium paratuberculosis* in faeces from cattle. *Zentralblatt für Veterinärmedizin B 46*, 137-140.

Uit het verleden

Miniatuur (via Wikipedia commons) uit 'Livre de Chasse' (1387-1389) van Gaston III, graaf van Foix (Béarn) bijgenaamd Phoebus (de schitterende), opgedragen aan hertog Filips de Stoute. Jagen was zijn passie naast, zoals de graaf zelf verklaarde, le combat et l'amour. Meerdere exemplaren van dit werk bleven bewaard, onder andere in de Brusselse Koninklijke Bibliotheek. We zien hierin meerdere vormen van de verzorging van gekwetste jachthonden.

Luc Devriese