Introduction
Mastitis caused by *Escherichia coli* is common in lactating dairy cows. *E. coli* is considered to be an opportunistic pathogen and originate from a contaminated environment (*Nemeth et al.* 1994). The severity and course of the disease vary greatly and mainly depend on cow’s response (*Hirvonen et al.* 1990). However, the virulence of the bacterial strain involved may also play a role. Bacteria require virulence factors to colonize, multiply and survive in the udder. These include toxins, adhesins, invasins, capsule production and the ability to resist serum complement and scavenge iron. Dominant virulence factors have not been identified in mastitis-derived *E. coli* isolates (*Kaipainen et al.* 2002). In this study we investigated the association between virulence factors of the isolated bacteria and the clinical course in bovine *E. coli* mastitis.

Materials and methods
A total of 155 *E. coli* isolates from acute bovine mastitis was collected during routine farm calls by the ambulatory clinic of the University of Helsinki. The attending veterinarian took aseptically a quarter milk sample for bacterial isolation and determination of milk N-acetyl-ß-D-glucosaminidase (NAGase) activity and recorded the clinical signs of the cow and the changes in milk appearance. Mastitis was classified as severe if the cow was depressed and had high fever no appetite, a swollen and painful udder, and substantial changes in the milk appearance. In mild mastitis, cows had no or a slight fever and only moderate changes in the udder and milk. The cows were divided into 3 lactation stage groups: 0-21, 22-210 and over 221 days post partum (p.p.).

After 3 to 4 weeks, the follow-up samples were taken for bacterial and milk NAGase analysis; 26 cases missed the resampling. The initial sample was classified as persistent if the second sample had growth of *E. coli*. The bacteria from the milk samples were identified as *E. coli* by colony morphology on the blood agar, gram stain, typical growth on eosin-methylene blue (EMB) agar, and by API 20E test (bioMérieux, Marcy l’Etoile, France). Milk NAGase activity was measured using a method described by *Mattila & Sandholm* (1986). Virulence factors and serum resistance were determined as previously described (*Kaipainen et al.* 2002). The association of F17-related fimbriae, S and P fimbriae (grouped together), cytotoxic...
necrotising factors 1 and 2 (CNF1 and CNF2), aerobactin, TraT, and serum resistance with clinical course of mastitis, NAGase activity, and lactation stage were analysed using the Fisher's exact test, ANOVA, and Pearson's chi square test, respectively. ANOVA and Pearson's chi square test were used also to test associations between the clinical course of mastitis, milk NAGase activity and lactation stage.

Results and discussion
Most of the isolates contained several of the analysed virulence factors. Isolates positive to S and P fimbriae (p<0.001), CNF1 (p<0.001), and CNF2 (p<0.05) were significantly associated with persistent mastitis (Table 1). S and P fimbriae and CNF1, usually found in extraintestinal sources, mostly appeared together in the same isolates (Kaipainen et al. 2002). Existence of udder-adapted E. coli strains has been suggested, as the same genotype of E. coli has been isolated in cases of recurrent mastitis (Döpfer et al. 1999, Bradley & Green 2001). The recurrent cases tended to be milder than non-recurrent cases, which could favour chronicity (Bradley & Green 2001). In our study, the acute stage severity of persistent and non-persistent mastitis did not differ significantly.

None of the virulence factors was significantly associated with the severity of mastitis. Milk NAGase activity was significantly (p<0.01) lower in severe than in mild mastitis (221 ± 23 arbitrary units (AU) vs 291 ± 21 AU). The isolates positive to S and P fimbriae and CNF1 caused significantly (p<0.01) higher NAGase (839 ± 48 AU), and F17-positive isolates lower (146 ± 34 AU) NAGase activity as compared with the cases caused by bacteria without these virulence factors, (245 ± 17 AU), and (268 ± 17 AU).

The lactation stage was not associated with the presence of any virulence factors or persistence of mastitis, but was significantly (p<0.05) asso-

| Number of isolates with the virulence factor | F17  | SP1  | CNF12 | CNF22 | AER3 | TraT  | Serum resistant | None  |
|--------------------------------------------|------|------|-------|-------|------|-------|-----------------|-------|
| Mild n=78                                   | 5    | 6    | 6     | 10    | 8    | 25    | 46              | 18    |
| Severe n=77                                 | 9    | 6    | 6     | 13    | 10   | 32    | 45              | 19    |
| Persistent n=16                             | 1    | 6    | 6     | 6     | 1    | 3     | 9               | 2     |
| Non-persistent n=113                        | 10   | 4    | 4     | 12    | 14   | 44    | 64              | 31    |
| ND4 n=26                                    | 3    | 2    | 2     | 5     | 3    | 10    | 18              | 4     |

1 S and P fimbriae
2 Cytotoxic necrotising factor 1 and 2
3 Aerobactin
4 Not determined
cialed with severity. The clinical signs tended to be milder in early (0-21 d p.p.) than in later lactation, which is contrary to previous studies (Hill 1979, Pyörälä & Pyörälä 1998). In field conditions the severity of the signs of mastitis is difficult to determine, as the time of the onset of the mastitis is not known, and the clinical signs change rapidly in E. coli mastitis.

In conclusion, some evidence of the association between bacterial factors and different types of E. coli mastitis was shown, but more research is needed.

References

Bradley AJ, Green MJ: Adaptation of Escherichia coli to the bovine mammary gland. J. Clin. Microbiol. 2001, 39, 1845-1849.

Döpfer D, Barkema HW, Lam TJGM, Schukken YH, Gaastra W: Recurrent clinical mastitis caused by Escherichia coli in dairy cows. J. Dairy Sci. 1999, 82, 80-85.

Hill AW, Shears AL & Hibbit KG: The pathogenesis of experimental Escherichia coli mastitis in newly calved dairy cows. Res. Vet. Sci. 1979, 26, 97-101.

Hirvonen J, Eklund K, Teppo AM, Huszenicza G, Kulcsar M, Salonieri M, Pyörälä S: Acute phase response in dairy cows with experimentally induced Escherichia coli mastitis. Acta vet. Scand. 1999, 40, 35-46.

Kaipainen T, Pohjanvirta T, Shpigel NY, Shwimmer A, Pyörälä S, Pelkonen S: virulence factors of Escherichia coli isolated from bovine clinical mastitis. Vet. Microbiol. 2002, 85, 37-46.

Mattila T, Sandholm M: Milk plasmin, N-acetyl-ß-D-glucosaminidase and antitrypsin as determinants of bacterial replication rates in whey. J. Dairy Sci. 1986, 69, 670-675.

Nemeth J, Muckle CA, Gyles CL: In vitro comparison of bovine mastitis and fecal Escherichia coli isolates. Vet. Microbiol. 1994, 40, 231-238.

Pyörälä S, Pyörälä E: Efficacy of parenteral administration of three antimicrobial agents in treatment of clinical mastitis in lactating cows: 487 cases (1989-1995). J. Am. Vet. Med. Assoc. 1998, 212, 407-412.

Reprints may be obtained from: Tanja Lehtolainen, Department of Clinical Veterinary Science, Faculty of Veterinary Medicine, University of Helsinki, Saari Unit, Pohjoisen pikatie 800, FIN-04920 Saarentaus, FINLAND. E-mail: tanja.lehtolainen@helsinki.fi, tel: +358-19-5295307, fax: +358-19-6851181.