Seroprevalence of anti-\textit{Yersinia} antibodies in meat juice samples of pigs

Konstantinos Nikolaou\textsuperscript{1}, Andreas Hense\textsuperscript{2}, Hermann Meyer\textsuperscript{1}, Claus P. Czerny\textsuperscript{3}, Heinrich K. J. Neubauer\textsuperscript{1}

\textsuperscript{1} Institute for Microbiology, Sanitätsakademie der Bundeswehr, Neuherbergstr. 11, 80937 München, Phone: 0049-89-3168-3113, Fax: 0049-89-3168-3292, E-mail: 320027945810-0001@t-online.de. \textsuperscript{2} Institute of Animal Hygiene and Public Veterinary Health, An den Tierkliniken 17, 04103 Leipzig, Germany. \textsuperscript{3} Tiergesundheitsdienst Bayern e.V., Senator-Gerauer-Str. 23, 85586 Poing, Germany.

\textbf{Abstract}: This investigation was intended to provide actual data on the seroprevalence of \textit{Yersinia} antibodies in German slaughtering pigs. The recomBlot western blot assay based on five recombinantly produced \textit{Yersinia} Outer Proteins (Yop) is well evaluated for human Yersiniosis and proved to be also valuable for the detection of porcine anti-Yop antibodies. 970 out of 1014 meat juice samples collected in 145 Bavarian pig farms contained anti-Yop antibodies. As other animal pathogens like \textit{Salmonella} express the proteins of secretion apparatus III as well, we propose that only blots with three and more detectable signals should be considered as positive. This means that at least 45.2\% of the pigs and 82\% of the pig farms respectively had a previous history of \textit{Yersinia} infection. \textit{Yersinia} infection in pigs is still a severe hygienical problem. Furtheron, pigs may not only be asymptomatic carriers but may also suffer from disease leading to economical losses.

\textbf{Keywords}: \textit{Yersinia enterocolitica}, \textit{Y. pseudotuberculosis}, \textit{Yersinia} outer proteins, seroprevalence, western blot

\textbf{Introduction}: \textit{Y. enterocolitica} infection is commonly associated with a selflimiting enterocolitis in man and seldomly post infectious sequalae like arthritis or erythema nodosum. Hence, economic losses may be enormous due to a two or three day period when diseased employees must stay at home and the time lost in production when parents nurse their children, which is the main group to be affected (Tauxe et al., 1987). \textit{Y. enterocolitica} and \textit{Y. pseudotuberculosis} infections in German pig herds were often reported in the 1980s from both German states as well as high seroprevalence rates in the human population (Bockemühl et al., 1979; Weber and Lembke, 1981; Wuthe et al., 1982; Nattermann et al., 1986). In a recent seroprevalence study, 40\% of German blood donors were positive and
*Y. enterocolitica* is still reported to be the third most important cause of gastroenteritis in many European countries (Neubauer et al., 2000; Neubauer et al., 2001a). However, no current data on the epizootiology of the disease or its life cycle in the pig, which is believed to be the animal reservoir and the main source of human infection are available.

**Materials and Methods:** In the course of a Bavarian monitoring programme for *Salmonella* in slaughtering pigs (Czerny et al., 2001; Osterkorn et al., 2001) 1014 meat juice samples were collected from pigs originating from 145 fattening pig farms. These samples were analysed using the recomBlot *Yersinia* Western Blot Kit according to the recommendations of the manufacturer (Mikrogen, Munich, Germany) with the exception that anti-porcine IgG-HRP conjugate (Cytomed, Berlin, Germany) was used. This kit contains recombinantly produced proteins namely the *Yersinia enterocolitica* outer proteins (Yop) D, H, M, E and the V-antigen. A clearly visible band shaped signal on the blot stripes was considered to be positive. A hyperimmune serum of a pig immunised i.m. with induced and heat inactivated *Y. enterocolitica* (O:3 / 4) cells served as positive control.

**Results:** The recomBlot western blot assay proved to be valuable for the detection of porcine anti-Yop and anti-V-antigen antibodies. From 1014 mucosal fluids collected of pigs from 145 Bavarian farms 970 contained antibodies (D: 5.6%; D+1 additional band (a.b.): 35.4%; D+2 a.b.: 27.1%; D+3 a.b.: 14.6%; D+4 a.b.: 3.5%; M: 9.4%). In each pig farm at least one seropositive animal was found.

**Discussion and Conclusions:** This investigation was intended to provide actual data on the seroprevalence of *Yersinia* antibodies in German slaughtering pigs. As detection system a western blot assay was used which is already well evaluated for human Yersiniosis (Heesemann et al., 1990; Neubauer et al., 2001b). It makes use of highly immunogenic antigens which are part of the secretion III system or involved in its regulation thus only expressed in pathogenic bacteria. It proved to be also valuable for the detection of porcine anti-*Yersinia* antibodies. However, it is not applicable for distinguishing between *Y. enterocolitica* and *Y. pseudotuberculosis* induced antibodies. In humans the demonstration of anti-Yop D antibodies alone or the existence of antibodies reactive to two other blot antigens is sufficient for a safe diagnosis. However, it is reported that other animal pathogens like *Salmonella* express the proteins of the secretion apparatus III, too (Hueck, 1998). We therefore propose that only blots with three or more detectable signals should be considered as indicative for a positive diagnosis. This means that at least 45.2% of the pigs and 119 of the pig farms respectively had a previous history of *Yersinia* infection. The presence of antibodies against proteins associated
with virulence indicate that pigs are not only asymptomatic carriers but may also suffer from disease leading to economic losses. The fact that *Y. enterocolitica* infections can lead to clinical syndromes in laboratory pigs as well as in farm animals has been shown in the past (Neubauer et al., 2001c). *Yersinia* infection in pigs is still a hygienical problem and might be the main source for human infections in Germany and thus in all other countries where pigs and pork are exported. The high seroprevalence of anti-*Yersinia* antibodies revealed in this investigation demonstrated the need for further epidemiological and animal studies.

**Acknowledgements:** The authors would like to acknowledge the excellent technical assistance given by S. Scholz and C. Lodri.

**References:**


