EXPERIMENTAL REPRODUCTION OF ATROPHIC RHINITIS WITH PASTEURELLA MULTOCIDA ON GNOTOBIOTIC AND CONVENTIONAL PIGLETS*

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Atrophic rhinitis (AR) is generally considered as an infectious disease with as major causative agents are B.bronchiseptica (Bb) and P.multocida (Pm).However,if the role of Bb has been demonstrated since a long time, we have emphasized that the role of Pm is less evident (4).Recently,de Jong et al.(1) have showed that only heat-labile toxine (HLT) producing strains of Pm were able to cause turbinate atrophy in SPF piglets.In our knowledge,some data are available relative on the experimental reproduction of AR with Pm on SPF piglets (1,2,9,10),but non on gnotobiotic piglets.In this paper,we report results of experiments with Pm alone or associated with Bb on gnotobiotic and conventional piglets.

MATERIAL and METHODS.

A-Animals:24 germfree colostrum-deprived piglets (5) and 32 conventional piglets naturally reared under 3 gilts purchased from one herd free from clinical AR.Before the trial,3 nasal swabs were taken from the gilts and they receive antibiotic during the week before farrowing.
The piglets were killed between 27 and 42 days post-inoculation.

B-Strains:
1-a toxine producing strain of Pm Carter's type D (TPmD) (CDI 40456),
2-a dubious toxine producing strain of Pm Carter's type A (T?PmA) (CDI 04041),only tested on conventional piglets,
3-a AR pathogenic strain of Bb (73B2) previously used (3).

C-Experimental groups.
Inoculation were performed at days 5,6 and 7 by a gently instillation with a syringue.
1-On gnotobiotic piglets (only the TPmD was tested):
Group A:0.5ml/nostril of a saline suspension of Pm at different concentrations (9.10^2 to 9.10^{11} CFU/ml). After growth on a solid medium,Pm was harvested and then suspended in saline.
Group B:0.5ml/nostril of a BHI broth culture,24h at 37°C,countered approx.10^{12} CFU/ml.
Group C:0.5ml/nostril of a cell-free broth-filtrate. Heat-labile toxine concentration was estimated by a dilution test in mice (DL50).
Group D:0.5ml/nostril of a Pm suspension (9.10^{11} CFU/ml) and 0.5ml/nostril of a Bb suspension (3.10^4CFU/ml).
Group E:0.5ml/nostril of a Pm broth (approx.10^{12} CFU/ml) and a Bb suspension (3.10^4CFU/ml).
Group F:control germfree piglets instillated with 0.5ml/nostril of sterile saline.
2-On conventional piglets:
Group G:0.5ml/nostril of a saline T?PmA suspension (9.10^{11}CFU/ml).
Group H:0.5ml/nostril of a saline TPmD suspension (9.10^{11}CFU/ml).

D-Examinations:
For each snout,the gradation of the lesions were performed on 6 cross-sections between the incisor tooth and the 3rd premolar tooth (5).Further histological examination were performed on each section.Clinical,bacteriological and serological examinations were also performed.

RESULTS

A-On Gnotobiotic piglets:
1-with a saline Pm suspension (9.10^5 to 9.10^{11}CFU/ml) (group A),no macroscopic or microscopic lesions were noted.Pm was recovered in 10/11 piglets from the tonsils but only in 1 case (1/11) from the nasal cavities.
2-with a Pm broth culture (group B),important turbinate atrophy are seen.Histologically,we did not observe cartilagineous metaplasia in spite of the extensive macroscopic lesions.Pm was recovered from the nasal cavities and tonsils.
3-with the cell-free broth-filtrate (group C),the lesions were also very important but predominant in the rostral part of the nasal ventral turbinate (NVT),before the first promolar tooth. Microbiological germfree status was confirmed.
4-with Pm and Bb suspension (group D),there were no macroscopic or microscopic lesions.Pm was only recovered from the tonsils and Bb was not detected.
5-with Pm broth culture and Pm suspension (Group E), the lesions were important and more extensive in the rostral part of the NVT.Pm and Bb were recovered from the nasal cavities.

B-On conventional piglets:
1-with a dubious toxin producing strain Pm (T?PmA) inoculated in saline suspension (9.10^{11} CFU/ml), only one piglet (1/10) showed a turbinate atrophy and also in the rostral part of the NVT.Pm was not recovered from the nasal cavities and,in 7 cases from the tonsils (7/10).
2-with the toxin producing strain (TPmD) inoculated in saline suspension (9.10^{11}CFU/ml),11 piglets (11/22) showed turbinate atrophy from which 8 have gross lesions only in the rostral part of the NVT.Pm was recovered from the nasal cavities in 8/22 piglets.

CONCLUSIONS:
1-Pm alone is able to cause severe turbinate atrophy on gnotobiotic piglets when a broth medium only was used as inoculum,even though experimental AR is easy with a Bb suspension (3).
2-The cell-free broth-filtrate of the TPmD instillated 3 consecutive days is also able to cause severe turbinate atrophy on gremfree piglets.This observation is different from those reported with Bb from which the cell-free extract needs to be instillated during several weeks to induce conchae atrophy(8).
3-In conventional piglets,bacteria,mycoplasms or viruses can help the colonization by Pm as previously suggested(10).Indeed,we think that nasal mucus may be important for the first step of colonization.
4-The classical examination at the level of the first premolar tooth to score the gross lesions is often normal whereas rostral cross-sections of the nose showed very important turbinate atrophy.Consequently two cross sections must be recommended for the field diagnosis.
5-Our results,in particular those with the cell-free broth-filtrate should be in relation with the more prominant osseous turn-over of the NVT during the two first weeks of life (6,7).

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