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Microbiological Studies of Wild Rodents in Farms as Carriers of Pig Infectious Agents

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ABSTRACT

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In 15 breeding and fattening pig herds, 85 mice (*Mus musculus*) and 40 rats (*Rattus norvegicus*) were captured and bacteria and viruses looked for. *Bordetella bronchiseptica*, *Pasteurella* sp., *E. coli*, *Campylobacter jejuni* and *Treponema* sp. were isolated from different samples. Rotavirus was also identified and neutralizing transmissible gastroenteritis antibodies were detected in the serum of one rat and mice from three different farms. Wild rats were also orally infected with Aujeszky's disease virus (ADV) and classical swine fever (CSF) virus. All the rats survived the ADV experimental infection and some of them showed ADV neutralizing antibodies in their sera. No multiplication of the SF virus was obtained.

INTRODUCTION

Although the responsibility of rodents for the deterioration of pig buildings is well known, limited information exists on their role in the transmission of pig-specific infectious agents. The rodent habitat and way of life has often given rise to the suspicion that they are responsible for spread of disease. Several authors have shown that rodents can be carriers of numerous pig pathogens, such as *Salmonella typhimurium* (Davis, 1948), *Erysipelothrix rhusiopathiae* (Boulton, 1979), *Leptospira* sp. (Sullivan, 1974), *Treponema hyodysenteriae* (Joens and Kinyon, 1982) and *Campylobacter* sp. (Vandenberthe and Marsboom, 1982); rodents do not seem to be susceptible to *Pasteurella multocida*, in contrast to *P. pneumotropica* (Mraz et al., 1980).

Serological and virological investigations have also demonstrated that mice and rats could be infected by porcine parvovirus (Joo et al., 1976), encephalomyocarditis virus (Boulton, 1978) and the Aujeszky's disease virus (ADV) (Maes et al., 1979). Results of the different studies carried out on Aujeszky's

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disease in rodents are often inconsistent. Nikitin (1959) found ADV in the brain of rats, captured in farms in which ADV affected the pigs, but some researchers, such as Aldasy and Mate (1969), MacFerran and Dow (1970) and, lastly, Maes et al. (1979), did not. In addition, Nikitin (1959) reported that surviving orally and intranasally infected rats could be carriers of ADV. This suggests that the rodents could play an important role in the spread of ADV.

In this study, the results of bacteriological, virological and serological investigations on rats and mice captured in intensive pig farms are reported. Even though the presence of different pig infectious agents was identified in rodents, the role of rodents in the transmission of disease could not be evaluated. The results of experimental oral infections of *Rattus norvegicus* by ADV and classical swine fever (CSF) virus are also reported.

MATERIALS AND METHODS

Farms

Fifteen breeding and fattening pig herds were chosen in Brittany, west of France, in an area with a high density of pig farms. Considering their management and their size, these farms are representative of the general level of the intensive pig farms of the region.

Trapping

On each farm, trapping of rats and mice was carried out. The traps used were classical wire-cage traps for the rats and I.N.R.A. tunnel-traps for mice. These two trapping systems allowed the rodents to be captured alive, permitting a good quality of samples to be obtained. On this occasion, different baits were tested to increase the probability of rat capture: corn soaked for a few hours in red wine or in anise-flavoured cocktail, as well as pieces of meat. The efficacy of mice trapping was enhanced by distributing some oat grains in front of the entrance and at the bottom of the tunnel-traps.

Samples, bacteriological and virological investigations

Each day, the traps were visited and the captured rodents killed with ether. Blood was collected and the serum of three or four mice, captured in the same farm, was pooled. Two oral swabs were taken before the necropsy. The following organs were collected in sterile flasks: lungs, liver, spleen, kidneys, large intestine and gut contents, feces, tonsils and salivary glands.

A part of the lungs, spleen, one kidney and an oral swab were frozen for ADV studies. The samples were inoculated three successive times on PK15 cells.

A part of the large intestine and its contents, with fecal material, were tested to search for the rotavirus by the enzyme-linked immunosorbent assay (ELISA) technique (Scherrer and Bernard, 1977) and the transmissible gastro-enteritis virus (TGE virus) by inoculating on secondary pig kidney cells. Another part of the lungs, tonsils, salivary glands and the second oral swab were placed on selective medium and cultured for isolation of *Pasteurella* and *Bordetella* according to the techniques described by Smith and Baskerville, (1979, 1983).

Isolation of *Escherichia coli* and *Salmonella* was attempted from liver, half of the spleen and the other kidney. Large intestine contents and feces were cultured for isolation of *E. coli* and *Salmonella*. *Treponema hyodysenteriae* and *Campylobacter* were also looked for according to the techniques described by Joens and Kinyon (1982) and Skirrow (1977).

Serological investigations

Neutralizing antibodies to ADV, CSF virus, TGE virus, foot and mouth disease virus and swine vesicular disease virus were looked for in the sera of the collected rodents (Mornet et al., 1982). Antibodies to porcine parvovirus and swine influenza (H_1N_1) were detected by the haemagglutination inhibition technique (Kundin and Easterday, 1972; Vannier et al., 1984).

Experimental studies

The rats used for experimental studies were raised in isolated conditions and had no previous contact with pigs.

Two groups of wild rats (*Rattus norvegicus*) were kept in two different isolated units.

In a first unit, three groups of three rats were established. At the beginning of the experiment (*D*0), the nine rats were orally inoculated with 0.5 ml of an ADV suspension of the virulent Kojnok strain. The initial titer of the suspension was $10^{5.3}$ tissue culture infectious dose $(TCID)_{50}$ ml^{-1}, but Group 1 was inoculated with a suspension diluted at 10^{-8}, Group 2 with a suspension diluted at 10^{-7} and Group 3 with a suspension diluted at 10^{-6}. Fourteen days later (*D*14) one animal from each group was killed and the two other animals of each group were re-inoculated with the same initial viral suspension diluted at 10^{-5}, 10^{-4}, 10^{-3}, respectively, for Groups 1, 2 and 3; i.e., the same remaining animals were inoculated a second time with a virus dose 1000 times higher than in the previous experimental infection 14 days earlier. At *D*28, another animal was killed in each group and the remaining rats were once again inoculated with the viral suspension diluted at 10^{-2} and 10^{-1}, respectively, for Groups 1 and 2. The Group 3 rat was infected with an undiluted suspension. At *D*40, these three remaining animals were killed (Table III).

Each inoculation was performed using a small rubber tube pushed into the bottom of the mouth, as far as possible behind the tongue of the rat, which was partially anesthetized with ether. Immediately, a swab of cotton around a small wooden stick was roughly rotated in the mouth to spread the inoculum on the mucosae.

At necropsy, brain, tonsils, salivary glands, lungs, spleen, kidney and feces were collected for viral isolation.

In the second unit, 10 rats (*Rattus norvegicus*) of the same origin as the previous batch, were inoculated under the conditions previously described with a highly virulent CSF strain which was only propagated in pigs from which virus-containing blood was collected after the rise of the thermic curve. The virus-containing blood was stored at $-70°C$ and constituted the stock virus used in this experiment. The titer of this strain was $10^{8.5}$ LD_{50} ml^{-1} for pigs. Each rat received, by the oral route, 0.5 ml of the 2-fold diluted viral suspension. Two animals were killed at Days 3, 13 and 26. Only one was killed at Day 31, as one rat died with metritis at the beginning of the experiment. At necropsy, oral swab, tonsils, lungs, liver, spleen, kidneys and feces were collected for virus isolation.

For the two experiments, blood samples could not be collected before inoculation as it was quite impossible to obtain a sufficient volume of blood without killing the wild animals, which were highly susceptible to stress and particularly to the conditions of etherization. However, as the two batches of 19 rats had the same origin, the blood samples of the group inoculated with CSF virus were used as controls for the group inoculated with ADV and conversely.

RESULTS

The field study

Eighty-five mice (*Mus musculus*) gathered in 34 different batches and 40 grey rats (*Rattus norvegicus*) were captured in the 15 herds.

Bacteriological investigations

Pasteurella pneumotropica was often isolated, particularly from *Rattus norvegicus*, being isolated from 75% of the captured rats. Afterwards, this agent was identified in 10 of the 34 batches of captured mice.

Bordetella bronchiseptica was isolated from 11 rats, but never from the mice and this bacteria was present only when one *Pasteurella* was identified.

Salmonella typhimurium was identified in two batches of captured mice from the same farm. One *Salmonella* of the C1 serotype was isolated from the feces of one rat.

Several serotypes of pig-pathogenic *E. coli* were identified in rats and mice.

TABLE I

Isolation frequency of bacteria

Agent	Number of carrier animals		Number of farms concerned		
	Rats	Mice batches	Rats	Mice	Total
Bordetella bronchiseptica	11/40[a]	0/34	5/15[b]	0/15	5/15
Pasteurella multocida	0/40	0/34	0/15	0/15	0/15
Pasteurella pneumotropica	30/40	10/34	6/15	5/15	8/15
Salmonella sp.	1/40	2/34	1/15	1/15	2/15
Pathogenic *E. coli*	9/40	6/34	2/15	4/15	5/15
Campylobacter jejuni	16/40	4/34	4/15	3/15	5/15
Treponema sp.	22/40	7/34	8/15	6/15	10/15

[a] = Number of animals with isolated bacteria/Number of tested animals.
[b] = Number of farms in which infected animals were found/Number of farms in which bacteria were looked for on rodents.

The isolated strains that are associated with neonatal diarrhea of piglets are: O8 K 87, O138 K 88, O141 K. 85, O10 KV 50, O119 KV 113; others are frequently associated with edema disease: O139 K 82, O45 KE 65. Serotype O10 K 50 was isolated from one rat used in the CSF experimental study. This animal was never in any contact with a pig and was apparently healthy.

Campylobacter jejuni was identified in 16 rats and in four batches of mice. This agent was frequently associated with *Treponema*, which was isolated from the gut contents of 22 rats and from five rat fecal samples collected in the farms. They were also isolated from the feces of a rat used in the CSF experiment, which was apparently healthy. Moreover, this agent was also identified in seven of the 34 batches of mice.

These *Treponema* did not have the same cultural and biochemical characteristics as *T. hyodysenteriae*, but were similar to those associated with recurrent diarrhea of fattening pigs (Jestin and Le Menec, 1984).

Table I shows the isolation frequency of the studied bacteria according to each bacterial genus and farm.

Serological and virological investigations

These results are presented in Table II.

Neutralizing antibodies against TGE were demonstrated on three different farms; in one herd, both captured mice and rats showed TGE antibodies in their sera. On two different farms, rotavirus was identified by ELISA in feces from rats or mice, but no antibody was found.

No antibody against other viral diseases could be detected in the sera of the captured rodents in the 15 farms.

TABLE II

Number and distribution of animals infected by pig viruses

Viral disease	Number of infected animals		Number of farms concerned		
	Rats	Mice batches	Rats	Mice	Total
Aujeszky's disease	0/40[a]	0/3	0/15[b]	0/15	0/15
Classical swine fever	0/40	0/3	0/15	0/15	0/15
Swine influenza	0/40	0/3	0/15	0/15	0/15
Porcine parvovirus	0/40	0/3	0/15	0/15	0/15
Rotavirus					
Serology	0/40	0/3	1/15	1/15	2/15
Virology	1/16	1/26			
Transmissible gastroenteritis	1/40	3/3	1/15	3/15	3/15
Foot and mouth disease	0/40	0/3	0/15	0/15	0/15
Swine vesicular disease	0/40	0/3	0/15	0/15	0/15

[a] = Number of animals with viral infection/Number of animals tested for viral infection.
[b] = Number of farms in which infected animals were found/Number of farms in which viral infection was looked for on rodents.

Experimental studies

Aujeszky's disease experimental infection

Table III shows the results of the Aujeszky's disease experimental infection.

No rat showed any clinical signs during the observation period and all the rats survived. All the sera of the CSF group rats were free of neutralizing ADV antibodies. Table III shows that neutralizing ADV antibodies were found in the serum of the rats inoculated with the lowest dose of virus in contrast to the other animals infected with higher titers of virus, except for Rat 3b. Nevertheless, no virus was isolated from the samples taken after killing the animals on Days 14, 28 and 40.

CSF experimental infection

Experimental oral infection of the rats did not induce any clinical reaction. CSF virus could not be isolated from all the samples collected from the killed animals, neither could CSF neutralizing antibodies be detected in the sera of the infected rats.

DISCUSSION

The efficacy of trapping was less satisfactory than was expected at the beginning of the study. Therefore, the number of captured rats and mice is relatively low. A total number of 10 rodents could be trapped in only six of the 15 farms.

TABLE III

Results of the ADV experimental infection

Group of rats	Number of inoculations	Dilutions of the viral suspension[a] for each inoculation at D[b]0-D14-D28	Antibody titer	Presence of virus
1 a	1	10^{-8}	4[c]	Neg.[e]
1 b	2	10^{-8}, 10^{-5}	0	Neg.
1 c	3	10^{-8}, 10^{-5}, 10^{-2}	±[d]	Neg.
2 a	1	10^{-7}	⩾8	Neg.
2 b	2	10^{-7}, 10^{-4}	0	Neg.
2 c	3	10^{-7}, 10^{-4}, 10^{-1}	0	Neg.
3 a	1	10^{-6}	⩾8	Neg.
3 b	2	10^{-6}, 10^{-3}	2	Neg.
3 c	3	10^{-6}, 10^{-3}, 1	0	Neg.

[a]Initial titer = $10^{5.3}$ $TCID_{50}$ ml^{-1}.
[b]D0 = Day 0 (day of inoculation).
[c]Reciprocal of the serum dilution neutralizing 100 $TCID_{50}$ 50 μl^{-1}.
[d]Doubtful result.
[e]Negative.

The traps were not always well adapted to the rodent species present in the farms. The tunnel-traps were relatively efficient in capturing the mice (*Mus musculus*), but the fykes or wire-cage traps were not so efficient for *Rattus norvegicus* as, on several farms, observation of signs of the presence of these rodents allowed a more or less accurate estimation of the rat population density which was not confirmed by the number of captured animals. Therefore, the rat population was certainly underestimated compared to the values obtained for mice. This point is important as it could be considered that the rats would be epidemiological vectors by their possible movements between the farms, which is not the case for the mice, which are sedentary.

The trapping was carried out during the summer time, which is favorable in relation to sanitary problems in the piggeries, but it is not optimal for finding the highest number of infectious agents that can be present in carriers.

Ultimately, as the manipulation of these wild animals was not very easy, it was not possible to obtain all the samples necessary for a good interpretation of the results. Indeed, in the experimental study, blood samples could not be obtained before the experimental infections; therefore, the results need to be compared with those collected from the other group of rats which had the same origin. Before the inoculation, the rats of the two groups had been raised together in close contact. Afterwards, when they were inoculated, they were slightly etherized and some animals did not ingest the whole volume of the viral suspension.

The results obtained show that the rodents on a farm can be considered as carriers of several bacteria involved in complex and multifactorial respiratory and digestive disorders of the pig. Indeed, in the farms, at least one of the following agents was isolated from the rodents: *E. coli*, *Campylobacter* sp., *Treponema* sp., *Salmonella* and rotavirus.

Since *Treponema* and pig-pathogenic *E. coli* (serotype O10 KV 50) were isolated from the gut of rats raised in isolated units without any contact with pigs and used for the CSF experimental study, these bacteria can be considered to belong to the normal digestive flora of the rodents. Nevertheless, it is difficult to assess whether the rotavirus identified in the feces of a mouse and a rat has a porcine origin; it has been demonstrated that rotaviruses have a common group antigen (Woode et al., 1976) and murine rotaviruses could have been detected by the ELISA with reagents made from porcine rotaviruses.

The isolation of *Bordetella bronchiseptica* in the lungs of $>25\%$ of the captured rats shows that the rodents can be considered as a reservoir of these bacteria and could be responsible for the infection of the pigs. On the other hand, the absence of *P. multocida* and the high frequency of isolation of *P. pneumotropica* confirms the concept of a high specificity of the *Pasteurella* (Mraz et al., 1980).

Neutralizing TGE antibodies were detected in the serum of one rat and three batches of five mice each. These rodents came from three farms in which, in the first one, there had been a recent TGE infection. The animals of the second farm were not affected by TGE, but the disease appeared in the neighbouring farms some weeks before. In the third, no TGE had been observed previously. It can be supposed that the antibodies detected could be induced by murine coronaviruses, but the hypothesis that the rodents could be a reservoir of TGE virus cannot be rejected and additional studies are necessary.

No infection of the rodents by ADV could be proved by the field investigations. On the other hand, experimental studies showed that *Rattus norvegicus* could survive the infection, even with probable viral multiplication. These results had only been reported by Nikitin (1959). Several comments have to be made about that assay. No virus could be detected from the different samples collected from the killed animals, but the collection was done late after the inoculation (minimum 14 days after). The highest neutralizing ADV antibody titers were obtained in the serum from rats inoculated with the lowest viral dose. It is difficult to explain such results, particularly because the antibodies appeared in the serum of animals inoculated with dilutions below the threshold of viral titer detectable by the titration technique. It is possible that very few viral particles were inoculated into rats which were not detected by the titration technique because of a lack of sensitivity of the cells. It is necessary to emphasize that these dilutions of the inoculum had been voluntarily chosen a priori on account of the well known extreme sensitivity of the rats to ADV. Nevertheless, it remains difficult to explain the absence of antibodies in

the serum of rats inoculated several times with increasing doses. These results could also be due to individual variations in susceptibility of rats to the viral infection as well as to variations in the experimental dose of virus as some animals regurgitated part of the inoculum. It seems unlikely that ADV antibodies, detected in the sera of these rats, could be due to non-specific reaction or to murine herpes viruses cross-reacting with ADV. Indeed, the antibody titers are relatively high and no ADV neutralizing antibody was detected in the CSF group and in the field investigation. The ADV strain used in the experimental studies certainly plays a role in the results obtained. The strain, used in this study, for the experimental infection is virulent for the pig, but has been multiplied numerous times on cell cultures, which decreases its virulence. This point could be important, as it was shown that rats could not survive inoculation with whatever virulent strain was used (Aldasy and Mate, 1969) and could explain the contradictory results reported in the literature. Nevertheless, these results suggest that the pig could not be the only carrier of ADV and further studies should be conducted to clarify this observation.

In the second experimental study with swine fever, no positive result was obtained and the specificity of the CSF virus is probably too high for it to be adapted to other mammalian species.

Results of this study confirm those from other investigations which showed that rodents in farms are really undesirable. Rodents have to be eliminated by active measures to prevent infection of pigs by disease agents present in the murine population.

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