5. Discussion

The sampling method from this study was not planned to support statistical analysis but aimed to achieve MAIC isolates as representatives of various regions of Germany.

From 703 lymph nodes without any typical pathoanatomic lesion of mycobacteriosis, in 35 positive samples (4.97%) acid-fast bacilli (AFB) were detected in low concentration. In addition, 10 (1.43%) smears were in doubt. On the other hand ABF were detected in 94 out of 135 (69.6%) lymph nodes with pathoanatomic lesions, from which 78 showed ABF in moderate to high concentration (2+ to 4+).

From the same group of lymph nodes without and with lesions, respectively, 80 and 78 mycobacterial isolates were obtained from lymph nodes. The sensitivity of Ziehl-Neelsen (ZN) staining method from this study was 81.16 % (129 of 158), which is similar to the report from Selvakumar et al. in 2002. Nevertheless, lower sensitivity of ZN has been reported by some authors in various degree (Nagesh et al., 2001; Mukherjee et al., 2002;). On the other hand ZN is recommended as one of the most practical screening test for AFB particularly in field practice because it is non-complicated, fast and economically reasonable.

Each of 842 lymph nodes from 175 pigs were decontaminated with NALC-NaOH and inoculated onto 3 culture media. During the period of 3 months, the bacterial growth was observed in 327 inoculated tubes, belonging to 175 lymph nodes from 118 pigs. The frequency of bacterial isolates on Ogawa (OG) culture medium is greater than on Löwenstein-Jensen (LJ) or on Stonebrink (S; 144, 99, 80 tubes, respectively). This might partly be related to the pH of culture media as described by Portaels and Pattyn in 1982 that

culture medium with relatively low pH is more suitable for the first isolation than neutral pH medium. The first colony was observed on OG and LJ media after 2 weeks but the time required to observe first colony was predominantly 5-6 weeks for OG and S and 7–8 weeks for LJ medium.

By means of PCR, 17 isolates were determined as non-mycobacterial strains and all of them were observed within first 4 week, 10 of 17 non-mycobacterial isolates were obtained from LJ medium. The contamination rates were calculated from the number of non-mycobacterial isolates / number of total isolates obtained from each culture medium. The contamination rate of LJ medium is 10.10%, which is greater than from OG and S media (3.44 % and 2.50 %, respectively).

Although LJ medium is reported as one of the best culture media for *M. tuberculosis* isolation (Liu et al., 1973), for the isolation of mycobacteria from pigs lymph nodes in this study, LJ medium showed lower efficiency than OG and S media both in term of contamination rate and time consumed.

147 isolates were confirmed by PCR as MAIC. Of the total of 77 isolates from lymph nodes with pathoanatomic lesions, 8 isolates were *M. avium* ssp. *avium* 69 were *M. avium* ssp. *hominissuis* and no *M. intracellulare* isolate was confirmed. The remaining 70 isolates were from lymph node without lesion, five of them were *M.avium* ssp. *avium*, 60 were *M. avium* ssp. *hominissuis* and five were *M. intracellulare* isolates. Comparing the prevalence of MAIC among the group of pigs without lesion, this study showed that pigs from an “ecological” farm had about two times higher prevalence than pigs from conventional farm

both in terms of positive lymph node- and positive animal ratio (24.18% to 10.51% and 60.45% to 32.05%). The high prevalence of MAIC infection in the “ecological“ farms could be explained as follows:

1. Pigs kept in house with outdoors facilities may contact with mycobacteria contaminated farm environments and they have a great chance to contact directly or indirectly with infected animals such as birds or wild animals.

2. Using organic materials such as wood shaving, sawdust or straw as the bedding material, could play a role not only as source of mycobacterial infection but also as the reservoir of contaminated dropping, which was first detected in artificially infected pig after 20-23 days post infection (Ellsworth et al., 1980). The studies from Fischer et al. in 2000a and Pavlik et al. (2000) proved, that mycobacterial strains can be isolated from organic bedding materials in pig farms.

Results from bacterial culture and PCR confirmed that the member of MAIC can be isolated from one or more different organ-associated lymph nodes. This indicates that localized to generalized mycobacteriosis may occur without any clinical sign.

High resolution genotyping by RFLP and PFGE revealed a considerable degree of heterogeneity within the species and within the different animal groups.

Based on a system for standardizing the interpretation of PFGE patterns in relation to determining strain relationship proposed by Tenover et al. in 1995, two large groups of DNA band patterns were observed. The first group was similar to DNA pattern from cattle isolates from Cloppenburg (Lower Saxony) in 2000. This DNA pattern was seen in pigs

from different origins (Lower Saxony, North Rhine-Westphalia and the Netherlands), which were slaughtered in the slaughterhouse in Essen (near Cloppenburg), Meiningen (Thuringia) and some wild animals from Thuringia. The second group was DNA band patterns other than Cloppenburg-like. In contrast, by RFLP genotyping this phenomenon –the generation of two major groups- could not be observed. These dendrogramme showed that nearly every single isolate was characterized by a unique band pattern. This may be an indication that the evolutionary clock regarding the insertion patterns of mobile IS element goes faster than it goes for the changes in the residual chromosomal reflected by the recognition sites of the restriction endonucleases. In some cases (data not shown) even between isolates from different organs of one single animal different RFLP patterns were detected, while at the same time from isolates identical PFGE profiles were generated. This indicates a hyperdiscrimination of IS 1245 RFLP to analytic relatedness among the strains when compared with PFGE. Moreover, since RFLP need more steps to detect DNA band patterns and some DNA bands may get lost during the detection step, it shows a worse DNA band discrimination, computer recognition and analysis.

Since it is the first confirmation of Cloppenburg-like PFGE band pattern in large scale after 2000 (Moser and Werner, 2004), the presence of this band pattern in pigs and wild animals from this study showed the epidemiological data that the transmission of mycobacteriosis from cattle to other host species has occurred during 2000-2004 in parts of Germany, at least from Lower Saxony to Thuringia. This could be explained as follows:

1. Most of pig farms do not produce piglets in their own farms but they have to buy piglets from other farms. Not only animal transportation but also feed stuffs and bedding materials transportation could play an important role as a fast and country-wide transmission mode.

2. Free living animals may play a role as the reservoir and/or carrier from cattle farm to wild animals and pigs farms.

Another possibility is that this Cloppenburg-like MAIC clone may have excised in mentioned area for a long time but it had never been detected before.

The 2-band pattern was identified in avian isolates from various countries by Guerrero et al. in 1995, and the 3-band pattern was described by Ritacco et al. in 1998 as “bird type” pattern. Isolates with these band patterns were found only in very few animals during this investigation. They were found in wild animals in higher percentage than in domestic pigs. This may indicate that wild animals contribute only to minor extent to mycobacterial infection in pigs.