Molecular Characterization of Pneumococcal Isolates from Pets and Laboratory Animals

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Abstract

**Background:** Between 1986 and 2008 *Streptococcus pneumoniae* was isolated from 41 pets/zoo animals (guinea pigs (n = 17), cats (n = 12), horses (n = 4), dogs (n = 3), dolphins (n = 2), rat (n = 2), gorilla (n = 1)) treated in medical veterinary laboratories and zoos, and 44 laboratory animals (mastomys (multimammate mouse; n = 32), mice (n = 6), rats (n = 4), guinea pigs (n = 2)) during routine health monitoring in an animal facility. *S. pneumoniae* was isolated from nose, lung and respiratory tract, eye, ear and other sites.

**Methodology/Principal Findings:** Carriage of the same isolate of *S. pneumoniae* over a period of up to 22 weeks was shown for four mastomys. Forty-one animals showed disease symptoms. Pneumococcal isolates were characterized by optochin sensitivity, bile solubility, DNA hybridization, pneumolysin PCR, serotyping and multilocus sequence typing. Eighteen of the 32 mastomys isolates (56%) were optochin resistant, all other isolates were optochin susceptible. All mastomys isolates were serotype 14, all guinea pig isolates serotype 19F, all horse isolates serotype 3. Rats had serotypes 14 or 19A, mice 33A or 33F. Dolphins had serotype 23F, the gorilla serotype 14. Cats and dogs had many different serotypes. Four isolates were resistant to macrolides, three isolates also to clindamycin and tetracyclin. Mastomys isolates were sequence type (ST) 15 (serotype 14), an ST/serotype combination commonly found in human isolates. Cats, dogs, pet rats, gorilla and dolphins showed various human ST/serotype combinations. Lab rats and lab mice showed single locus variants (SLV) of human STs, in human ST/serotype combinations. All guinea pig isolates showed the same completely new combination of known alleles. The horse isolates showed an unknown allele combination and three new alleles.

**Conclusions/Significance:** The isolates found in mastomys, mice, rats, cats, dogs, gorilla and dolphins are most likely identical to human pneumococcal isolates. Isolates from guinea pigs and horses appear to be specialized clones for these animals. Our data redraw attention to the fact that pneumococci are not strictly human pathogens. Pet animals that live in close contact to humans, especially children, can be infected by human isolates and also carriage of even resistant isolates is a realistic possibility.

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Introduction

*Streptococcus pneumoniae* is the major causative pathogen of community-acquired respiratory tract infections (RTIs) in humans, including community-acquired pneumonia, acute otitis media and acute maxillary sinusitis. It is also the cause of severe invasive infections like meningitis and bacteremia in both children and adults. Most humans, especially young children (0–5 years of age), are likely to be colonized at least once during their lifetime with pneumococci that are spread by droplet infection [1]. Pneumococcal-related disease is usually preceded by colonization of the nasopharynx. Therefore, pneumococcal carriage is believed to play an important role in horizontal spread of *S. pneumoniae* in the community.

In general, *S. pneumoniae* is considered to be a human pathogen only, despite there being established mouse and rat models for various *S. pneumoniae*-caused diseases [2]. For example, BALB/c mice are used as models for pneumococcal pneumonia and meningitis [3], and rats for pneumococcal otitis media [4]. Transmission of other streptococcal species from animals to humans is well-documented, in particular for *S. equi subsp. zooepidemicus, S. canis, S. suis, S. porcinus* and *S. phocae* [5,6,7,8,9]. Pneumococci are naturally competent organisms and can easily exchange DNA in their natural habitat, the human mouth and...
throat. This habitat is populated by various streptococcal species, which form a ‘gene pool’ out of which the pneumococci can recruit their resistance genes. Gene transfer and mosaic genes have been intensively reported for *S. pneumoniae*, *S. oralis*, *S. sanguis* and *S. mitis*.[10]

Multilocus sequence typing (MLST) produces unambiguous molecular typing data.[11,12] The MLST technique is highly portable as any laboratory can compare the sequences of the seven loci in their isolates with those in a central database (www.mlst.net) and thereby obtain the allelic profile of each isolate. MLST results in data giving insight into the clonal relatedness of bacterial isolates.

In the present study, we characterized a large collection of *S. pneumoniae* from pets and laboratory animals including mastomys, guinea pigs, cats, mice, rats, horses, dogs, dolphins and a gorilla. Our findings show that *S. pneumoniae* is capable of colonizing and infecting animal hosts and raises the question whether these animals, which as pets are often in close contact to children, serve as an extra-human reservoir for *S. pneumoniae*.

**Results**

During routine checks of animals in the animal facilities of the German Cancer Research Center (DKFZ) in Heidelberg, Germany, β-hemolyzing streptococci were isolated from multimammate mouse (*Mastomys coucha*, *n* = 32), rats (*Rattus norvegicus* F344, *n* = 4), mice (*Mus musculus* C57BL/6, *n* = 6) and guinea pigs (*Cavia porcellus*, *n* = 2). Isolates from 41 pets comprising guinea pigs (*n* = 17), cats (*Felis domesticus*, *n* = 12), dogs (*Canis lupus familiaris*, *n* = 3), horses (*Equus caballus*, *n* = 4) and rats (*Rattus rattus*, *n* = 2) were received from a veterinary medical laboratory (Vet Med Labor GmbH) in Ludwigsburg, Germany and the Department of Infectious Diseases and Immunology (VMDC) of the Veterinary Medicine of the University of Utrecht, the Netherlands. Two isolates from dolphins (*Tursiops truncatus*) were obtained from a zoo in Nuremberg, Germany and one isolate from a gorilla (*Gorilla gorilla*) was from a zoo in Kerkrade, the Netherlands.

Two mastomys suffered from conjunctivitis. Two guinea pigs (laboratory animals) were C4-immune deficient, and one of them suffered from peritonitis and sepsis and died. All (*n* = 17) pet guinea pigs were obviously ill: RTI (*n* = 10), otitis media (*n* = 1), abscesses (*n* = 2), undefined illness (*n* = 4). Both pet rats were ill (one with RTI, one with unknown disease). Seven pet cats and three pet dogs suffered from RTI. The five other pet cats suffered from otitis media, otitis externa, conjunctivitis, polyarthritis and meningitis respectively. The latter animal died. Three horses had RTI, for one there were no detailed diagnostic data available. The two dolphins were zoo animals that died without showing clear symptoms of disease, but autopsy showed pneumococci in lung biopsies. The gorilla suffered from acute infection with inflamed tonsilli with purulent excretion.

Mastomys isolates were obtained from swabs taken from nose (*n* = 14), eye (*n* = 10), nose and eye (*n* = 2), lung (*n* = 1) and trachea (*n* = 1). Carriage isolates were obtained from eye swabs. Guinea pig isolates were from swabs from nose (*n* = 10), ear (*n* = 2) and wound, abscess, lung, trachea, abdomen, bulla tympanica (one each). For one animal the origin of the material could not be traced anymore. Isolates from laboratory mice an rats were all from nose swabs. For the two pet rats isolates were obtained from lung and wound swabs (one animal each). For the cat isolates were obtained from swabs from nose (*n* = 5), thorax (*n* = 2), and ear, wound, throat, synovia (one animal each). For the cat that suffered from meningitis and died the isolate was obtained from a brain biopsy. The three dog isolates were obtained from nose swabs.

Horse isolates were obtained from nose swabs (*n* = 3) and BAL (*n* = 1). The two isolates from dolphins were obtained from lung-tissue biopsies. The gorilla isolate was obtained from purulent secretion material (Table 1).

Further characterization of the streptococcal isolates at the German National Reference Center for Streptococci confirmed identification of all isolates as *Streptococcus pneumoniae*. All isolates were bile soluble, showed hybridization with a species-specific gene probe and had positive pneumolysin PCRs. Eighteen isolates, all from mastomys, were optochin-resistant.

All mastomys isolates (*n* = 32) were serotype 14. Guinea pig isolates were 19F, mice isolates were 33A or 33F, laboratory rat isolates were 14, pet rat isolates were 19A, horse isolates were 3, dolphin isolates were 23F and the gorilla isolate was 14. Cats and dogs had a large variety of different serotypes (3 (*n* = 2), 6A, 6B, 10A, 14 (*n* = 2), 19A, 19F (*n* = 3), 22A, 22F, 23F, 31) (Table 1).

To exclude a possible transfer of *S. pneumoniae* from the animal facility staff members to the laboratory animals, throat swabs of animal facility staff members of the German Cancer Research Center (DKFZ) were taken. None of the seven staff members were found to carry pneumococci. All obtained isolates belonged to normal throat flora (*Streptococcus oralis*, *Streptococcus mitis*, *Streptococcus parasanguis* and *Gemella morbillorum*). No indications for the presence of pathogenic species were found.

To determine whether mastomys could carry pneumococcal isolates asymptptomatically, four animals were followed over periods of several weeks. *S. pneumoniae* could be isolated from the same obviously healthy animal over periods of 6, 7, 17 and 22 weeks respectively (Table 2). All isolates were serotype 14.

MIC analysis of the isolates showed that four isolates were resistant to clarithromycin, three of them were also resistant to clindamycin and tetracyclin. The isolates were from two cats and two pet rats. The three multiresistant isolates showed an iMLSb phenotype and an ermB genotype, the single resistant isolate had the M-phenotype, and mecA subclass mecA (Table 3). All other isolates were susceptible for all tested substances (penicillin G, amoxicillin, cefotaxime, cefpodoxime, cefuroxime, clarithromycin, clindamycin, tetracycline, levofloxacin, trimethoprim-sulfamethoxazole and vancomycin).

MLST analysis revealed that all mastomys isolates were sequence type (ST)15. All 19 guinea pigs had STs with the same new combination of existing alleles, which has not been found previously, nor have single or double locus variants (Table 4). Mice isolates showed two new sequence types, both single locus variants (SLV) of ST100, one harbouring a variant *spi* allele, with 99% identity to allele 9, and the other a variant *rep* allele (99% identity to allele 12). The four laboratory rats had the same SLV of ST124, showing a variant *gki* allele (99% identity to allele 1). The cats and dogs showed a large variety of STs (9, 124, 176, 177 (*n* = 2), 180 (*n* = 2), 311, 473, 819, 1815, 1994, 3017, 3705 and an SLV of 1551). Three horses had the same new ST, with new alleles (ST 9/14, ST 176/6B, ST 177/19F, ST 180/3, ST 311/23F, ST 440/23F found in mastomys, mice, rats, dog, gorilla and dolphins respectively are common sequence type/serotype combinations found in human pneumococcal isolates (www.mlst.net). This is also the case for the combinations ST 9/14, ST 176/6B, ST 177/19F, ST 180/3, ST 311/23F, ST 473/6A, ST 819/22F, ST 1551/10A, ST 1015/19F, ST 1994/31, and ST 3017/19A.
| Isolate number | Animal | Year of isolation | country | origin | Diagnosis | Site of isolation | Opto Test | Sero-type | MLST |
|----------------|--------|-------------------|---------|--------|-----------|------------------|-----------|-----------|-------|
| 24073          | mastomys 1 | 2003             | Germany | DKFZ   | no clinical symptoms | nose     | R         | 14 15 |
| 24074          | mastomys 2 | 2003             | Germany | DKFZ   | no clinical symptoms | nose     | S         | 14 15 |
| 24080          | mastomys 3 | 2003             | Germany | DKFZ   | no clinical symptoms | nose     | S         | 14 15 |
| 22887          | mastomys 4 | 2003             | Germany | DKFZ   | no clinical symptoms | nose     | R         | 14 15 |
| 22878          | mastomys 5 | 2003             | Germany | DKFZ   | no clinical symptoms | nose     | R         | 14 15 |
| 22879          | mastomys 6 | 2003             | Germany | DKFZ   | no clinical symptoms | trachea  | R         | 14 15 |
| 22875          | mastomys 7 | 2004             | Germany | DKFZ   | no clinical symptoms | nose     | R         | 14 15 |
| 22876          | mastomys 8 | 2004             | Germany | DKFZ   | no clinical symptoms | nose     | R         | 14 15 |
| 22881          | mastomys 9 | 2004             | Germany | DKFZ   | no clinical symptoms | nose     | R         | 14 15 |
| 22882          | mastomys 10 | 2004         | Germany | DKFZ   | no clinical symptoms | lung     | S         | 14 15 |
| 22883          | mastomys 11 | 2004           | Germany | DKFZ   | no clinical symptoms | nose     | R         | 14 15 |
| 22884          | mastomys 12 | 2004         | Germany | DKFZ   | no clinical symptoms | nose     | R         | 14 15 |
| 27110          | mastomys 13 | 2005           | Germany | DKFZ   | no clinical symptoms | nose     | S         | 14 15 |
| 22955          | mastomys 14 | 2005          | Germany | DKFZ   | no clinical symptoms | nose     | R         | 14 15 |
| 22956          | mastomys 15 | 2005          | Germany | DKFZ   | no clinical symptoms | nose     | R         | 14 15 |
| 26267          | mastomys 16 | 2005          | Germany | DKFZ   | no clinical symptoms | eye      | R         | 14 15 |
| 27072          | mastomys 17 | 2006          | Germany | DKFZ   | no clinical symptoms | nose/eye | S         | 14 15 |
| 27673          | mastomys 18 | 2006          | Germany | DKFZ   | no clinical symptoms | eye      | R         | 14 15 |
| 27857          | mastomys 19 | 2006          | Germany | DKFZ   | no clinical symptoms | eye      | R         | 14 15 |
| 28293          | mastomys 20 | 2006          | Germany | DKFZ   | no clinical symptoms | nose/eye | R         | 14 15 |
| 28417          | mastomys 21 | 2006          | Germany | DKFZ   | no clinical symptoms | eye      | R         | 14 15 |
| 28418          | mastomys 22 | 2006          | Germany | DKFZ   | no clinical symptoms | eye      | S         | 14 15 |
| 28419          | mastomys 23 | 2006          | Germany | DKFZ   | no clinical symptoms | eye      | R         | 14 15 |
| 28420          | mastomys 24 | 2006          | Germany | DKFZ   | no clinical symptoms | eye      | S         | 14 15 |
| 28421          | mastomys 25 | 2006          | Germany | DKFZ   | no clinical symptoms | eye      | S         | 14 15 |
| 28422          | mastomys 26 | 2006          | Germany | DKFZ   | no clinical symptoms | eye      | S         | 14 15 |
| 29791          | mastomys 27 | 2006          | Germany | DKFZ   | conjunctivitis     | eye      | S         | 14 15 |
| 33432          | mastomys 28 | 2008          | Germany | DKFZ   | conjunctivitis     | nose     | R         | 14 15 |
| 27703          | mouse (C57BL/6) 1 | 2006 | Germany | DKFZ   | no clinical symptoms | nose     | S         | 33A SLV100 |
| 28448          | mouse (C57BL/6) 2 | 2006 | Germany | DKFZ   | no clinical symptoms | nose     | S         | 33A SLV100 |
| 33645          | mouse (C57BL/6) 3 | 2008 | Germany | DKFZ   | no clinical symptoms | nose     | S         | 33F SLV100 |
| 32276          | mouse (C57BL/6) 4 | 2007 | Germany | DKFZ   | no clinical symptoms | nose     | S         | 33A SLV100 |
| 32969          | mouse (C57BL/6) 5 | 2007 | Germany | DKFZ   | no clinical symptoms | nose     | S         | 33A SLV100 |
| 32970          | mouse (C57BL/6) 6 | 2007 | Germany | DKFZ   | no clinical symptoms | nose     | S         | 33A SLV100 |
| 24072          | rat (F344) 1 | 2003            | Germany | DKFZ   | no clinical symptoms | nose     | S         | 14 SLV 124 |
| 24075          | rat (F344) 2 | 2003            | Germany | DKFZ   | no clinical symptoms | nose     | S         | 14 SLV 124 |
| 24077          | rat (F344) 3 | 2003            | Germany | DKFZ   | no clinical symptoms | nose     | S         | 14 SLV 124 |
| 24079          | rat (F344) 4 | 2003            | Germany | DKFZ   | no clinical symptoms | nose     | S         | 14 SLV 124 |
| 32965          | rat 5        | 2007            | Austria  | VML   | respiratory problems, sudden death | lung     | S         | 19A 3546 |
| 34828          | rat 6        | 2008            | Monaco   | VML   | no data | wound     | S         | 19A SLV 3546 |
| 24070          | guinea pig 1 | 1986           | Germany | DKFZ   | peritonitis, sepsis | lung     | S         | 19F new |
| 24071          | guinea pig 2 | 1986           | Germany | DKFZ   | peritonitis | abdomen | S         | 19F new |
| 28614          | guinea pig 3 | 2006           | The Netherlands | VML | severe respiratory problems | nose     | S         | 19F new |
| 28976          | guinea pig 4 | 2006           | Germany | VML   | head wound | wound     | S         | 19F new |
| 31598          | guinea pig 5 | 2007           | Germany | VML   | umbilical abscess, jaw abscess | abscess | S         | 19F new |
| 32066          | guinea pig 6 | 2007           | Germany | VML   | respiratory problems, clotted nose | nose     | S         | 19F new |
### Table 1. Cont.

| Isolate number | Animal       | Year of isolation | country       | Diagnosis                                      | Site of isolation | Opto Test | Sero-type | MLST |
|----------------|--------------|-------------------|---------------|------------------------------------------------|-------------------|-----------|-----------|------|
| 32449          | guinea pig 7 | 2007              | Germany       | purulent efflux nose, eyes                     | nose              | S         | 19F       | new  |
| 32668          | guinea pig 8 | 2007              | Germany       | coughing, nasal discharge, swollen lymphnodes | nose              | S         | 19F       | new  |
| 32669          | guinea pig 9 | 2007              | Germany       | nasal discharge                                | ear               | S         | 19F       | new  |
| 33098          | guinea pig 10| 2007              | France        | tired, tilted head                             | ear               | S         | 19F       | new  |
| 33099          | guinea pig 11| 2007              | Germany       | otitis media                                   | ear               | S         | 19F       | new  |
| 34603          | guinea pig 12| 2008              | The Netherlands| nasal discharge, tiredness                     | nose              | S         | 19F       | new  |
| 34719          | guinea pig 13| 2008              | Germany       | sneezing, nasal discharge                      | nose              | S         | 19F       | new  |
| 34728          | guinea pig 14| 2008              | Germany       | abortion of offspring, dead                    | Trachea, uterus, liver | S    | 19F       | new  |
| 35456          | guinea pig 15| 2008              | Peru          | no data                                        | nose              | S         | 19F       | new  |
| 34950          | guinea pig 16| 2008              | Germany       | recidiving rhinitis                            | nose              | S         | 19F       | new  |
| 35100          | guinea pig 17| 2008              | Germany       | coughing, purulent nasal discharge             | nose              | S         | 19F       | new  |
| 35489          | guinea pig 18| 2006              | The Netherlands| no data                                        | no data           | S         | 19F       | new  |
| 35494          | guinea pig 19| 2007              | The Netherlands| respiratory problems                           | bulla tympanica  | S         | 19F       | new  |
| 28615          | cat 1        | 2006              | The Netherlands| severe respiratory problems                    | nose              | S         | 3         | 180  |
| 31057          | cat 2        | 2007              | Germany       | otitis media                                   | wound             | S         | 19F       | 177  |
| 32317          | cat 3        | 2007              | Germany       | conjunctivitis, strong sniffing                | throat            | S         | 6A        | 473  |
| 32667          | cat 4        | 2007              | Germany       | rhinitis, conjunctivitis                       | nose              | S         | 19F       | 1815 |
| 32827          | cat 5        | 2007              | Germany       | dyspnoea, lungoedema, thorax puncture         | S                 | 22F       | 819       |
| 33211          | cat 6        | 2008              | Germany       | purulent otitis externa                        | ear               | S         | 14        | 9    |
| 34829          | cat 7        | 2008              | Deutschland   | nasal efflux                                   | nose              | S         | 31        | 1994 |
| 35135          | cat 8        | 2008              | The Netherlands| sneezing, nasal discharge                      | nose              | S         | 19A       | 3017 |
| 35488          | cat 9        | 2003              | The Netherlands| purulent meningitis, died                      | brain             | S         | 23F       | 311  |
| 35490          | cat 10       | 2003              | The Netherlands| chronic rhinitis                              | nose              | S         | 6B        | 176  |
| 35492          | cat 11       | 1997              | The Netherlands| polyarthritis                                  | synovia           | S         | 22A       | 3705 |
| 35493          | cat 12       | 2004              | The Netherlands| liquothorax, respiratory problems             | thorax fluid      | S         | 19F       | 177  |
| 32966          | dog 1        | 2007              | Netherlands   | coughing, sneezing, nasal discharge           | nose              | S         | 14        | 124  |
| 33437          | dog 2        | 2008              | Germany       | chronic recidivating rhinitis                  | nose              | S         | 3         | 180  |
| 33770          | dog 3        | 2008              | Germany       | sinusitis                                      | nose              | S         | 10A SLV1551 |
| 29800          | horse 1      | 2006              | Germany       | sinusitis                                      | nose              | S         | 3         | new  |
| 32450          | horse 2 (pony)| 2007              | Germany       | coughing, nasal discharge                      | nose              | S         | 3         | new  |
| 34368          | horse 3      | 2008              | Germany       | no data                                        | nose, trachealavage S | 3         | new  |
| 34604          | horse 4 (racing horse)| 2008| Germany       | coughing, nasal discharge, swollen lymphnodes | BAL               | S         | 3         | new  |
| 24186          | dolphin 1    | 1986              | Germany       | died without disease symptoms                  | biopsy lung       | S         | 23F       | 440  |
| 24187          | dolphin 2    | 1986              | Germany       | died without disease symptoms                  | biopsy lung       | S         | 23F       | 440  |
| 35491          | ape (gorilla)| 2008              | The Netherlands| acute infection, inflammed tonsilli with pus excretion | pus    | S         | 14        | 124  |

*DKFZ: German Cancer Research Center (DKFZ), Heidelberg, Germany; VML: Vet Med Labor GmbH, Ludwigsburg, Germany; VMDC: Department of Infectious Diseases and Immunology (VMDC) of the Faculty of Veterinary Medicine of the University of Utrecht, the Netherlands, ZN: Zoo Nuremberg, Germany, ZK: Zoo Kerkrade, The Netherlands.

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found in cats and dogs. The combination ST 3705/22A found in one cat has so far never been encountered in humans.

**Discussion**

It is generally accepted that *Streptococcus pneumoniae* is strictly a human pathogen despite there being animal models (mouse, rat) available to study pneumococcal infections. However, on the other hand, recent literature describing naturally occurring carriage of or infections by pneumococci in animal hosts is scarce [13,14]. A report from 1988 describes the isolation of *S. pneumoniae* serotype 3 from a racchorse [15]. Other pneumococcal isolates from racehorses also appear to have serotype 3 [16,17]. In a recent report from Chi et al. *S. pneumoniae* isolated from Chimpanzees are described [13]. Notably, anecdotal reports of *S. pneumoniae* isolated from animals can be found as early as in the 1940s (Table 5).

Outbreaks of infections with *S. pneumoniae* of serotypes 2, 3, 8 and 19 in laboratory rats in the US have been described in several publications [18,19,20,21]. Furthermore, veterinary textbooks describe pneumococcal infections to be not only present in rodents (rats, mice, guinea pigs) but also in larger domestic animals (calves, horses) [22,23].

Since all pneumococcal isolates obtained from animals in this study were bile soluble, and showed hybridization with a species specific DNA probe, they were unambiguously identified as *S. pneumoniae*. Moreover, the fact that serotyping and MLS typing gave positive results confirms that the isolates are pneumococci. However, 18 out of 32 mastomys isolates were optochin-resistant. Since pneumococcal isolates are almost always optochin susceptible and optochin resistant is usually a trait of other viridance group streptococci, this could be one of the reasons why pneumococci are being overlooked and therefore not reported in animal isolates, thereby leading to underrepresentation of information on the extent of pneumococci colonization in the literature. Finally, it is noteworthy that optochin-resistant pneumococci are being increasingly reported from human sources [24].

The most obvious cause of infection for the laboratory animals would be the animal facility staff. For the mastomys colony in the German Cancer Research Center this could be verified. None of the staff carried pneumococci at the moment of sampling, making it less likely that animals had been infected recently. However, it cannot be excluded that the colony got infected at an earlier point in time since carriage in humans is transient. For the other laboratory animals, which came from large commercial facilities, a possible infection by facility staff members could not be tested.

Most ST/serotype combinations identified in the isolates in this study are commonly found among human pneumococcal isolates (www.mlst.net). The combination ST 15/serotype 14, found in 28 mastomys is commonly found among human isolates in association with meningitis and bacteremia and has been reported from the UK, the Netherlands, Portugal, Germany, Italy, Brazil and Hong Kong (www.mlst.net).

The combination ST 100/33F is commonly encountered among human isolates and has been reported from Spain, Germany, Poland and USA. The combination with serotype 33A has not been reported so far (www.mlst.net). This could indicate that a serotype switch from 33F to 33A has occurred in mice, as well as a mutation in the *spi* or the *recP* allele.

The combination ST 124/serotype 14 found in the gorilla, a dog and, as an SLV, in the laboratory rats has been reported from Germany, Sweden, UK, Denmark, Finland, Norway, The Netherlands, Australia, Canada, and Poland and is associated with meningitis, bacteremia, pneumonia and otitis media in human isolates, as well as with carriage (www.mlst.net).

The combination ST 3546/19A found in two pet rats has been reported only once, from Norway, and was isolated from blood (www.mlst.net).

**Table 2. Carriage of *S. pneumoniae* by mastomys.**

| Isolate number | Animal | 1st sample (week of life) | last sample (week of life) | Carriage length (weeks) | Year of isolation | country | origin | Diagnosis | Site of isolation | Opto Test | Serotype | MLST |
|---------------|--------|--------------------------|---------------------------|------------------------|-------------------|---------|--------|-----------|---------------|-----------|----------|------|
| 31578         | mastomys #18 | 52                       | 74                       | 22                     | 2007              | Germany | DKFZ   | no clinical symptoms | eye          | S        | 14 | n.d. |
| 32161         | mastomys #29 | 66                       | 73                       | 7                      | 2007              | Germany | DKFZ   | no clinical symptoms | eye          | S        | 14 | n.d. |
| 31467         | mastomys #33 | 55                       | 72                       | 17                     | 2007              | Germany | DKFZ   | no clinical symptoms | eye          | S        | 14 | n.d. |
| 31580         | mastomys #168 | 45                       | 51                       | 6                      | 2007              | Germany | DKFZ   | no clinical symptoms | eye          | S        | 14 | n.d. |

*DKFZ: German Cancer Research Center (DKFZ), Heidelberg, Germany; n.d.: not done.

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**Table 3. Macrolide resistant isolates found in pet cats and rats.**

| Animal | Diagnosis                  | MLST | Serotype | Macrolide resistance genotype | Macrolide resistance phenotype | CLA (µg/ml) | CLI (µg/ml) | TET (µg/ml) | PEN (µg/ml) | CEF (µg/ml) |
|--------|----------------------------|------|----------|-------------------------------|-------------------------------|-------------|-------------|-------------|-------------|--------------|
| cat 4  | rhinitis, conjunctivitis   | 1815 | 19F      | ermB                          | MLS2                         | 16          | 16          | 16          | 0.015       | 0.015        |
| cat 6  | otitis externa eitrig      | 9    | 14       | mefA                          | M                            | 8           | 0.12        | 0.25        | 0.015       | 0.015        |
| rat 5  | Breathing problems, sudden death | 3546 | 19A      | ermB                          | MLS2                         | 16          | 8           | 16          | 0.015       | 0.015        |
| rat 6  | no data                   | SLV 3546 | 19A      | ermB                          | MLS2                         | 16          | 16          | 16          | 0.06        | 0.015        |

CLA: clarithromycin, CLI: clindamycin, TET: tetracycin, PEN: penicillin, CEF: cefotaxime.
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**Pneumococci from Animals**

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**Table 3. Macrolide resistant isolates found in pet cats and rats.**

| Animal | Diagnosis                  | MLST | Serotype | Macrolide resistance genotype | Macrolide resistance phenotype | CLA (µg/ml) | CLI (µg/ml) | TET (µg/ml) | PEN (µg/ml) | CEF (µg/ml) |
|--------|----------------------------|------|----------|-------------------------------|-------------------------------|-------------|-------------|-------------|-------------|--------------|
| cat 4  | rhinitis, conjunctivitis   | 1815 | 19F      | ermB                          | MLS2                         | 16          | 16          | 16          | 0.015       | 0.015        |
| cat 6  | otitis externa eitrig      | 9    | 14       | mefA                          | M                            | 8           | 0.12        | 0.25        | 0.015       | 0.015        |
| rat 5  | Breathing problems, sudden death | 3546 | 19A      | ermB                          | MLS2                         | 16          | 8           | 16          | 0.015       | 0.015        |
| rat 6  | no data                   | SLV 3546 | 19A      | ermB                          | MLS2                         | 16          | 16          | 16          | 0.06        | 0.015        |

CLA: clarithromycin, CLI: clindamycin, TET: tetracycin, PEN: penicillin, CEF: cefotaxime.
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The combination serotype ST 440/23F (UK, Poland, Czech Republic, Switzerland) found in the two dolphins is also commonly encountered among human isolates where it is associated with meningitis and bacteremia as well as being found in carriage (www.mlst.net).

Table 4. MLSTs of 81 Streptococcus pneumoniae isolates from pets and laboratory animals in Germany.

| Animal       | number of animals | Year          | Serotype | MLST | aroE | gdh | gki | recP | spi | xpt | ddf |
|--------------|------------------|---------------|----------|------|------|-----|-----|------|-----|-----|-----|
| mastomys     | 28               | 2003–2006     | 14       | 15   | 1    | 5   | 4   | 5    | 5   | 3   | 8   |
| guinea pig   | 19               | 1986/2006/2007/2008 | 19F | new | 2    | 5   | 4   | 5    | 27  | 20  | 5   |
| mouse (C57BL/6) | 5             | 2006          | 33A/33F  | new (SLV 100) | 5   | 12  | 29  | 12   | 9 (99%) | 39  | 18 |
| mouse (C57BL/6) | 1             | 2006          | 33A     | new (SLV 100) | 5   | 12  | 29  | 12   | 9 (99%) | 39  | 18 |
| rat (F344)   | 4               | 2003          | 14      | new (SLV 124) | 7   | 5   | 1   | 9 (99%) | 8    | 14  | 11  |
| rat (pet)    | 1               | 2007          | 19A     | 3546 | 1    | 5   | 41  | 5    | 10  | 28  | 8   |
| cat          | 1               | 2008          | 19A     | new (SLV 3546) | 1   | 5   | 41  | 5    | 10  | 99% 28 | 8 |
| cat          | 1               | 2003          | 6B      | 176  | 7    | 13  | 8   | 6    | 10  | 6   | 14  |
| cat          | 1               | 2004/2007     | 19F     | 177  | 7    | 14  | 4   | 4    | 12  | 1   | 14  |
| cat          | 1               | 2006          | 180     | 177  | 7    | 15  | 2   | 4    | 10  | 6   | 1   | 22  |
| cat          | 1               | 2003          | 23F     | 311  | 7    | 8   | 9   | 1    | 6   | 4   | 6   |
| cat          | 1               | 2007          | 6A      | 473  | 7    | 25  | 4   | 4    | 15  | 20  | 28  |
| cat          | 1               | 2007          | 22F     | 819  | 1    | 1   | 4   | 1    | 18  | 58  | 18  |
| cat          | 1               | 2007          | 19F     | 1815 | 1    | 5   | 4   | 12   | 5   | 3   | 159 |
| cat          | 1               | 2008          | 31      | 1994 | 1    | 2   | 29  | 1    | 111 | 14  | 18  |
| cat          | 1               | 2008          | 19A     | 3017 | 8    | 11  | 14  | 1    | 17  | 230 | 14  |
| cat          | 1               | 1997          | 22A     | 3705 | 2    | 194 | 53  | 18   | 10  | 1   | 1   |
| dog          | 1               | 2007          | 14      | 124  | 7    | 5   | 1   | 8    | 14  | 11  | 14  |
| dog          | 1               | 2008          | 3       | 180  | 7    | 15  | 2   | 10   | 6   | 1   | 22  |
| dog          | 1               | 2008          | 10A     | SLV 1551 | 5   | 7   | 4   | 6    | 10  | 1   | 6   |
| horse        | 3               | 2006          | 3       | new  | 10   | 9   | 4   | 12   | 10 (99%) | 10 (99%) | 6 (99%) |
| horse (pony) | 1               | 2007          | 3       | new  | 10   | 9   | 4   | 12   | 10 (99%) | 10 (99%) | 6 (99%) |
| dolphin      | 2               | 1986          | 23F     | 440  | 7    | 5   | 1   | 1    | 13  | 31  | 14  |
| gorilla      | 1               | 2008          | 14      | 124  | 7    | 5   | 1   | 8    | 14  | 11  | 14  |

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The combination ST 180/serotype 3 found in a cat and in a dog isolate has been reported from several countries (UK, Spain, Poland, Portugal, Italy, Taiwan, Canada, Denmark, Netherlands) and is associated with invasive disease (meningitis, bacteremia) but also with community acquired pneumonia, otitis media and...

Table 5. Anecdotal reports of Streptococcus pneumoniae isolated from different mammalian species.

| Animals       | Number of infected animals | Outbreak | Serotypes | Laboratory animals | Year | Source                          |
|---------------|----------------------------|----------|-----------|--------------------|------|---------------------------------|
| guinea pigs   | 39                         | yes      | 19        | yes                | 1945 | Homburger et al. [33]            |
| rats          | 71                         | yes      | 2         | yes                | 1950 | Mirick et al. [18]              |
| rats          | 254                        | yes      | 8         | yes                | 1965 | Ford [19]                       |
| rats          | 156                        | no       | 2, 3, 19  | yes                | 1969 | Weisbroth and Freimer [20]      |
| rats          | 32                         | no       | 3         | yes                | 1971 | Mitraka [21]                    |
| mice, rats    | 9,10                       | yes      | 35        | yes                | 1988 | Fallon et al. [34]             |
| horses        | 10                         | no       | 3         | no                 | 1988 | Huber and Willoughby [15]       |
| horses        | 11                         | no       | 3         | no                 | 1999 | Whatmore et al. [16]           |
| cat           | 1                          | no       | n.d.      | no                 | 2006 | Zhang et al. [14]              |

n.d: no data.
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Carriage (www.mlst.net). The combinations ST 9/14, ST 176/6B, ST 177/19F, ST 311/23F, ST 473/6A, ST 819/22F, ST 1551/10A, and ST 1994/31 found in cats and dogs are all well known human ST/serotype combinations associated with both invasive disease and carriage (www.mlst.net). Two isolates, ST 1815/19F and ST 3017/19A, both found in cats have been reported only once to the MLST database (www.mlst.net). ST 1815/19F was from a case of bacteremia from the Czech Republic. ST 3017/19A was isolated from blood from a patient from the Netherlands, which is also the country of origin of the cat. The combination ST 3705/22A has not been reported to the MLST database. The only isolate with ST 3705 is a carriage isolate from the Netherlands with serogroup 19. Again this isolate is from the same country as the cat in this study.

eBURST analyses revealed that STs 15, 124, 177, 180, 473 and 1994 are all predicted founders of clonal complexes (CC). ST 124 is the predicted founder of a group of 78 STs and is one of the original penicillin-resistant clones described in South Africa [25]. CC124 also comprises STs 440, ST9 and ST1815 belong to CC15. STs 176, 311, 819, 1551, and 3705 are members of different clonal complexes. STs 3546 and 3017 are singletons, i.e. they are not related to any other STs in the database. Taken together, our data show that the pneumococcal isolates from mastomys, mice, rats, cats, dogs, dolphins and gorilla analysed in this study are highly similar, if not identical, to human isolates related to invasive pneumococcal disease and carriage.

Serotype 19F, found in all four guinea pig isolates, is a common serotype found in human isolates (www.mlst.net), where it is associated with invasive pneumococcal disease as well as carriage. It occurs in combination with large numbers of different STs. However, the STs of all 19 guinea pig isolates consist of a new allele combination never encountered in human isolates. From these findings it may be interpreted that the guinea pigs did not pick up the pneumococcal infections from humans. Moreover, the fact that the same new allele combination has been found in isolates from guinea pigs from different locations (pets, laboratory animals), different countries (France, Germany, Peru, the Netherlands) and more widely separated in time (1986, 2006) seems to indicate that this clone is typical for guinea pigs and that these animals are a reservoir for *S. pneumoniae* of this specific sequence type. As apparent from our diagnostic data, this clone is clearly pathogenic for guinea pigs.

The four horse isolates described here have serotype 3 and a new ST, with three or two new alleles. Whatmore et al. report on a collection of horse pneumococcal isolates of serotype 3 and conclude on the basis of RFLP analyses that these isolates are closely related to, but distinguishable from human pneumococcal isolates [16]. Showing a new sequence type, our data confirm that the horse isolates form a population different from human isolates. Pneumococci found in guinea pigs and horses seem to belong to separate host specific populations. The isolates found in guinea pigs from different locations (pets, laboratory animals), different countries (France, Germany, Peru, the Netherlands) and widely separated in time (1986, 2006) seems to indicate that this clone is typical for guinea pigs and that these animals are a reservoir for *S. pneumoniae* of this specific sequence type. As apparent from our diagnostic data, this clone is clearly pathogenic for guinea pigs.

Four animals, two cats and two rats, had macrolide resistant isolates. One cat isolate showed moderate resistance to clarithromycin and carried the *mefA* subclass *mef(A)* gene. The isolate had ST13 combined with serotype 14, which is the most common macrolide resistant clone (PMEN England1-9) in Germany [26,27]. The other isolates all contained the *ermB* resistance marker, which results in higher MICs for clarithromycin and resistance to clindamycin. These isolates were also resistant to tetracyclin. The ST/serotype combinations were 1815/19F and (SLV/3546/19A. As discussed above these are rather rare combinations. However, serotypes 19A and 19F are strongly associated with multidrug resistance.

Our findings are unequivocal proof that pneumococci are capable of causing severe disease in a number of different animal species. One of the pet guinea pigs was a member of a larger colony in which the animals suffered from respiratory problems and of which several animals died. Further, the cats, dogs and horses also suffered from severe respiratory problems, and the two dolphins were likely to have died from pneumococcal infections.

On the other hand, laboratory animals (mastomys, mice and rats) from which pneumococci were isolated showed no disease symptoms. For two animals from the mastomys colony that suffered from conjunctivitis, another potential causative agent for the clinical signs (*Pasteurella pneumotropica* biotype Heyl) was found in large numbers in addition to *S. pneumoniae*. This finding indicates that the rodents may carry *S. pneumoniae* in their normal flora without suffering from pneumococcal disease, much like in the case of humans. Indeed, for four individual mastomys, we were able to isolate the same isolate over a period of up to 22 weeks, showing that the animals are carriers.

This is the first time that a larger collection of *S. pneumoniae* isolated from a range of animals has been characterized on a molecular basis using MLST, enabling a solid comparison to human isolates present in the MLST database. The results show that one on hand animals can be infected with human pneumococcal clones. On the other hand animals like horses and guinea pigs seem to be affected by their ‘own’ specific pneumococcal clones. The fact that pets like cats, dogs, guinea pigs, rats and mice are possible carriers of pneumococcal strains, and in many cases of the same ST/serotype combinations as human isolates, is worrisome. Even more alarming is the fact that antibiotic resistant strains, on one occasion even the most common macrolide resistant clone in Germany (England1-9), could be isolated from pets. Our data imply that there is an infection route for pneumococci from humans to pet animals that live in close contact to humans, especially children. By residing in animals, pneumococci would not only enlarge their gene-pool with other streptococcal species found more commonly in animals, but would also be able to avoid the negative selective pressure of vaccination in children. The question remains if pneumococci residing in animals can re-infect humans. On basis of the present data no conclusion on this can be drawn and further research is needed.

**Materials and Methods**

**Study material**

The German National Reference Center for Streptococci (GNRCS) received a total of 85 bacterial isolates from animals in Austria, France, Germany, Monaco, The Netherlands and Peru in 1986 (n = 4) and during 2003–2008 (n = 81). Forty-four isolates were from the animal facility of the German Cancer Research Center (DKFZ) in Heidelberg, Germany, 32 isolates were from pets treated at a commercial veterinary medical laboratory (Vet Med Labor GmbH) in Ludwigsburg, Germany, 6 isolates were obtained from the Department of Infectious Diseases and Immunology (VMDC) of the Faculty of Veterinary Medicine of the University of Utrecht, the Netherlands, two isolates were from a zoo in Nuremberg and one from a zoo in Kerkrade, the Netherlands. All samples were sent to the GNRCS as isolated bacteria, no primary material was received.

**Testing of animal facility staff**

Throat swabs were obtained in May 2006 from seven members of the animal facility staff of the German Cancer Research Center.
and tested for bacterial content using routine microbiological methods. Throat swabs were taken by the institute medical officer of the DKFZ, after verbal consent of each of the animal facility staff members. Isolated bacteria were analysed both at the DKFZ as well as at the GNRCs.

Characterization of isolates

*Streptococcus pneumoniae* isolates were characterized by optochin susceptibility and bile solubility testing. Optochin susceptibility testing was carried out in a 5% CO₂ atmosphere on sheep blood agar [29]. Bile solubility testing was performed by preparing a bacterial suspension in 1 mL 0.85% NaCl (McFarland standard 1.0) adding four drops of 10% sodium deoxycholate. Complete lysis after incubation for 2 h at 35°C was taken as a positive result. A commercially available DNA hybridization test (AccuProbe® *Streptococcus pneumoniae* identification test; bioMérieux, Germany) was used according to the manufacturer’s instructions.

Serotyping

Pneumococcal strains were serotyped by the Neufeld Quellung reaction using type and factor sera provided by the Statens Serum Institut, Copenhagen, Denmark [29].

Susceptibility testing

Minimal inhibitory concentration (MIC) testing was performed using the broth microdilution method as recommended by the Clinical Laboratory Standards Institute [30]. Microtiter plates containing penicillin G, amoxicillin, cefotaxime, cefpolodoxime, cefuroxime, clarithromycin, clindamycin, tetracycline, levofloxacin, trimethoprim-sulfamethoxazole and vancomycin with cation-adjusted Mueller-Hinton broth (Oxoid, Wesel, Germany) plus 5% lysed horse blood (Oxoid) were used. The final inoculum was 5 x 10⁵ CFU/mL. MICs were determined following incubation for 24 h at 35°C in ambient air. *S. pneumoniae* ATCC 49619 was used as a control strain. Current Clinical Laboratory Standards Institute interpretive criteria were used to define antimicrobial resistance [30].

DNA extraction

Isolates were inoculated from agar plates (5% sheep blood) into a sterile culture tube containing 10 mL Todd-Hewitt broth (Oxoid, Limited Basingstoke, Hampshire, UK) and incubated overnight at 37°C. After centrifugation, the chromosomal DNA was isolated using a DNA extraction kit (Qiagen, Hilden, Germany).

Pneumolysin PCR

Analysis of the pneumolysin gene (ply) was performed using real-time PCR on a Light Cycler (Roche Diagnostics GmbH, Penzberg, Germany) according to the LightCycler Operator’s Manual Version 3.5. PCR was carried out using the Light Cycler Faststart DNA Master SYBR Green I — kit according to the manufacturer’s instructions. The reaction mixture of 20 µl contained 2 µl SYBR Green I, 2 µl MgCl₂, 12 µl H₂O, 1.5 µl of each primer and 200 ng of DNA. The oligonucleotide primers pair was ply 5' TGG GGA GCG TCC TTT GTT GGT CTA T 3' and plyrev 5' TCT TTA CTC GTT TTC ACG TTG A 3' [31]. PCR cycling comprised initial denaturation for 10 min at 95°C and 35 amplification cycles for 0 s at 95°C, 2 s at 62°C and 4 s at 72°C with temperature transition rates of 20°C/s. This was followed by a melting programme of 30 s at 95°C, 30 s at 67°C and 0 s at 95°C at rates of 20, 20, and 0.1°C/s, respectively, and cooling at 40°C for at least 30 s.

Multilocus sequence typing

Multilocus sequence typing (MLST) of 81 isolates was carried out as described previously. Briefly, internal fragments of the *aroE, gdh, gki, recP, spi, xpt* and *dly* genes were amplified by PCR from chromosomal DNA with the primer pairs described by Enright and Spratt [12]. The alleles at each of the seven loci provide the allelic profile of each isolate and also define their sequence type (ST). Allelic profiles are shown as the alleles at each of the seven loci, in the order *aroE, gdh, gki, recP, spi, xpt* and *dly*.

MLST analysis

STs were compared to the pneumococcal MLST database on www.mlst.net. The STs were analysed using the program eBURST. This program is able to predict and display the relationships between closely-related isolates of a bacterial species or population. eBURST, unlike cluster diagrams, trees or dendrograms, uses a simple but appropriate model of bacterial evolution in which an ancestral (or founding) genotype increases in frequency in the population, and while doing so, begins to diversify to produce a cluster of closely-related genotypes that are all descended from the founding genotype. This cluster of related genotypes is referred to as a ‘clonal complex’ (eburst.mlst.net) [32].

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Author Contributions

Conceived and designed the experiments: MvdL AAL WN. Performed the experiments: MvdL AAL RRR. Performed the data analysis: MvdL AAL WN. Wrote the paper: MvdL.

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