Resistance to Infection of Guinea Pigs with a Rat *Streptobacillus Moniliformis*

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Summary

A rat *S. moniliformis* strain was dosed, orally and nasally, to two groups of 12 guinea pigs aged 10-15 weeks and 4-5 weeks respectively. In the first experiment 3 animals were sacrificed 1, 2, 4 and 6 weeks after infection and *S. moniliformis* was detected by culture in one animal at 2 weeks and by PCR in two animals at 4 weeks. In the second experiment 4 animals were sacrificed 2, 4 and 6 weeks after infection and *S. moniliformis* was not detected by culture and PCR in any animal. Seroconversion to *S. moniliformis* was found from 2 weeks after infection in 3 of 12 and 7 of 12 guinea pigs respectively. Clinical signs and gross lesions in the ventral cervical area and the lungs were not observed in any animal. We conclude that guinea pigs seem difficult to infect with a rat *S. moniliformis* strain.

Introduction

Streptobacillus moniliformis is a Gram negative bacterium found in various laboratory animal species and is the causative agent of rat bite fever (RBF) and Haverhill fever (HF) in man (*Wullenweber,* 1995). *S. moniliformis* infection has been detected in conventional and barrier-maintained colonies of laboratory mice, rats and guinea pigs in the last 20 years (references in *Boot et al.,* 2002). In guinea pigs *S. moniliformis* has been reported from cases of cervical lymphadenitis (*Smith,* 1941; *Aldred et al.,* 1974; *Fleming,* 1976) and pneumonia (*Kirchner et al.,* 1992). Using the ELISA we occasionally detect seropositive guinea pigs but have been unable to confirm the infection by culture and/or PCR.

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However, *S. moniliformis* strains from guinea pigs have not been kept and their infectivity to other animal species has not been studied. It is also unknown if rat strains of the bacterium are infective to guinea pigs. Therefore we experimentally infected guinea pigs with a rat strain of *S. moniliformis* and monitored the animals by culture, PCR, serology and (histo)pathology.

Materials and Methods

Bacteria

S. moniliformis strain KUN-3 isolated from a rat (*Koopman et al.,* 1991) was grown on 5 % sheep blood agar (SBA) (Oxoid) for 48 h at 37 °C under 7.5 % CO_2. This bacterial strain has been used in studies on the validation of an ELISA for detection of antibodies to the bacterium (*Boot et al.,* 1993) and of a PCR (*Boot et al.,* 2002)

Experimental infection

We used HSD.Poc:D.H.Rivm (Dunkin Hartley) guinea pigs supplied by the Dutch Central Animal Laboratory of the National Vaccine Institute in two experiments.

Published in the Scandinavian Journal of Laboratory Animal Science - an international journal of laboratory animal science

Experiment 1 was carried out with hysterectomy-derived isolator-reared animals which had been associated with an enteric flora to normalize intestinal abnormalities such as the enlarged cecum in germfree animals. The flora originated from rats and mice from a rederived barrier-maintained breeding colony to which the isolator-reared guinea pigs were to be transferred after health monitoring had been completed.

Experiment 2 was carried out with animals from this barrier-maintained colony about 4 weeks after transfer. Mice, rats and guinea pigs from the colony were free from inadvertent infections listed in the FELASA recommendations for the monitoring of colonies of rodents and rabbits (*Nicklas et al.*, 2002).

In experiment 1 we orally (0.2 ml) and nasally (0.2 ml) dosed with a *S. moniliformis* suspension containing 10^{4-5} cfu / ml to 6 male and 6 female, 10 - 15 weeks old animals and housed them according to sex.

In experiment 2 we similarly dosed the bacterium to 6 male and 6 female, 4 – 5 wks old animals.

The animals were kept on the floor of a Gustaffson type stainless steel isolator, and were fed 2.5 Mrad gamma irradiated pelleted diet *ad libitum* (LKK 20, Hope Farms, Woerden NL). Sterilised tap water was available *ad libitum*. All animals were inspected twice daily for clinical signs.

All animal experiments were approved by the institute's Ethical Committee on Animal Experiments and were conducted in compliance with national legislation which is based on European Community Council directive 86/609/EEC.

Detection of S. moniliformis infection

Orally/nasally infected animals: in the first experiment at 1, 2, 4 and 6 weeks post infection (p.i.), 3 of the 12 guinea pigs were sacrificed and the remaining guinea pigs were blood sampled by higly competent technicians through puncture of the retro-orbital venous plexus under (KRA) [Ketamine (Alfasan, Woerden NL) 90 mg/kg i.p., Rompun (Bayer AG, Leverkusen Germany) 10 mg/kg i.p., atropine (Vetinex Animal Health, Bladel NL) 0.05 mg/kg i.p.) anesthesia. In the second experiment at 2, 4 and 6 weeks p.i., 4 of the 12 guinea pigs were sacrificed and the remaining guinea pigs were blood sampled as described above. All sacrificed animals were inspected for gross abnormalities of the lungs, the larynx and the *lymphonodulus (lnn) cervicalis profundus cranialis* and *lnn mandibularis*. The pharynx was swabbed for culture and PCR and the left cervical and mandibular lymph nodes were excised and used for all detection methods whereas the nodes from the right side and the right lung were fixed in 4 % buffered formaldehyde for histology. Finally the left lung tissue was sampled for culture and PCR. All sampling was carried out in a disinfected class II biohazard cabinet and strict precautions were taken to avoid cross-contamination between animals and samples.

Culture was carried out on 20 % horse serum agar containing 16 mg / l trimethoprim and 304 mg / l sulfamethoxazole (selective agar) incubated 48 h at 37 °C under 7.5 % CO_2 and *S. moniliformis* suspected growth was pure-cultured and identified by conventional morphological and biochemical tests as described (*Koopman et al.,* 1991) and by *S. moniliformis* PCR. Tissue samples for PCR analysis were kept at - 20 °C until testing and the assay was carried out as described (*Boot et al.,* 2002). Sera were kept at - 20 °C until testing in the 1: 50 dilution for antibodies to *S. moniliformis* by ELISA using strain KUN 3 as the antigen (*Boot et al.,* 1993). Animals were considered to seroconvert if the ELISA activity measured after infection exceeded 30 % of the activity of the positive control serum. For histo-pathological evaluation 4-5 µm sections of the *lnn cervicalis profundus cranialis*, the *lnn mandibularis*, pharynx, larynx and lung were stained by H&E.

Negative control animals: immediately after sampling the experimentally infected guinea pigs, we sacrificed and sampled one (in experiment 1, totalling 4) or two (in experiment 2, totalling 6) uninfected age-matched control guinea pigs in

order to exclude *S. moniliformis* infection in the hysterectomy-derived and the barrier-maintained colonies respectively, to monitor possible carry over effects during sampling and (considered of primary interest) as background pathology controls. Absence of *S. moniliformis* infection was determined by culture, PCR and ELISA as indicated.

Statistical evaluation

Differences in the number of culture/PCR positive and seroconverting guinea pigs between both experiments and in histology scores between infected and control guinea pigs in the second experiment were evaluated by Fischer's exact test.

Results

Experiment 1

S. moniliformis was cultured from the pharynx and lung of one infected guinea pig killed 2 weeks p.i. (Table 1) and PCR detected the infection in the cervical lymphnode (n =1), the mandibular lymphnode (n=1) and the pharynx (n=2) of 2 infected animals 4 weeks after infection. Seroconversion to *S. moniliformis* was detected from 2 wks p.i. in 3 guinea pigs including a PCR positive animal, but not in the culture positive animal. Control guinea pigs were negative by all methods applied to detect *S. moniliformis* infection. Clinical signs and gross lesions in the ventral cervical area and the lungs were not observed in any of the animals in this experiment.

Experiment 2

S. moniliformis was not detected by culture and PCR in any of the infected guinea pigs. Seroconversion to the bacterium was detected from 2 wks p.i. (Table 1) and 7/12 guinea pigs became ELISA positive, but clinical signs and gross lesions were not observed in any of the animals in this experiment. Control guinea pigs were negative by all methods applied to detect *S. moniliformis* infection.

The only remarkable finding on histopathological examination was moderate mixed submucosal infiltration in the larynx, and this abnormality was more often seen in infected guinea pigs than in controls (Fischer's exact test $p = 0.03$). Submucosal infiltration in the larynx was detected in some infected guinea pigs but, not in control animals, accompanied by minimal mononuclear cell infiltration in lung tissue. Infected and control guinea pigs did not differ in abnormality scores for lung tissue ($p = 0.44$) and in the number of follicles in cervical and mandibular lymph nodes ($p \geq 0.1$). The number of guinea pigs found positive by direct methods (culture and PCR) in the first (3/12) and the second experiment (0/12) did not differ ($p = 0.10$). The number of *S. moniliformis* ELISA serocoversions in the first (3/12 young adults from which 4/30 positive samples) and the second experiment (7/12 young from which 15/24 positive samples) did not differ significantly ($p = 0.11$).

Table 1: Monitoring *S. moniliformis* infection in orally and nasally infected guinea pigs by culture, PCR and ELISA

			Sampling week post infection					Total number positive	
Experiment	Age (weeks)	Method		1	2	4	6	samples	animals
1	10-15	culture	0/3*	1/3	0/3	0/3		1/12	1/12
		PCR	0/3	0/3	2/3	0/3		2/12	2/12
		ELISA	0/12	1/9	2/6	1/3		4/30	3/12
2	4-6	culture		0/4	0/4	0/4		0/12	0/12
		PCR		0/4	0/4	0/4		0/12	0/12
		ELISA		4/12	7/8	4/4		15/24	7/12

*: number pos. / number examined.

Discussion

The fact that in the first experiment *S. moniliformis* was detected in 3/12 guinea pigs at 2- 4 wks after oral and nasal dosing the bacterium proves that guinea pigs can occasionally be infected by a rat strain of *S. moniliformis*. In previous studies in orally dosed and exposed rats the ELISA appeared superior to culture and PCR in detecting *S. moniliformis* infection (*Boot et al.*, 1993, 2002), so it might be argued that the actual number of infected guinea pigs was higher than detected by direct methods. It can however not be ruled out that seroconversion (without persistent colonization) is due to a high infectious dose of *S. moniliformis.* It might also be possible that the development of specific immunity to *S. moniliformis* has rendered the bacterium in a cellwall deficient state that is difficult to culture (*Domingue & Woody*, 1997), but this would not *per se* have precluded detection of the bacterium by PCR. Why only some of the experimentally infected guinea pigs seroconverted to the bacterium is unclear. In studies in which rats were exposed to *S. moniliformis* infected counterparts for 6 weeks, we observed clear differences in seroconversion even between cage mates (*Boot et al.*, 2002), suggesting genetically based differences in colonization and subsequent antibody development in rats. Genetically based differences in susceptibility to experimental *S. moniliformis* infection have also been described in mice (*Wullenweber et al,* 1990).

The fact that *S. moniliformis* was detected by direct methods in the first experiment but not in the second experiment seems to suggest that guinea pigs aged 10-15 weeks might be more susceptible to infection than animals aged 4-5 weeks. The number of positive animals however did not differ significantly between both experiments. Further, experiments were not carried out simultaneously so different preparations of infectious material, be it of the same bacterial strain, were used. Finally the background flora present in the guinea pigs likely slightly differed as the first experiment was carried out with hysterectomy-derived animals kept in isolators and the second experiment after counterparts were transfered to a barrier-maintained colony.

Histopathology did not yield any additional evidence for persistent *S. moniliformis* infection in our guinea pigs. The moderate mixed submucosal infiltration in the larynx, which in our animals was the only remarkable histological finding, was not reported earlier. The few earlier reports on streptobacillosis in guinea pigs described cervical lymphadenitis (*Smith,* 1941; *Aldred et al.,* 1974; *Fleming,* 1976) or granulomatous pneumonia (*Kirchner et al.,* 1992), but these abnormalities were not seen in our animals. Various infectious agents have been implicated in cervical lymphadenitis and granulomatous pneumonia in guinea pigs so, especially in the older reports which were (in contrast to our guinea pigs) on conventional animals, the contribution of other infections to the development of the lesions seems likely.

Our experiments suggest that guinea pigs are difficult to infect with a *S. moniliformis* strain of rat origin but this does not exclude the existence of clones among rat *S. moniliformis* strains that show higher infectivity to guinea pigs.

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