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Efficacy of an oral live vaccine for veterinary use against pseudotuberculosis

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ABSTRACT

Pseudotuberculosis, an infection caused by the ubiquitous enteropathogenic bacterium *Yersinia pseudotuberculosis*, is a recurrent veterinary problem in livestock and zoo animals. The only vaccine currently available in zoos is Pseudovac (a mixture of killed strains of various serotypes), but its efficacy is not well established. We show here that Pseudovac does not protect guinea pigs against a severe *Y. pseudotuberculosis* infection. We thus evaluated the possibility of using a live attenuated *Y. pseudotuberculosis* strain (IP32680) as an oral vaccine against animal pseudotuberculosis. We report that IP32680 is avirulent for guinea pigs and induces a strong IgG response against various serotypes of *Y. pseudotuberculosis*. One and two oral inoculations of IP32680 provided 50% and 83% protection, respectively against a severe infection with a highly pathogenic strain. The avirulent *Y. pseudotuberculosis* IP32680 is therefore much more protective than Pseudovac and may represent a valuable oral vaccine against pseudotuberculosis in zoo animals.

1. Introduction

The Gram-negative enteropathogenic bacterium *Yersinia pseudotuberculosis* is widely distributed in countries with temperate or cold climate and human cases of pseudotuberculosis are commonly reported in Europe, North America, Japan and Eastern Russia [1]. Such cases are most often sporadic, although outbreaks have been reported in Japan, Finland and Russia [2–4]. Ubiquitous in the environment, *Y. pseudotuberculosis* is usually contracted primarily through the consumption of contaminated water or greeneries. *Y. pseudotuberculosis* is enzootic in various mammalian and bird species, and rodents are the main reservoir of infection [5]. Contamination of livestock animals such as cattle [6], buffaloes [7], deers [8] and sheep [9] with *Y. pseudotuberculosis* also occurs and has a significant economic impact. Pseudotuberculosis is also a recurrent health problem for precious animals in zoological gardens [10–13] and wildlife parks [14] where the density of populations is high. This infection may thus be of major concern for the preservation of endangered species.

The bacterial cells absorbed orally disseminate from the gastrointestinal tract to the mesenteric lymph nodes, causing diarrhea, abdominal pain and fever. In weakened individuals, bacteria disseminate to visceral sites such as the spleen and liver, and septicemia is often observed in humans [15] and animals. Infection is treated by means of antibiotherapy, however some *Y. pseudotuberculosis* strains naturally resistant to several antibiotics have recently been observed ([16] and our unpublished observations). In terms of prevention, hygiene measures are useful but are not always sufficient to prevent pseudotuberculosis. Vaccination could thus be valuable to prevent the lethality

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caused by this microorganism in animals living in captivity. The veterinary vaccine mainly used in European Zoos [13], named Pseudovac (Utrecht Veterinary Faculty, the Netherlands) is a killed whole-cell vaccine composed of serotype I–VI isolates. Produced for zoological gardens and wildlife parks, its use in birds [17–19] and small primates [20,21] has been reported. However, its efficacy has never been really evaluated. Furthermore, pseudotuberculosis cases were observed in animals vaccinated with Pseudovac in zoos (unpublished observations from T. Petit and A. Maillot at the La Palmyre and Amnéville zoos, France, respectively).

In the present study, we examined whether Pseudovac could efficiently protect guinea pigs (which are highly susceptible to the disease) against a *Y. pseudotuberculosis* infection. Furthermore, we compared the protection conferred by Pseudovac to that of a live attenuated strain of *Y. pseudotuberculosis* (IP32680) administered orally. This strain was previously shown to be avirulent in mice [22]. We show here that Pseudovac is inefficient in protecting against a severe *Y. pseudotuberculosis* infection and that oral vaccination with IP32680 is much more protective and may represent a valuable alternative to the currently used veterinary vaccine.

2. Materials and methods

2.1. Yersinia strains and culture conditions

The serotype II *Y. pseudotuberculosis* strain IP32680 was isolated from a dead hare. IP32680 lacks genetic determinants coding for known virulence factors such as the High Pathogenicity Island or the super antigens, but harbors the pYV virulence plasmid or the psa fimbriae [22]. This strain was found to be avirulent in the mouse model since oral or sc inoculation of high doses (10^9 cfu) of IP32680 failed to kill or to cause detectable signs of disease in these animals. All *Y. pseudotuberculosis* isolates (serotypes I–V) used in this study were taken from the collection of the *Yersinia* Research Unit (Institut Pasteur) and were positive by PCR for the presence of the pYV virulence plasmid and the Psa fimbriae (pH6 antigen). Bacteria were usually grown at 28 °C in Luria–Bertani agar plates supplemented with 0.2% hemin (LBH) for 48 h before use.

2.2. Guinea pig immunization and infection conditions

Female Hartley guinea pigs (6–8 weeks old) were purchased from Charles River (L'Arbresle, France). All animal care and experimentations were conducted according to the guidelines of the Institut Pasteur and Ecole Nationale Vétérinaire (France). Anesthesia was obtained by intramuscular (im) injection of Zoletil 100 (Virbac, France) or Xylazine (Rompun® from Bayer; 2.5 mg/kg) plus Ketamine (Imalgene 1000® from Mérial; 125 mg/kg). Bacterial suspensions (200 μl in saline) of the serotype II strain IP32680 were inoculated via the intragastric (ig) route using a curved feeding needle. To evaluate the virulence of the IP32680 strain, guinea pigs were inoculated ig with a high bacterial dose (10^9 cfu) and survival was followed for 3 weeks. At the end of the experiment, animals were euthanized by intraperitoneal (ip) injection of Pentobarbital (200 mg/kg) and the presence of lesions in the intestine, mesenteric lymph nodes, liver, spleen and lungs were searched for. Peyer's patches were also examined and the number and size of enlarged patches were recorded. In order to evaluate the clearance of the vaccine bacteria from the body, various organs (2 swollen Peyer's patches, mesenteric lymph nodes, spleen, liver and lungs) were sterily taken, cut into pieces and minced using glass beads (VWR, USA) and an electric mill (Retsch, Germany). Bacterial counts were obtained by plating dilutions of homogenates on LB agar (Table 1).

To determine the 50% lethal dose (LD_{50}) of strain IP32953 for guinea pigs inoculated ig, four animals per dose were infected with 10-fold serial dilutions of IP32953 and survival was followed for 3 weeks. Vaccination with the IP32680 strain consisted in one or two ig inoculation of 10^9 cfu in saline at 40-day interval. The Pseudovac vaccine was obtained from the Microbiology Laboratory of the Zoo and Exotic animals section of the Utrecht Veterinary Faculty. Vaccination using Pseudovac consisted in two subcutaneous (sc) injections (0.25 ml each) in the back of the guinea pigs at 40-day interval, as recommended for animals below 1 kg of weight. Although the name "Pseudovac" has been used for this vaccine for years, it is not a registered trademark and a distinct vaccine against *Pseudomonas aeruginosa* now also has this name.

In order to evaluate guinea pigs protection against pseudotuberculosis after vaccination, animals received a suspension of 10^9 cfu ($2200 \times LD_{50}$) of the virulent *Y. pseudotuberculosis* strain IP32953 inoculated ig 30 days after the last vaccine dose. Thereafter, animal mortality, behavior and fur appearance were recorded daily for 3 weeks. Body weight was measured every 3–4 days as a marker of health. At the end of the experiment, animals were euthanized and the presence of lesions in the

Table 1
Clearance of the *Y. pseudotuberculosis* strain IP32680 after oral vaccination[a].

Guinea pig	*Y. pseudotuberculosis* counts[b]				
	Peyer's patches	Mesenteric LN	Liver	Lungs	Spleen
1	70	0	0	0	0
2	93	0	0	0	0
3	0	0	0	0	0

[a] Guinea pigs were orally inoculated with 10^9 cfu of *Y. pseudotuberculosis* strain IP32680 and were sacrificed 21 days later.
[b] Organs were sterily minced and bacterial counts were obtained by plating dilutions of homogenates on LB agar. Due to the size of these organs, 1/10th of liver and lungs or 1/5th of spleen homogenates was plated.