Schweiz. Arch. Tierheilk.
© 2009 by Verlag Hans Huber, Hogrefe AG, Bern
M. Jäggi et al., Band 151, Heft 8, August 2009, 369–375
DOI 10.1024/0036-7281.151.8.369

Retrospective study on necrotizing enteritis in piglets in Switzerland

M. Jäggi[1], N. Wollschläger[2], C. Abril[3], S. Albini[3], C. Brachelente[4], M. Wyder[1], H. Posthaus[1]

[1]Institute of Animal Pathology, [2]Swine Clinic and [3]Institute of Veterinary Bacteriology, University of Bern, Switzerland, [4]Department of Biopathological Sciences, University of Perugia, Italy

Abstract

The re-emergence of necrotizing enteritis (NE) in Swiss pig breeding farms raised concern that, besides *C. perfringens* type C strains, additional *C. perfringens* toxinotypes might cause this disease. Therefore we retrospectively investigated the association of NE with C. *perfringens* type C or different *C. perfringens* toxinotypes. We evaluated pathological lesions, routine diagnostic bacteriology results, and multiplex real-time PCR analyses from DNA extracts of archived intestinal samples of 199 piglets from our diagnostic case load. 96.5 % of NE cases and 100 % of herds affected by NE were positive for *C. perfringens* type C genotypes. Animals without necrotizing enteritis revealed a significantly lower detection rate of type C genotypes. Non affected piglets showed a high prevalence for beta-2-toxin positive *C. perfringens* type A strains. Collectively, our data indicate that outbreaks of NE in piglets in Switzerland cannot be attributed to newly emerging pathogenic toxinotypes, but are due to a spread of pathogenic *C. perfringens* type C strains.

Keywords: *Clostridium perfringens* type C, necrotizing enteritis, beta-toxin, porcine

Retrospektive Studie über die Nekrotisierende Enteritis der Saugferkel in der Schweiz

Das wiederkehrende Auftreten der Nekrotisierenden Enteritis (NE) bei Saugferkeln in Schweizer Zuchtbetrieben hat zu Bedenken geführt, dass neben *C. perfringens* Typ C Stämmen weitere *C. perfringens* Toxinotypen diese Erkankung auslösen können. Deshalb wurde eine retrospektive Studie über die Assoziation von NE mit *C. perfringens* Typ C oder weiteren Toxinotypen durchgeführt. Hierfür wurden pathologische Läsionen, diagnostisch-bakteriologische Resultate und multiplex real-time PCR Analysen von DNA Extrakten aus archiviertem Darmgewebe von 199 Saugferkeln ausgewertet. In 96.5 % alle Ferkel mit NE und 100 % der von dieser Krankheit betroffenen Betriebe wurden *C. perfringens* Typ C Genotypen nachgewiesen. In nicht betroffenen Tieren lag eine signifikant tiefere Detektionsrate von Typ C Genotypen vor. Diese Tiere zeigten eine hohe Prävalenz von Beta-2-toxigenen Typ A Stämmen. Zusammenfassend zeigen unsere Daten, dass Ausbrüche von NE der Saugferkel in der Schweiz nicht mit dem Auftreten neuer, *C. perfringens* Toxinotypen sondern mit der Verbreitung pathogener *C. perfringens* Typ C Stämme erklärbar sind.

Schlüsselwörter: *Clostridium perfringens* Typ C, Nekrotisierende Enteritis, Beta-Toxin, Schwein

Introduction

Clostridium perfringens are gram-positive, anaerobic, endospore-forming bacteria which are wide-spread in the environment. They are normal inhabitants of the intestinal tract of mammals, but certain toxigenic strains frequently cause severe enteric diseases in animals and humans (Songer, 1996). A large array of exotoxins and other virulence factors have been discovered (Petit et al., 1999; Smedley et al., 2004). Individual pathogenic isolates, however, only secrete a fraction of this toxin repertoire. The production of 4 major toxins α, β, ε, and ι allows the categorization into 5 toxinotypes (A–E). The *cpa* gene encoding α-toxin is located on the bacterial chromosome, whereas those encoding β- (*cpb*), ε- (*etx*) and ι-toxin (*iap/iab*) are located on plasmids (Petit et al., 1999). Individual strains can secrete a variety of additional toxins such as enterotoxin *(cpe)* or β_2-toxin *(cpb2)*. The β_2-toxin has recently been associated with gastrointestinal diseases in animals and humans (Schotte et al., 2004; Fisher et al., 2005). Two alleles of the *cpb2* gene have been described (Gibert et al., 1997; Fisher et al., 2005; Jost et al., 2005). Whereas the consensus *cpb2* gene is found in porcine *C. perfringens* isolates, an atypical gene, which has 62.3 % identity and 80.4 % similarity to the former one, is mainly present in non-porcine isolates (Bueschel et al., 2003; Jost et al., 2005).

Clostridium perfringens types A and C are principally involved in enteric disease in swine (Songer and Uzal,

2005). Type A strains produce α-toxin, type C strains α- and β-toxin. Porcine isolates belonging to both toxinotypes are frequently β_2-toxin (*cpb2*) positive (Bueschel et al., 2003; Waters et al., 2003; Jost et al., 2006). *C. perfringens* type C strains are known to cause necrotizing enteritis (NE) in neonatal piglets. This disease occurs worldwide and represents an economically important problem (Songer and Uzal, 2005). Typically, neonatal piglets from their first day of life until approximately 3 weeks of age are affected. Peracute to acute disease affects piglets within the first days post partum. Depression is followed by rapid death and in a proportion of cases hemorrhagic diarrhea. Piglets showing signs at 1–3 weeks of age often have a more protracted clinical course with non-hemorrhagic diarrhea. The hallmark lesion is a deep, segmental mucosal necrosis of the small intestine, mainly the jejunum, with massive hemorrhage in the intestinal wall. More protracted cases may show a segmental, fibrino-necrotizing enteritis (Songer and Uzal, 2005).

Immunization of pregnant sows with *C. perfringens* type C toxoid based vaccines and antibiotic treatment of newborn piglets reduces the incidence of fatal NE (Springer and Selbitz, 1999). However, vaccination does not always protect from disease (Springer and Selbitz, 1999; Luginbühl, 2002). Failure of piglets to receive adequate amounts of protective colostral antibodies, trypsin secretion deficiencies, colostral protease inhibitors and the involvement of further toxins in the pathogenesis of the disease are possible factors which contribute to disease outbreaks (Songer and Uzal, 2005). *C. perfringens* type A strains are generally considered to be normal, non-pathogenic inhabitants of the intestinal tract in pigs (Songer, 1996). Since the discovery of β_2-toxin (Gibert et al., 1997), an association of β_2-toxigenic type A strains with diarrhea and enteritis in piglets was however reported in pigs and other species (Herholz et al., 1999; Klaasen et al., 1999; Waters et al., 2003; Schotte et al., 2004). Frequent isolations of such strains from porcine fecal samples in the Institute of Veterinary Bacteriology, Bern, raised questions whether they could contribute to a re-emergence of necrotizing enteritis in Switzerland.

To address this we investigated whether outbreaks of NE in piglets in Switzerland were solely associated with a *C. perfringens* type C infection or whether β_2-toxigenic *C. perfringens* type A strains could have played a role in this disease. We thus retrospectively evaluated our routine diagnostic case material from piglets submitted to the Institute of Pathology from January 2000 until July 2006. For a limited number of animals, which were necropsied in parallel to an epidemiological study by Wollschläger (2007), available herd management data allowed us to evaluate the status of *C. perfringens* type C infections on herd level. All animals were examined macroscopically. Confirmation of the diagnosis of NE was achieved by histopathology and/or routine diagnostic bacteriology which included genotyping of isolated cultures. In cases where these examinations were inconclusive or incomplete, multiplex real time PCR analyses of intestinal tissue DNA extracts was performed to identify clostridial infection.

Animals, Material and Methods

Animals

199 one day to four weeks old suckling piglets submitted for routine diagnostic necropsy to the Institute of Animal Pathology from January 2000 to July 2006 were included in this study. 142 of these were classified as NE cases based on macroscopical findings. 57 animals, which died of other causes, were not affected by NE and served as reference cases. In the following these cases are referred to as NE cases and reference cases, respectively.

Herds

57 of the 199 piglets, necropsied during 2005 and 2006, derived from herds which took part in a parallel epidemiological study at the Swine Clinic, Vetsuisse Faculty, University of Bern (Wollschläger, 2007). Thus reliable information about the occurrence of NE within the herd was available.

Histopathology

Formalin-fixed, paraffin-embedded small intestinal tissue samples were available from 104 NE and 57 reference cases. In the remaining animals a conclusive diagnosis was achieved through the combination of macroscopical and bacteriological findings. Small intestinal tissue samples were routinely processed for histology and stained with hematoxylin and eosin (H & E).

Routine bacteriological examination and genotyping

Routine bacteriological culturing for *C. perfringens* was performed on intestinal samples of 125 NE and 53 reference cases at the Institute of Veterinary Bacteriology, Vetsuisse Faculty, Bern. *C. perfringens* culture growth was achieved from 118 necrotizing enteritis and 53 reference animals. Genotyping data achieved by a conventional PCR based genotyping protocol (Albini et al., 2008; Herholz et al., 1999) were available from 105 NE and 38 reference cases. Briefly, 5 individual bacterial colonies, which showed the typical morphology of *C. perfringens*, were pooled. Template DNA was obtained by direct lysis and PCR using specific primers for *cpa, cpb,* and *cpb2* was performed.

DNA extraction and multiplex real-time-PCR from tissue samples

C. perfringens culture genotyping results were not available in 37 cases of NE and 19 reference animals. These cases were thus examined by multiplex-real-time PCR on

intestinal tissue DNA extracts. Additionally, all cases negative for either *cpb* or *cpb2* were re-evaluated using this method to exclude false negative results due to limitation of culture genotyping to 5 colonies.
Paraffin embedded tissue blocks or small intestinal tissue samples, frozen at necropsy, were investigated by direct multiplex real time PCR. Three 20 µm thick slices of paraffin-embedded small intestinal tissue blocks were cut and transferred to 1.5 ml microcentrifuge tubes. To avoid cross contamination during sample processing, microtome blades were changed after cutting each tissue block. Additional, control samples from empty paraffin blocks (empty controls) were cut before each new tissue block was processed. DNA was extracted using the Quiagen DNeasy Kit (Quiagen, Basel, Switzerland). Additionally, during necropsies in 2005 and 2006 samples of the small and large intestine of each animal were separately frozen at –20 °C. After thawing, DNA was extracted from each individual sample using the Quiagen mini stool kit (Quiagen; Basel, Switzerland). 2.5 µl of template DNA of either paraffin embedded or frozen tissues was used for gene amplification applying a recently described multiplex real-time PCR protocol for detection of *cpa, cpb, cpb2, etx* and *cpe* (Albini et al., 2008). Finally, the results of culture genotyping and direct gene amplification from the intestinal samples were combined. The combination of individual toxin genes thus indicated presence (*cpb* positivity in the absence of *etx*) or absence (*cpb* negative) of *C. perfringens* type C strains.

Statistical analysis

Animals were defined as affected or non-affected by NE according to the morphological findings. Statistical analysis was done by cross tabulation of the disease status to the final bacterial genotype. The respective frequencies were compared using Chi-Square and Fisher's Exact Tests. All statistical analyses were run in the statistical software package NCSS 2007 (www.ncss.com). The level for statistical significance was set to $p < 0.05$.

Results

NE cases

Macroscopically, 142 piglets showed a typical segmental, necro-hemorrhagic jejunitis (Fig. 1). The histological evaluation of 104 of these cases revealed acute lesions in 60 cases. These were characterized by deep coagulation necrosis of the mucosa, vascular necrosis and massive hemorrhage in the lamina propria, submucosa and occasionally the muscular layers (Fig. 2). Inflammation was mostly absent and large numbers of gram positive rods were visible in the necrotic zones. Subacute lesions were present in 44 cases and were characterized by an additi-

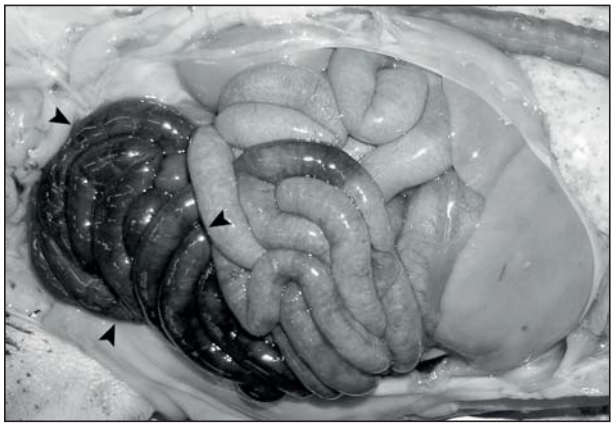

Figure 1: Small intestine of a 2 day old piglet showing the characteristic segmental necro-hemorrhagic enteritis (arrowheads).

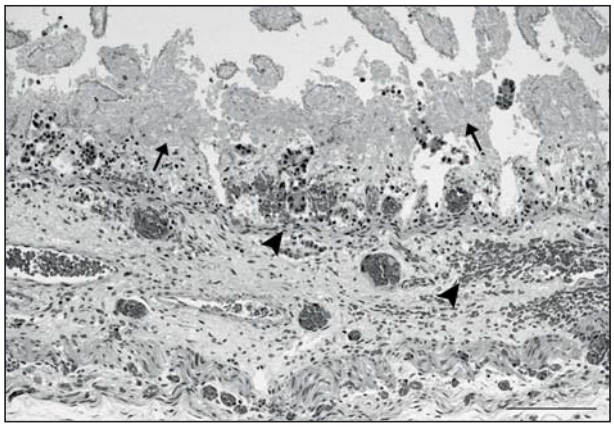

Figure 2: Histopathology of acute NE in a 2 day old piglet. Deep necrosis of the lamina mucosae (arrows), underlying hemorrhage (arrowheads) without inflammatory reaction (H&E stain, bar 100 µm).

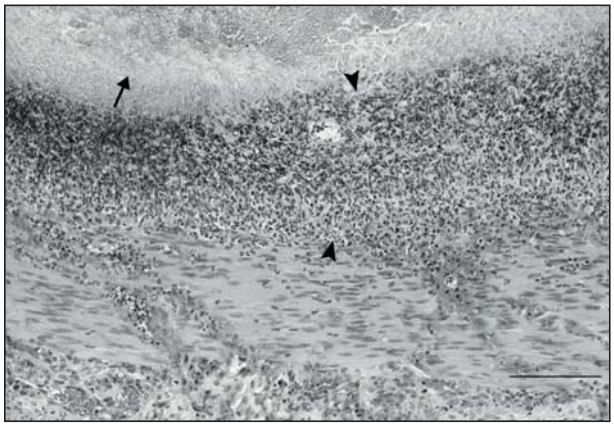

Figure 3: Histopathology of subacute NE in a 2 week old piglet. Deep necrosis of the lamina mucosae (arrow) and demarcation by a rim of large numbers of mainly neutrophilic granulocytes (arrowheads) (H&E stain, bar 100 µm).

onal moderate to massive infiltration with neutrophilic granulocytes at the margin of the necrotic zone (Fig. 3). Colonies of small, gram-negative, coccoid bacteria were present in necrotic areas and clostridium-like organisms were visible in smaller numbers. The acute form of the disease was mainly present in neonatal piglets, which died during the first 3 days of life. Subacute lesions predominated in older animals (Fig. 4).

Retrospective evaluation of routine diagnostic genotyping data revealed the genotypes *cpa/cpb/cpb2* in 95/105 (90.4 %), *cpa/cpb2* in 5/105 (4.8 %), and *cpa* in 5/105 (4.8 %) cases. 50 cases of NE, where either no culture genotyping results were available or where these were negative for *cpb* or *cpb2*, were investigated. No gene amplification was achieved from empty control blocks which excluded contamination during tissue processing. All intestinal samples tested positive for *cpa*, confirming the presence of *C. perfringens*, even in culture negative cases. Amplification of *cpe* and *etx* was not achieved in any of the samples, indicating that neither *C. perfringens* type B or D strains were present in detectable quantities. Genotyping revealed *cpa/cpb/cpb2* in 42 (84 %), *cpa/cpb* in 4 (8 %), *cpa/cpb2* in 2 (4 %), and *cpa* in 2 (4 %) cases. Thus, compared to culture genotyping results we were able to detect *C. perfringens* type C genotypes in 40 additional animals. The combined results of both methods on the individual animal level are depicted in Figure 5.

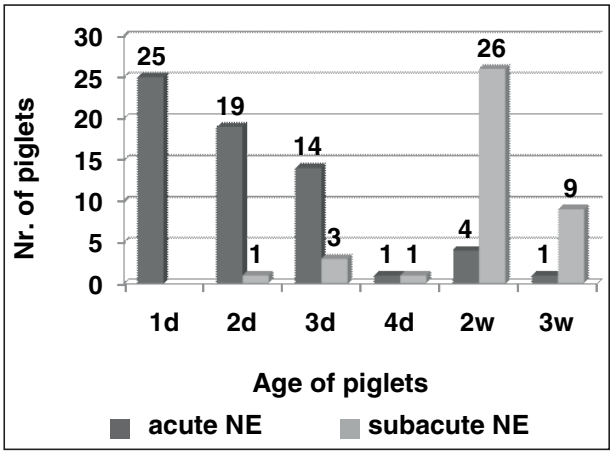

Figure 4: Age distribution of piglets with necrotizing enteritis.

Reference cases

Fifty-seven animals neither had macroscopical nor histological lesions of NE. Retrospective evaluation of necropsy reports revealed that the cause of death was *E. coli* diarrhea in 15, coccidiosis in 3, inanition in 9, accidental crushing by the mother in 11, bacterial septicaemia in 2 and unresolved in 17 cases.

Routine diagnostic genotyping revealed the genotypes *cpa/cpb/cpb2* in 4/38 (10.5 %), *cpa/cpb2* in 29/38 (76.3 %), and *cpa* in 5/38 (13.2 %) cases. 52 samples were tested. Genotyping revealed *cpa/cpb/cpb2* in 11 (21.2 %), *cpa/cpb2* in 28 (53.8 %), and *cpa* in 13 (25 %) cases. The combined results of culture genotyping and direct real-time PCR on the individual animal level are depicted in Figure 5. The predominant gene combination detected was *cpa/cpb2* (61.4 %), which corresponds to the presence of *cpb2* positive type A strains.

Overall, 96.5 % of animals with NE were positive for a genotype indicative of *C. perfringens* type C infection (*cpa/cpb/cpb2 or cpa/cpb*), in contrast to 26.3 % of reference

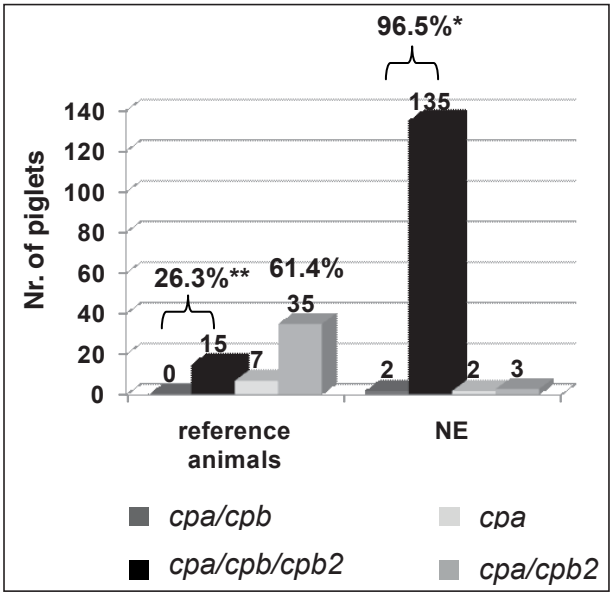

Figure 5: Association of necrotizing enteritis with *cpb* positive *C. perfringens*. Combination of culture genotyping and multiplex real-time PCR analyses revealed that necrotizing enteritis cases (NE) were significantly associated (*) with detection type C genotypes (*cpa/ cpb /cpb2; cpa/cpb*). In contrast to this, the frequency of type C genotype detection was significantly lower (**) in animals without necrotizing enteritis (reference animals) (Fisher's exact test, $p < 0.001$).

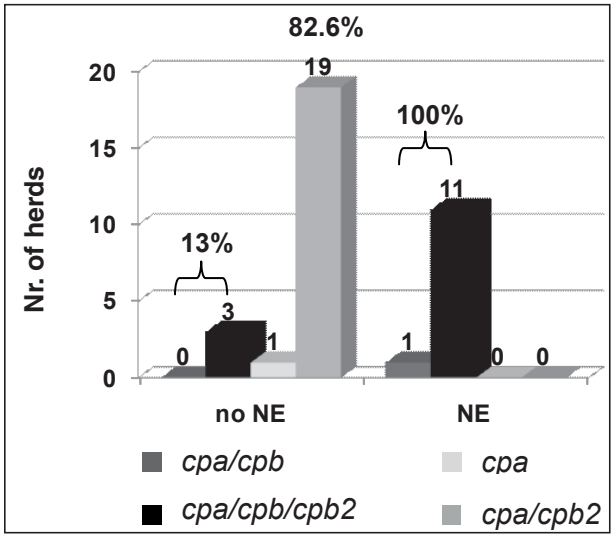

Figure 6. Genotyping results depicted on the herd level. 100 % of herds diagnosed with necrotizing enteritis (NE) were positive for *cpb* (*C. perfringens* type C). Most herds not affected by necrotizing enteritis (no NE) were positive for *cpb2* positive *C. perfringens* type A strains (*cpa/cpb2*).

animals (Fig.5). This difference was highly significant ($p < 0.0001$). Exclusive detection of *C. perfringens* type A genotypes (genotype *cpa* or *cpa/cpb2*) was significantly higher in reference cases.

Herd level data

Data on 57 animals which derived from 35 farms allowed us to evaluate *C. perfringens* type C detection on a herd level (Fig.6). All herds in which NE was diagnosed in at least one animal, also tested positive for *cpb*. In contrast to this, in herds not-affected by NE we mainly detected *cpa/cpb2* positive *C. perfringens* type A.

Discussion

C. perfringens type C strains are known to cause severe necrotizing enteritis in suckling piglets. In Switzerland, this disease remains to be a problem in swine production despite the availability of commercial *C. perfringens* type C vaccines (Gut et al., 2002; Luginbühl, 2002). Emerging reports of β_2-toxigenic *C. perfringens* as potential additional cause of enteric disease in piglets (Klaasen et al., 1999; Garmory et al., 2000; Bueschel et al., 2003), increasing concern about the spread of the disease to and also from Swiss stock breeding farms led us to re-evaluate the association of necrotizing enteritis with *C. perfringens* type C in Switzerland. To retrospectively evaluate cases where initial diagnostic culture genotyping results were not available we made use of a recently established multiplex-real-time PCR analysis (Albini et al., 2008) on intestinal DNA extracts. By combining these results with available data from the initial diagnostic bacteriology, we were able to evaluate which toxin genes were present in *C. perfringens* from the intestinal tract of each individual animal.

Our results demonstrate a significant association between the detection of *C. perfringens* type C strains and NE in piglets. The gene combinations *cpa/cpb/cpb2* and *cpa/cpb*, which in case of absence of *etx* indicate *C. perfringens* type C infection, were detected in 96.5 % of all NE cases. These results correspond to published reports from other countries (Songer and Uzal, 2005). Interestingly, all but 1 *cpb* positive cases were also *cpb2* positive. Due to the experimental setup chosen in our study (DNA extracts of intestinal samples or pooled colonies of *C. perfringens*) it was not possible to determine whether the *cpb2* gene derives from the same *cpa/cpb/cpb2* positive type C, or a combination of *cpa/cpb* positive type C and *cpa/cpb2* positive type A strains. Several studies (Bueschel et al., 2003; Waters et al., 2003; Jost et al., 2006) have shown that porcine type C strains are frequently *cpb2* positive. It is also known, that in acute cases, which represent more than half of our case material, *C. perfringens* type C can be isolated almost in pure culture (Songer and Uzal, 2005). It is thus conceivable that in our study most of the *C.* perfringens type C strains harbour the *cpb2* gene and account for a large proportion of *cpa/cpb/cpb2* positive results. Additional presence of *cpb2* positive type A strains can however not be excluded. Two studies (Sayeed et al., 2008; Vidal et al., 2008) recently demonstrated that *C. perfringens* β-toxin is both required and sufficient to induce experimental necrotizing enteritis in the rabbit ileal loop model. Although not proven experimentally for the pig intestine, it is conceivable that *C. perfringens* type C strains alone are the causative agent of NE in our cases. Data on the herd disease status were available through a parallel clinical study (Wollschläger, 2007) for 57 animals. When all data from one herd were combined, *C. perfringens* type C infection were detected in 100 % of herd affected by recent outbreaks of NE.

We also detected high prevalence of *cpb2* positive *C. perfringens* type A strains in piglets which were not affected by NE. This supports the conclusion that these strains are not associated with NE in Swiss pig herds but may be found frequently in our case material. Several reports indicate, that β_2-toxigenic type A strains might induce milder forms of enteritis in piglets, however these strains are also frequently isolated from healthy pigs (Songer and Uzal, 2005). Detailed studies on the prevalence of these strains in healthy pigs have however been lacking so far, which hampers statistical evaluations of suspected increased prevalence of these strains in clinically diseased animals.

The age distribution of necrotizing enteritis in our study corresponds to published data (Songer and Uzal, 2005). NE mainly affected animals within the first three days post partum. These animals showed acute lesions with large numbers of clostridium-like organisms. *C. perfringens* type C, when ingested early post-partum, rapidly outcompetes the developing intestinal flora and thus leads to extensive damage of the mucosa by secretion of β-toxin (Songer and Uzal, 2005). Because, β-toxin is rapidly degraded by trypsin, high trypsin inhibitor levels in the colostrums of the sow contribute to the disease progression (Songer and Uzal, 2005; Sayeed et al., 2008). In contrast to few days old animals, piglets dying after the first week of life predominantly showed subacute lesions. This could be explained by early post-partum infections with more protracted courses of the disease. Alternatively, when infection occurs after the first week post partum, the initially established physiological gut flora might suppress *C. perfringens* type C pathogenicity.

Taken together our data confirm that NE in piglets in Switzerland is caused by *C. perfringens* type C. Thus, outbreaks in Swiss pig breeding farms cannot be attributed to newly emerging pathogenic *C. perfringens* toxinotypes, such as β_2-toxigenic *C. perfringens* type A, but are due to a spread of pathogenic *C. perfringens* type C strains. Diagnosis of the disease during an outbreak can be achieved by demonstrating pathognomonic macroscopic lesions, even without identification of the causative agent. Likewise, the identification of *C. perfringens* type C strains, even in unaffected animals, should be regarded as indicative of a risk for disease outbreak in a herd.

Acknowledgements

We thank Prof. M. Doherr for help with statistical analysis. This project was supported by a grant from the Vetsuisse Faculty (BE/05, M. Jäggi) and the Swiss Federal Veterinary Office (N. Wollschläger, Project Nr. 1.04.09).

Etude rétrospective sur l'entérite nécrotisante des porcelets en Suisse

L'apparition répétée de l'entérite nécrotisante chez les porcelets dans les exploitations d'élevage suisses a conduit à la réflexion que, outre les souches de *C. perfringens* de type C, d'autres types de toxines de *C. perfringens* pouvaient déclencher cette maladie. C'est pourquoi une étude rétrospective sur l'association de l'entérite nécrotisante *C. perfringens* de type C ou d'autres types de toxines a été conduite. Pour cela les lésions pathologiques, les résultats bactériologiques et les analyses d'extraits d'ADN provenant des tissus intestinaux de 199 porcelets ont été étudiés. Chez 96,5 % de tous les porcelets souffrant d'entérite nécrotisante et dans 100 % des exploitations touchées par la maladie, Clostridium *perfringens* de type C a été mis en évidence. Chez les animaux non atteints, le taux de détection du type C était significativement plus faible. Ces animaux présentaient une forte prévalence de souche de type A, Beta-2-toxigène. En résumé, ces données montrent que les épisodes d'entérite nécrotisante des porcelets en Suisse ne sont pas liés à l'apparition de nouveaux types de toxines de *C. perfringens* mais à l'extension des souches de *C. perfringens* de type C.

Studio retrospettivo sull'enterite necrotizzante nei maialini da latte in Svizzera

Con la riapparizione nelle ditte di allevamento svizzere dell'enterite necrotizzante (EN) nei maialini da latte si è pensato alla possibilità che accanto ai ceppi di *C. perfringens* tipo C altri tossinotipi di *C. perfringens* potrebbero far ritornare questa malattia. Per questo motivo è stato eseguito questo studio retrospettivo sull'associazione dell'EN da *C. perfringens* tipo C o da altri tossinotipi. La valutazione comportava le lesioni patologiche, i risultati delle diagnosi batteriche e le analisi PCR multiples real-time provenienti da estratti di ADN di tessuti intestinali archiviati, originati da 199 maialini da latte. Sono stati ritrovati genotipi di *C. perfringens* tipo C, nel 96.5 % di tutti i maialini affetti da EN e nel 100 % delle aziende colpite da questa malattia. Negli animali non colpiti si è riscontrata una piccola ma significativa percentuale del genotipo di tipo C. Questi animali mostravano un'alta prevalenza di ceppi di tossina β2 di tipo A. Riassumendo i nostri dati possiamo affermare che la propagazione di EN nei maialini da latte in Svizzera non è dovuta alla comparsa di tossinotipi di *C. perfringens* bensì dalla propagazione patogena di ceppi di *C. perfringens* tipo C.

References

Albini, S., Brodard, I., Jaussi, A., Wollschlaeger, N., Frey, J., Miserez, R., Abril, C.: Real-time multiplex PCR assays for reliable detection of Clostridium perfringens toxin genes in animal isolates. Vet. Microbiol. 2008, 127: 179–185.

Bueschel, D. M., Jost, B. H., Billington, S. J., Trinh, H. T., Songer, J.G.: Prevalence of cpb2, encoding beta2 toxin, in Clostridium perfringens field isolates: correlation of genotype with phenotype. Vet. Microbiol. 2003, 94: 121–129.

Fisher, D. J., Miyamoto, K., Harrison, B., Akimoto, S., Sarker, M. R., McClane, B. A.: Association of beta2 toxin production with Clostridium perfringens type A human gastrointestinal disease isolates carrying a plasmid enterotoxin gene. Mol. Microbiol. 2005, 56: 747–762.

Garmory, H. S., Chanter, N., French, N. P., Bueschel, D., Songer, J. G., Titball, R. W.: Occurrence of Clostridium perfringens beta2-toxin amongst animals, determined using genotyping and subtyping PCR assays. Epidem. Inf. 2000, 124: 61–67.

Gibert, M., Jolivet-Reynaud, C., Popoff, M. R.: Beta2 toxin, a novel toxin produced by Clostridium perfringens. Gene 1997, 203: 65–73.

Gut, P., Luginbühl, A., Nicolet, J., Boerlin, P., Burnens, A. P.: The necrotizing enteritis by Clostridium perfringens type C in piglets: II. Molecular epidemiology study. Schweiz. Arch. Tierheilk. 2002, 144: 275–281.

Herholz, C., Miserez, R., Nicolet, J., Frey, J., Popoff, M., Giwbert, M., Gerber, H., Straub, R.: Prevalence of beta2-toxigenic Clostridium perfringens in horses with intestinal disorders. J. Clin. Microbiol. 1999, 37: 358–361.

Jost, B. H., Billington, S. J., Trinh, H. T., Bueschel, D. M., Songer, J. G.: Atypical cpb2 genes, encoding beta2-toxin in Clostridium perfringens isolates of nonporcine origin. Inf. Immun. 2005, 73: 652–656.

Jost, B. H., Billington, S. J., Trinh, H. T., Songer, J. G.: Association of genes encoding beta2 toxin and a collagen binding protein in Clostridium perfringens isolates of porcine origin. Vet. Microbiol. 2006, 115: 173–182.

Klaasen, H. L., Molkenboer, M. J., Bakker, J., Miserez, R., Häni, H., Frey, J., Popoff, M. R., van den Bosch, J. F.: Detection of the beta2 toxin gene of Clostridium perfringens in diarrhoeic piglets in The Netherlands and Switzerland. FEMS Immun. Med. Microbiol. 1999, 24: 325–332.

Luginbühl, A.: Die nekrotisierende Enteritis des Saugferkels (durch C. perfringens Typ C): I. Therapie- und Impferfahrungen sowie epidemiologische Beobachtungen. Schweiz. Arch. Tierheilk. 2002, 144: 263–273.

Petit, L., Gibert, M., Popoff, M. R.: Clostridium perfringens: toxinotype and genotype. Trends Microbiol. 1999, 7: 104–110.

Sayeed, S., Uzal, F. A., Fisher, D. J., Saputo, J., Vidal, J. E., Chen, Y., Gupta, P., Rood, J. I., McClane, B. A.: Beta toxin is essential for the intestinal virulence of Clostridium perfringens type C disease isolate CN3685 in a rabbit ileal loop model. Mol. Microbiol. 2008, 67: 15–30.

Schotte, U., Truyen, U., Neubauer, H.: Significance of beta 2-toxigenic clostridium perfringens infections in animals and their predisposing factors--a review. J. Vet. Med. B. 2004, 51: 423–426.

Smedley, J. G., 3rd, Fisher, D. J., Sayeed, S., Chakrabarti, G., McClane, B. A.: The enteric toxins of Clostridium perfringens. Rev. Physiol. Biochem. Pharmaco. 2004, 152: 183–204.

Songer, J. G.: Clostridial enteric diseases of domestic animals. Clin. Microbiol. Rev. 1996, 9: 216–234.

Songer, J. G., Uzal, F. A.: Clostridial enteric infections in pigs. J. Vet. Diagn. Invest. 2005, 17: 528–536.

Springer, S., Selbitz, H. J.: The control of necrotic enteritis in sucking piglets by means of a Clostridium perfringens toxoid vaccine. FEMS Immun. Med. Microbiol. 1999, 24: 333–336.

Vidal, J. E., McClane, B. A., Saputo, J., Parker, J., Uzal, F. A.: Effects of Clostridium perfringens beta-toxin on the rabbit small intestine and colon. Inf. Immun. 2008, 76: 4396–4404.

Waters, M., Savoie, A., Garmory, H. S., Bueschel, D., Popoff, M. R., Songer, J. G., Titball, R. W., McClane, B. A., Sarker, M. R.: Genotyping and phenotyping of beta2-toxigenic Clostridium perfringens fecal isolates associated with gastrointestinal diseases in piglets. J. Clin. Microbiol. 2003, 41: 3584–3591.

Wollschläger, N.: Diagnostik und Sarnierung der hämorrhagisch nekrotisierenden Enteritis (Clostridium perfringens Typ C) beim Saugferkel in der Schweizer Schweinepopulation. Dissertation, Universität Bern, 2007.

Corresponding address

Dr. H. Posthaus
Institute of Animal Pathology, Vetsuisse Faculty
University of Bern
PO-Box 8466
CH-3001 Bern, Switzerland
E-Mail: horst.posthaus@itpa.unibe.ch

Received: 13 October 2008
Accepted: 18 November 2008