Xylose-positive staphylococci as a cause of respiratory disease in immunosuppressed rats

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INTRODUCTION

Respiratory disease in laboratory rodents can be caused by different bacteria and viruses. One common bacterial finding in respiratory disease in laboratory rats is coagulase-positive, non-xylose fermenting strains of Staphylococcus aureus (Ohder & Wullenweber 1989). However, in routine health monitoring a coagulase-negative, xylose-fermenting staphylococcus species, Staphylococcus xylosus, is frequently found (*Møllegaard Breeding Center* 1990). It is considered non-pathogenic, but in the case-report described below it was shown to be the cause of severe respiratory disease in immunosuppressed rats.

MATERIALS AND METHODS

The affected animals were 163 Mol:SPRD rats used in two 16 weeks Cyclosporine A (CyA) nephrotoxicity studies. CyA was given by gavage once daily. Controls were given the vehicle without CyA. The test substance was administered with a stomach tube, which was used for all animals without disinfection. Study 1 consisted of 80 rats. Out of these, 51 rats were given CyA 25 mg/kg/day, and 29 were controls. Study 2 consisted of 83 rats. CyA 12,5 mg/kg/day was given to 63 rats, and 20 rats were vehicle treated controls. The animals were kept in a controlled environment (temp. 21 ± 1°C, rel. humidity 55 ±5 % and light/dark cycles of 12 hours), and fed Altromin 1324 rat chow and tap water ad libitum. All rats originated from a colony known to be free of Mycoplasma pulmonis, Sendai virus, coronaviruses, PVM, Reovirus 3 and parvoviruses (Møllegaard Breeding Center). However, BN/Mol rats with antibodies to parvovirus were kept in the same room.

Rats showing signs of severe respiratory disease were euthanized by inhalation of pure CO_2 and examined macroscopically and histopathologically. The lungs were fixed in 4 % buffered formalin. Parafinized sections of the lungs were stained with Hematoxylin-Eosin as well as with Giemsa. Smears of bronchial exudate were stained with Giemsa's method.

Sterile swabs were taken from the trachea and lungs of euthanized rats, and inoculated on nutrient agar[3] with 10 % horse blood and incubated for 24 hours at 37°C. All morphologically different colonies were inoculated on 10 % blood agar and incubated for further 24 hours. Hereafter all cultures were gram-stained, and tested for catalase and oxydase.

Gram-positive, catalase-positive cocci were identified by biochemical reactions according to table 2 using Api STAPH mediae[4] and Staphaurex latex agglutination[5].

A random sample of 9 rats (Mol:SPRD and BN/Mol) were bled two weeks after the onset of the disease and the serum was tested for the presence of antibodies to Mycoplasma pulmonis, Sendai virus, coronaviruses, PMV, Reovirus 3 and parvoviruses by the use of ELISA[6].

RESULTS

Two to eight weeks after the rats were enrolled in the study, almost all CyA treated rats started sneezing. Out of a total number of 114 CyA-treated rats, 66 again stopped sneezing and survived the last 8 to 14 weeks of the study without further symptoms. Approx-

Table I. Isolations of xylose-positive staphylococci from trachea and lungs of rats with signs of respiratory disease.

	Trachea		Lungs		Trachea+lungs*	
	Abs.	%	Abs.	%	Abs.	%
Examined rats in all	15	100.0	15	100.0	15	100.0
S. xylosus mono-infection	13	86.7	9	60.0	9	60.0
S. xylosus mixed infection**	1	6.7	1	6.7	6	40.0
Total number of rats infected with S. xylosus	14	93.3	10	66.7	15	100.0

* The findings of trachea and lungs from the same animal regarded as one sample.
** Other bacterial sp found:
Proteus sp, Enterobacter cloacae, Xanthomonas maltophilia, Staphylococcus simulans, Staphylococcus hominis.

imately half of the 48 severely diseased rats (25 from study 1 and 23 from study 2) died suddenly before symptoms apart from sneezing were observed. Three sneezing rats were euthanized for the purpose of pathological and serological examinations. The remaining 48 rats lost weight as a general symptom of distress, and were euthanized.

In the control groups some sneezing was observed but no animals lost weight or died, except three rats which were euthanized for the purpose of pathological and serological examinations.

The dead or euthanized rats all had rhinitis, bronchitis and interstitial pneumonia. Large amounts of broncial exudate was present, and

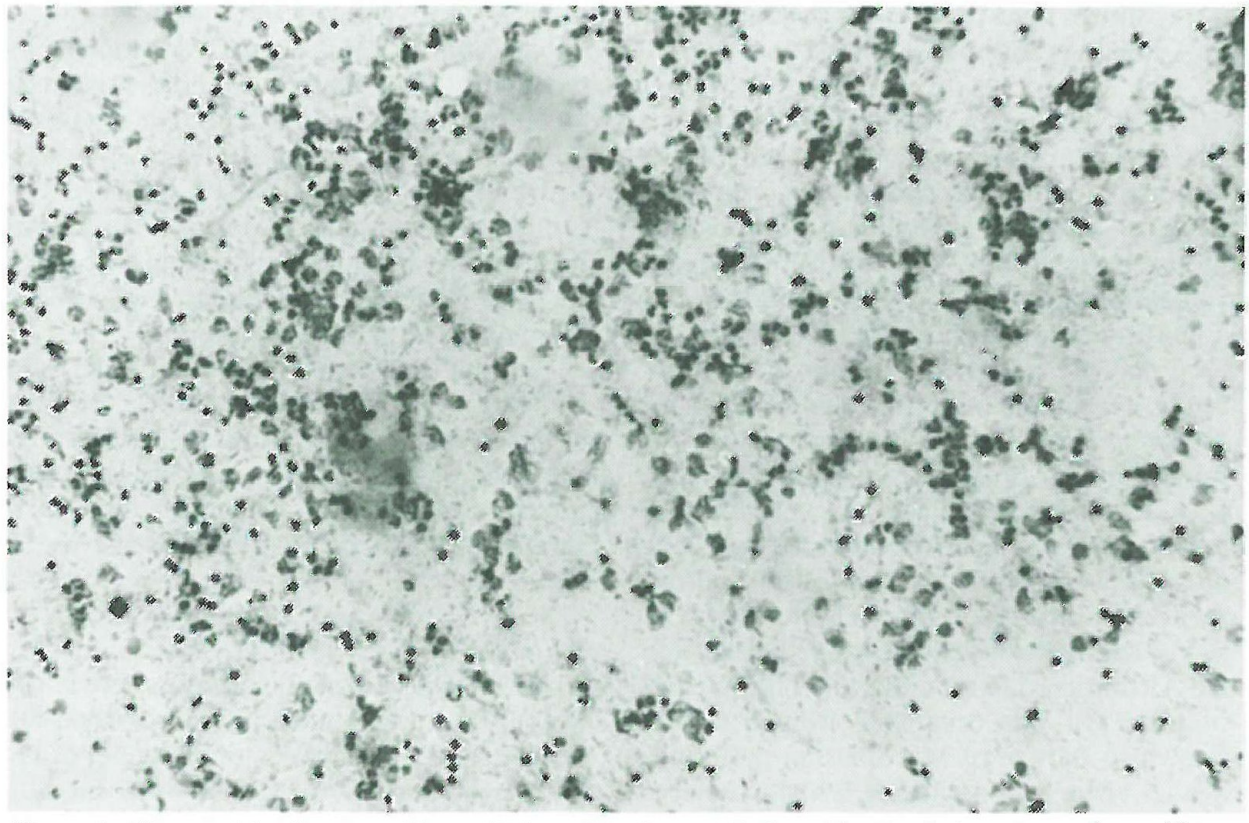

Figure 1. Giemsa stained smear of bronchial exudate from rat infected by Staphylococcus xylosus. Numerous cocci and polymorph nuclear neutrophils are present.

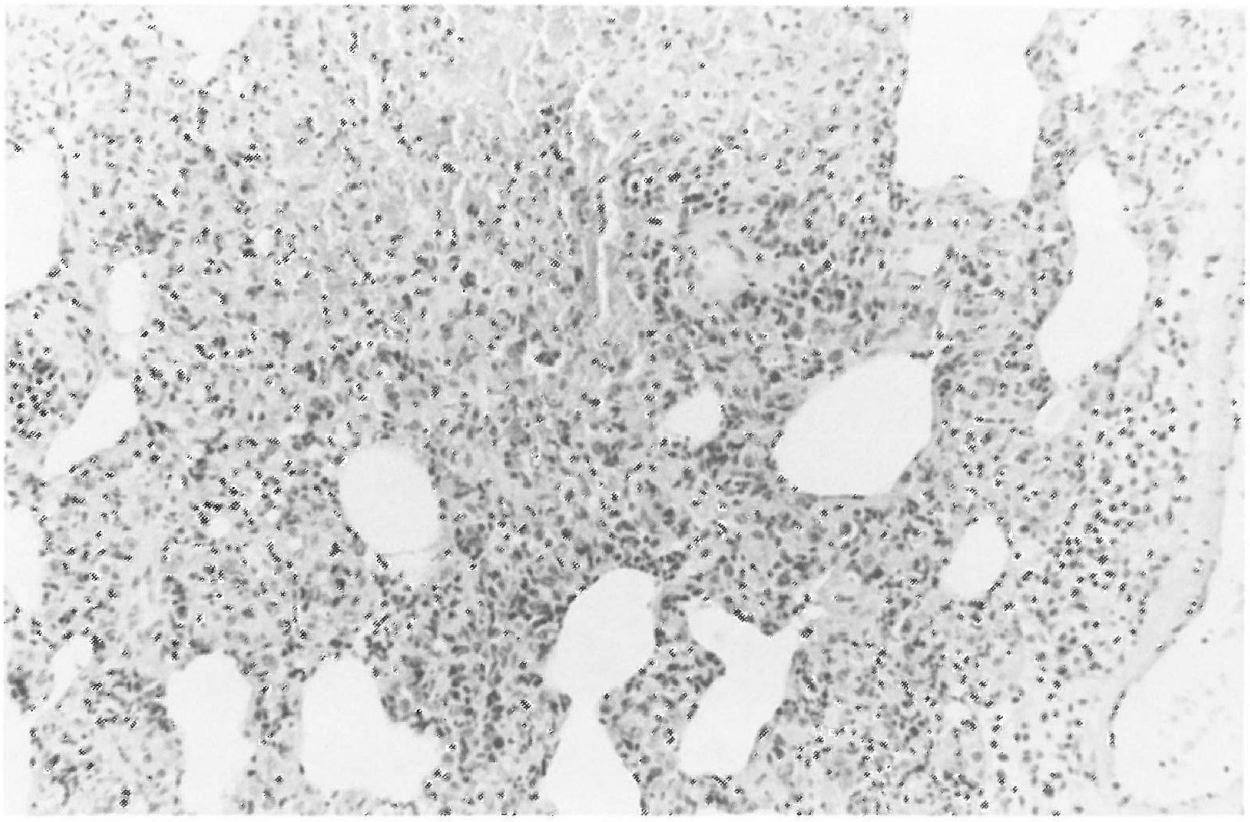

Figure 2. Haematoxylin-Eosin stained slide of lung tissue from rat infected by Staphylococcus xylosus. The tissue is consolidated and infiltrated with neutrophils and exudate.

the lungs appeared greyish-red and patchy with an increased density.

Giemsa stained smears of bronchial exudate revealed large amounts of big cocci and polymorph nuclear neutrophils (fig. 1). In Hematoxylin-Eosin stained slides from lungs there were large consolidated areas within the bronchi infiltrated with neutrophils and exudate (fig. 2).

Results of the bacteriological examinations are shown in table I, and biochemical reactions are given in table II. All xylose-positive staphylococci appeared as 1 mm white colonies after 24 hours on blood agar. Catalase reaction was constant but weak.

No antibodies to the antigen investigated were found in the nine sacrificed animals, except for one BN/Mol rat which had antibodies to parvovirus. This was considered of no importance to the disease outbreak since no other animal had seroconverted to parvovirus.

Table II. Biochemical reactions of isolates of xylose-positive staphylococci from trachea and lungs of rats with signs of respiratory disease.

Gram	+
Catalase	+
Oxidase	–
Hemolysis	–
Coagulase/protein A	–
D-glucose	+
D-fructose	+
D-mannose	+
Maltose	+
Lactose	–
D-trehalose	+
D-mannitol	–
Xylitol	–
Melibiose	–
Alkaline phosphatased	*
Voges-Proskauer	+
Raffinose	–
D-xylose	+
Sucrose	+
α-methyl-D-glucoside	–
N-acetyl-glucosamine	+
Arginin dihydrolase	+
Urease	–

* 30 % positive, 70 % negative.

DISCUSSION

CyA is an immunosuppressive drug with several adverse effects, but it has never been incriminated as a primary cause of interstitial lung disease. Furthermore, Mol:SPDR rats have previously tolerated equal or higher CyA dosages for prolonged periods, using similar protocols and in the same environment (*Dieperink et al.* 1988). Thus, because the disease process described is periodic and related to upper and lower respiratory distress, we conclude that it was caused by the microorganism Staphylococcus xylosus, which was found in pure cultures in the lungs and bronchi, where it was accompanied by exudation and neutrophil extravasation.

All affected animals were infected with coagulase-negative xylose-positive staphylococci and most of these were found as pure cultures. The biochemical reactions given in table II are quite uniform, but differ from isolates of S. xylosus from humans (*Schleifer & Kloos* 1975). This indicates that the isolated bacteria could be a certain pathogenic biotype, maybe with a strain specific relationship.

A virus or a mycoplasm could have been the primary agent, but there was no evidence that the unit was infected with normally occurring rodent respiratory viruses or mycoplasms.

It is not likely that staphylococcus species are of any great importance as pathogens in immunocompetent animals, but this case clearly shows the importance of very high hygienic standards when working with immunosuppressed animals. A microorganism that does not normally cause disease in healthy carrier animals may cause fatal disease in immunosuppressed animals. In case it seems most obvious to assume that the use of an immunosuppressive drug as well as a contaminated stomach tube played a major role in the disease outbreak. CyA suppresses the cellular immune response (*Bunjes et al.* 1981) and this response is important in the protection against stafylococcal infections.

Since it is probably not possible for any length of time to exclude stafylococcus species from the upper respiratory tract of rats, and as it is known that staphylococci of human origin easily colonize laboratory animals (*Blackmore & Francis* 1970), it is of importance to study the breeder's health-monitoring report and try to identify bacteria that might act as opportunists.

Footnotes

[1] Møllegaard Breeding Center, Ll. Skensved, Denmark.
[2] Altromin Denmark, Gentofte, Denmark.
[3] Gibco, Paisley, Scotland.
[4] Api International, Meyrin-geneve, France.
[5] Wellcome Diagnostics, Dartford, England.
[6] Microbiology Laboratories, Middlesex, England.

Summary

A severe outbreak of respiratory disease was diagnosed during a long-term toxicity study of Cyclosporine A in Sprague Dawley rats. Only rats dosed orally with cyclosporine A fell ill, whereas no control animals contracted the disease. The causative agent was found to be Staphylococcus xylosus, a normal inhabitant the the respiratory tract of rats. Under normal conditions this organism is not pathogenic. In the case of immunocompromised animals, however, Staphylococcus xylosus was able to cause disease. The severity of the outbreak could be explained by insufficient hygienic procedures when dosing the animals with a common stomach tube.

Sammendrag

Der beskrives et alvorligt udbrud af lungebetændelse hos sprague dawley rotter under et langtids toxikologisk forsøg med Cyclosporin A. Alle dyr, der doseredes dagligt med Cyclosporin A blev syge, medens kontroldyrene ikke blev angrebet. Sygdommen var forårsaget af Stafylococcus xylosus, der normalt forekommer i rottens respirationsveje. Under normale omstændigheder er denne organisme ikke patogen. Hos immunosuprimerede dyr kan S. xylosus imidlertid fremkalde sygdom. Udbruddets alvorlige karakter kan muligvis tilskrives manglende hygiejne i forbindelse med peroral dosering af dyrene, idet der blev anvendt en fælles mavesonde til alle dyr i forsøget.

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Poxvirus hos katt

Ett fall av poxvirusinfektion (kokoppvirus) hos människa har nyligen konstaterats i Sverige. Med stor sannolikhet har smittan erhållits efter nära kontakt med katt. Klinisk bild med hudutslag (koppor) och förekomst av hög antikroppstiter mot poxvirus hos misstänkt katt styrker antagandet.

År 1978 rapporterades det första fallet av koppvirusinfektion hos huskatt i Storbritannien. Fram til dags dato har över 160 fall rapporterats från Storbritannien (flertalet fall), Nederländerna, Österrike, Polen och USSR. Koppvirus på katt anses vara identisk med, eller mycket nära relaterat till koppvirus på nöt – kokoppvirus, ett virus som endast sällan isolerats från nöt. Mycket talar för att kokoppvirus förekommer som endemisk smitta bland vilda gnagare. Antikroppstitrar hos mus och sork i Storbritannien, virusisolering av nära relaterade koppvirus från vilda gnagare i Östeuropa, samt infekterade katters sjukdomshistorie styrker antagandet att vilda gnagare kan utgöra smittreservoir i naturen.

Den primära skadan består ofta av en bitsårsliknande skada i huden på hoved eller framben. Sekundära hudskador med utveckling av koppor uppkommer efter några dagar til några veckor. Smitta från katt till katt kan förekomma men ger enligt uppgift endast subklinisk infektion hos mottagaren. Hittills har åtta fall av smitta från katt till människa rapporterats varav två med dödlig utgång. Bakomliggande faktorer som nedsatt immunforsvar anses ha bidragit till fatal utgång på människa.

Med det nu rapporterade fallet av koppvirusinfektion på katt i Sverige, det första kända, kan förekomst av smitta med koppvirus bland vilda gnagare i Sverige inte uteslutas. En ökad vaksamhet mot koppvirusinfektion på katt är därmed befogad inte minst i försöksdjursbesättningar med uppfödning och djurhållning av katt respektive gnagare.

Virologiska laboratoriet vid Statens Veterinärmedicinska Anstalt står til tjänst med ytterligare information, råd och upplysningar samt analys av inskickade prover.

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