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Short Communication

Persistent occurrence of a single *Streptococcus equi* subsp. *zooepidemicus* clone in the pig and monkey population in Indonesia

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In the present study 41 mucoid growing *Streptococcus equi* subsp. *zooepidemicus* strains (37 strains isolated from healthy two from diseased pigs, two strains isolated from healthy monkeys) appeared to be phenotypically and genotypically identical to mucoid growing *S. equi* subsp. *zooepidemicus* strains isolated from a previously described outbreak among the pig and monkey population on the island of Bali, Indonesia. These findings indicate that the mucoid growing *S. equi* subsp. *zooepidemicus* clone was still present in the pig and monkey population in Indonesia.

Key words: *S. equi* subsp. *zooepidemicus*, pig, monkey, epidemiological relation

Streptococcus equi subsp. *zooepidemicus* is well known from infections of a wide variety of animals, including pigs, sheep, cows, goats, foxes, birds, rabbits, guinea pigs, and monkeys [11,15,16]. All these animals might be potential reservoirs for infections of humans. Cases of human infections with *S. equi* subsp. *zooepidemicus* have been reported, and such infections are frequently associated with the consumption of homemade cheese or unpasteurized milk [3,4,6]. The isolation of *S. equi* subsp. *zooepidemicus* from humans has been described in cases of endocarditis [13], pneumonia [14], meningitis [8,12], septic arthritis [2,9], and cervical lymphadenitis [10]. At the beginning of 1994, a disease outbreak among pigs and monkeys was reported on the island of Bali, Indonesia. The first cases were reported among animals of a pig owner in a small village on the island of Bali. In the following weeks and months, the outbreak spread rapidly to the surrounding districts in Bali, to other islands of Indonesia and into a monkey population. The diseased animals showed clinical symptoms such as painful swelling of the joint, respiratory disturbances, and diarrhea. Most of the animals died within a few days. The postmortem examination of the pigs and monkeys revealed signs of polyarthritis, bronchopneumonia, pleuritis, epicarditis, endocarditis, and meningitis [5]. The bacteriological examination resulted in the isolation of streptococci of Lancefield group C. The bacteria were identified as *S. equi* subsp. *zooepidemicus*. A DNA fingerprinting revealed identical profiles, indicating that a single virulent clone was the causative agent of the various pig and monkey infections on the island of Bali and the other islands of Indonesia [15]. These findings raises the question whether the bacterial clone discovered in 1994 remained to be present in the pig or monkey population. The present study was designed to further characterize *S. equi* subsp. *zooepidemicus* isolated from healthy and diseased pigs and monkeys on the islands of Bali and Java, Indonesia between the years 1995 to 1998.

A total of 49 β-hemolytic streptococci were investigated in this study. Thirty nine streptococci were isolated from tonsils of 39 healthy pigs in the slaughter house in Denpasar, Bali, Indonesia, during a period of 4 years (1995, one strain; 1996, three strains; 1997, two strains; 1998, 33 strains), two streptococci were isolated in 1997 from two diseased pigs in Yogyakarta, Central Java, Indonesia, and two streptococci were isolated from two clinically healthy monkeys from a Bali monkey resort in 1995. The isolates were compared with six *S. equi* subsp. *zooepidemicus* obtained from the original outbreak in the year 1994.

The bacteria were cultivated on sheep blood agar plates (Oxoid, Wesel, Germany) and in Todd-Hewitt broth (Gibco Europe, Karlsruhe, Germany) and identified biochemically according to Farrow and Collins [7] and Barnham and Cole [2], serologically with autoclaved extracts of the bacteria and group C specific antiserum in immunodiffusion

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reactions and with a commercial grouping kit (Slidex Strepto-kit bioMerieux, Nürtingen, Germany). The growth pattern of the bacteria in fluid media and the morphology of the bacterial colonies in soft agar was evaluated as described previously [17]. For analysis of restriction fragment length polymorphisms of the 16S ribosomal RNA gene of the cultures, the 16S rRNA gene was amplified by polymerase chain reaction (PCR) with oligonucleotide primers (primer 1, 5'-GAG AGT TTG ATC CTG GCT CAG CA-3', primer 2, 5'-CGG GTG TTA CAA ACT CTC GTG GT-3') and thermal cycler programs (Techne-Progene, Thermodux, Wertheim, Germany) described previously [1]. The PCR product was further investigated after restriction endonuclease digestion with the restriction enzyme *Hinc*II (Biolabs, Schwalbach, Germany) [1]. The preparation and *Sma*I digestion of the chromosomal DNA for macrorestriction analysis of the isolates by pulsed-field gel electrophoresis was performed as described by Soedarmanto *et al.* [15].

According to cultural, biochemical and serological properties, all 43 isolates (41 from pigs, two from monkeys) investigated in the present study and the six cultures of the original outbreak were β-hemolytic, belonged to Lancefield's serological group C and could be identified as *Streptococcus equi* subsp. *zooepidemicus*. Upon cultivation of the bacteria on blood agar, all 43 isolates and three strains from the original outbreak grew in large mucoid colonies. As already described [15] the remaining 3 *S. equi* subsp. *zooepidemicus* strains obtained from the original outbreak grew on solid media in small non mucoid colonies. All group C streptococci that showed mucoid growth on solid media grew with a uniform turbidity in liquid media and exhibited a broad diffuse colony morphology in soft agar. The remaining three *S. equi* subsp. *zooepidemicus*, obtained from the original outbreak, showing non mucoid growth on solid media, grew in liquid media as sediment with clear supernatant, and with compact colonies in soft agar. The growth properties of these three bacteria obtained from the original outbreak had already been described [15].

According to studies of Abdulmawjood and Lämmler [1] the V2 region of the 16S rRNA gene of *S. equi* subsp. *zooepidemicus* displayed intraspecific variations detectable by endonuclease restriction of the 16S rRNA gene with the restriction enzyme *Hinc*II. This allowed a molecular identification and typing of isolates of this species. The size of the amplified 16S rRNA gene product of all 43 strains of the present investigation and the six control strains investigated previously was approximately 1450 bp relative to the DNA size marker. After digestion with *Hinc*II two fragments with sizes of approximately 1250 bp and 200 bp could be observed for all strains investigated indicating no intraspecies variation in this gene segment (Fig. 1). To further investigate the relation of the *S. equi* subsp. *zooepidemicus* strains the 43 isolates and the six previously

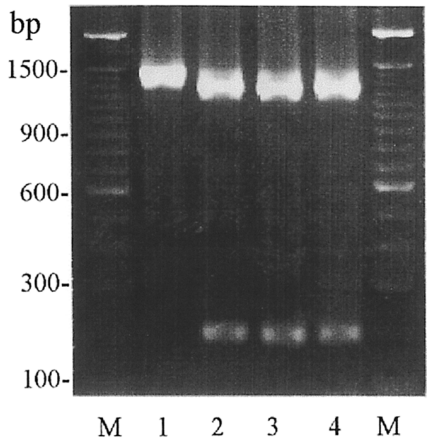

Fig. 1. Typical fragments of the PCR amplified 16S rRNA gene of the *S. equi* subsp. *zooepidemicus* strains; line 1 before and lines 2-4 after digestion with the restriction enzyme *Hinc*II. M = a 100-bp ladder (Gibco BRL Life Technologies, Eggenstein, Germany) served as size marker.

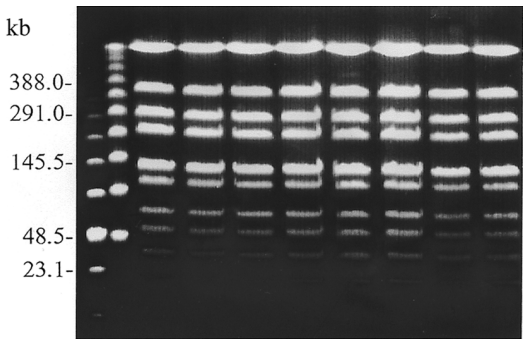

Fig. 2. PFGE analysis of chromosomal DNA of eight *S. equi* subsp. *zooepidemicus* strains after digestion with endonuclease *Sma*I. M = lambda DNA (*Hind*III fragments 0.1-200 kb), and lambda DNA concatemeres (50-1000 kb) (both Sigma, Deisenhofen, Germany).

investigated strains were subjected to macrorestriction analysis of their chromosomal DNA by pulsed-field gel electrophoresis. The *Sma*I restriction pattern of all 43 *S. equi* subsp. *zooepidemicus*, isolated from healthy and diseased pigs and monkeys on the island of Bali and Java were identical (Fig. 2) and corresponded to the PFGE pattern of four of the six *S. equi* subsp. *zooepidemicus* obtained from the original outbreak in 1994. As described previously [15] two *S. equi* subsp. *zooepidemicus*, obtained from the original outbreak, differed from the PFGE pattern of the other 45 *S. equi* subsp. *zooepidemicus* strains in two fragments (not shown data).

According to their phenotypic and genotypic properties all *S. equi* subsp. *zooepidemicus* cultures isolated between 1995 and 1998 appeared to be identical to the original outbreak strain from the year 1994. However, the 43 isolates of the present study were obtained from healthy pigs and monkeys from Bali, two isolates from Java from diseased pigs. These

results indicated that the mucoid *S. equi* subsp. *zooepidemicus* clone isolated during the pig and monkey disease in 1994 is, at least till the year 1998, still present in the pig and monkey population on the various islands in Indonesia. The isolation of the strains generally from animals without signs of the previous diseases might be caused by a specific immunity of the pig and monkey population towards this bacterial strain. However, at present nothing is known about the zoonotic potential of this mucoid ß-hemolytic streptococcal clone, possibly distributed by healthy pigs, for people who consume pork meat on the island of Bali. The future investigation of specimen from healthy and diseased humans, pigs and monkeys and also from other animals might elucidate the possibly existing epidemiological relation.

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