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RESPIRATORY PASTEURELLOSIS: INCIDENCE IN YOUNG RABBITS AND MECHANISMS OF TRANSMISSION

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Pasteurellosis is the number one disease problem associated with production and housing of rabbits all over the world. The organism, Pasteurella multocida, seems to be ubiquitous in commercial and research colonies. This debilitating disease has brought to an unsatisfactory demise numerous research projects utilizing rabbits and forced scores of rabbit producers into closing their rabbitries.

The transmission of P. multocida has been theorized to be primarily airborne and direct contact. These theories have never adequately explained the checkerboard-type of incidence observed in most rabbit colonies. Therefore, several studies have been conducted to try and elucidate alternative methods of transmission and the incidence of P. multocida in young rabbits.

INTRODUCTION

Experiment 1: The occurrence of P. multocida in newborn and young (weanling) rabbits.

A review of the scientific literature reveals a relative absence of recent information concerning the prevalence of P. multocida in newborn and young (4-10 weeks of age) rabbits. P. multocida was isolated from rabbits 11-17 days of age in 1958, but information on the does' nasal status was not given (7). In 1977 a four per cent incidence of otitis media caused by P. multocida was reported in 8-10 week old rabbits (6). A very recent report showed that regardless of the doe's nasal status (positive or negative for P. multocida), the nares of young rabbits produced by these does were not positive for P. multocida until after 12 weeks of age (4).

Young rabbits (4-10 weeks of age) had been observed to have clinical cases of rhinitis (snuffles) in our rabbitry. When it was detected in one rabbit of a litter, it was often observed in littermates, but not rabbits of another litter caged along side. In tracing back the parentage of these "snuffles" litters, it seemed that the doe was often found to have a clinical case of rhinitis. Consequently a study was started to examine the offspring of does that were nasal positive for P. multocida.

Experiment 2: The incidence and implications of pasteurella contaminated watering nipples.

Automatic watering systems are recommended for the progressive rabbit raiser with more than 6 does. There are several designs available, all of which are equally functional and result in monetary advantages, e.g. decreased labor costs and

increased weight gains (3). In general all automatic watering systems utilize a watering nipple or fount of some kind. These watering valves have the potential to be involved in the vertical transmission of P. multocida from the dam to her offspring.

A study was designed to examine the incidence of P. multocida contamination of the drinking nipples and its relationship to the involved rabbits.

MATERIALS AND METHODS:

Experiment 1: Newborn Group: All the does in this group were diagnosed as having clinical rhinitis which was positive for P. multocida, 1-2 weeks prior to kindling. Additional nasal swab cultures were obtained from these does on the same day that litters were cultured. Litters were cultured at 1-4 days of age. One litter, 5 days of age, was found dead in the nest box and was subsequently necropsied. Cultures of the lungs and liver were taken during the postmortem examination. Two of the three working does, from which P. multocida was recovered from their litters, were also necropsied.

Weanling Group: All working does in this group were clinically "normal" rabbits with dry external nares, but had culture positive (P. multocida) nasal passages. One litter was sacrificed and necropsied at 21 days of age. The doe was cultured on the day the litter was sacrificed. The remaining four litters of this group were weaned at 27-32 days of age. Does were again nasally cultured on the same day that weaning occurred. Litters were necropsied at intervals as indicated in Table 4.

Rabbits in both groups were housed in a conventional rabbitry with natural ventilation and natural lighting. All rabbits in this study were New Zealand White, Orrc:(NZW). They were housed in quonset-style wire mesh cages measuring 75cm x 75cm x 45cm. The weaned litters were moved to a laboratory animal facility with stainless steel cages measuring 60cm x 60cm x 35cm. Ventilation in this room was 20 air changes/hour with 12:12 lighting. The litters were maintained for a minimum of 12 days in the laboratory animal facility, so that mechanical transmission from their dams could be evaluated. Food and water were ad-libitum in both facilities.

Cultures were taken by inserting sterile cotton tipped swabs 2-3cm into the right or left external nares of does or into the oropharyngeal area of the newborn rabbits. Young rabbits (5-11 weeks of age) were cultured at necropsy. All culture swabs were streaked onto sheep blood agar plates and incubated at 37°C for 24 hours. P. multocida was identified by standard microbiological methods (1).

Experiment 2: Nasal cultures from 142 working New Zealand White does, Orrc:(NZW), (6 mo. - 2 yrs. of age) were obtained by inserting a small calcium alginate swab approximated 2-3cm into both external nares. Watering nipple cultures were obtained by using conventional sterile cotton tipped applicators and carefully sampling the outside surface of the valve. Three different styles of watering nipples were sampled during this study. All rabbits in this study were housed in a conventional rabbitry with quonset-tyle wire mesh cages measuring 75cm x 75cm x 45cm. The rabbitry had forced air ventilation (4-10 air changes

per hour) and 16:8 lighting.

Swabs were streaked onto sheepblood agar plates and incubated at 37°C for 24 hours. P. multocida was identified by standard microbiological methods (1).

RESULTS

Experiment 1: Three of five newborn litters were found to be P. multocida positive (Table 1.). Two litters contained oro-pharyngeal positive rabbits and the third litter was lung and liver positive. Thirty-five newborn rabbits (1-5 days of age) were cultured and 15 were P. multocida positive (42.8%). All five does in this group were nasal positive and four of five had positive drinking valves in their cages (Table 1.).

Two of the P. multocida working does of the newborn culture group were sacrificed and necropsied to allow microbiological examination of a number of organs including the vagina. The results of the cultures are shown in Table 2.

In the weanling group, three of five litters were positive for P. multocida (60%). It should be noted that one litter was sacrificed at 21 days of age while the other rabbits were killed between 39 and 74 days of age. The culture data from these necropsies are found in Tables 3 and 4. P. multocida was found in 11 of 24 rabbits for a 45.8% incidence. Table 5 shows the incidence of P. multocida recovered from various sites in the weanling rabbits.

Experiment 2: The incidence of nasal P. multocida in the 142 rabbits sampled in a commercial rabbitry was 51.4% (Table 6). Table 6 also shows the incidence of P. multocida contamination of drinking valve within the cages of these 142 rabbits (36.6%). While the incidence in watering nipple contamination is some 15% less than the nasal incidence, it is very revealing to look at watering nipple contamination in relationship to the incidence of nasal detection of P. multocida and then to further divide the pasteurella positive rabbits into categories depending on severity of the resultant clinical condition.

Table 7 shows the division of rabbits into nasal positive and nasal negative with their corresponding watering nipple results. Of the 73 nasal positive rabbits, 67.1% had positive P. multocida drinking valves, while only 4.3% of the 69 nasal negative rabbits had contaminated watering valves.

Table 8 compares three groups of rabbits based upon gross nasal appearances or snuffles severity. As demonstrated in this table, the recovery of P. multocida from a watering nipple is related to the apparent nasal condition of the rabbit, e.g. 90% of those rabbits exhibiting an active nasal discharge had water nipples contaminated with P. multocida, whereas only 54.1% of "clinically normal" (but pasteurella positive) rabbits with dry external nares had positive water nipples.

DISCUSSION

The results of Experiment 1 suggest that P. multocida transmission can occur very early in the life of a rabbit. Furthermore, it also suggests that contamination of the newborn may occur during the kindling process and that nasal positive does with an active nasal discharge may be the most infectious. Whether this contamination of the newborn rabbits establishes a colonizing level of P. multocida could not be determined from this study, but it is interesting that

the incidence of P. multocida in the weanling group and the newborn group is similar.

The purpose of necropsing two of the P. multocida positive does from the "newborn group" was to determine the vaginal status relative to P. multocida. A previous report suggested that verticle transmission via a pasteurella contaminated vagina may occur at kindling (8). This source of possible transmission was dismissed in these two cases since both rabbits were uterine and vaginal negative (see Table 2).

Experiment 2 showed an incidence of 67% contaminated drinking valves when does were found to be nasal positive for P. multocida. The 80% incidence of contaminated drinking valves of dams of the newborn group in Experiment 1, correlates nicely with that information. Therefore, another source for infecting young weanling rabbits could be the contaminated drinking valve. As young rabbits begin using the drinking valves at about 21-28 days of age, one could not rule out the possibility that positive rabbits in the weanling group were infected by the drinking valve and not the dam.

To show that it was, indeed, a colonization and not just mechanical contamination, the weanlings were removed from the doe and moved to a laboratory animal facility. A determination of their P. multocida status at weaning would have been helpful, but this determination was not made as the external nares at this age are extremely small. The fact that 47.4% (9/19) of these transferred rabbits were P. multocida positive after a minimum of 12 days at the new facility strongly suggests that they were colonized prior to removal. The possibility that only a few rabbits were colonized at weaning and by direct contact passed it to other members of the litter can not be ruled out as each litter was kept intact in a separate cage.

Table 3 shows the culture data of the 3 week-old litter that was sacrificed prior to weaning. Two of the five rabbits were pasteurella positive, but it should be noted that the P. multocida isolated from the nasal swabs were in extremely low numbers, e.g. only one or two colonies were observed on the entire plate. Whereas, the sinus and trachea cultures had numerous P. multocida colonies. These nasal positive cultures might have been overlooked, if strict attention had not been given to each and every colony on the plate, resulting in these rabbits being classified as nasal negative. In addition, it points out the possibility that the oro-pharyngeal area may become contaminated or colonized prior to the nasal passages of rabbits.

The necropsy data from the weanling rabbits that were sacrificed showed the pharyngeal area and the middle ears to have the highest incidence of P. multocida (62% and 50% respectively). This is in contrast to nasal cultures which detected only 37.5% (Table 5). This was quite unexpected as numerous investigators have reported the nasal passages as being the only site of consistent pathological findings and have suggested that P. multocida lives primarily in the nose (2, 4, 10, 11, 14). Furthermore, the deep nasal swab technique, as described by Webster is the generally accepted method to determine the P. multocida status of a given rabbitry (12, 13, 15, 16). The data presented in this paper suggests that the ability of the nasal swab technique to determine the incidence of P. multocida by itself is questionable and that a more appropriate use would be in conjunction with other methods of detecting the presence of P. multocida.

The results of Experiment 2 show very vividly that drinking nipples can become contaminated with P. multocida and that the incidence of this contamination seems to be related to the snuffles condition of the cage occupant. A plausable explanation for this situation is that the doe contaminates the valve while drinking and that does with an active nasal discharge contaminate the valve more often than those that are positive but have no discharge.

The observation of a few contaminated drinking nipples from nasal negative rabbits is very intriguing (Table 2). The most obvious explanation of this seemingly contradictory fact is that the rabbits were, in fact, nasal positive, but the bacteria were too low in numbers to be recovered from the nares with the culturing method used. This position can be supported by the recommendation of Webster, that three negative nasal cultures be obtained before a rabbit can be classified as truly nasal negative (14). In this study only one nasal culture was taken, so positive rabbits could have been missed. Conversely, the rabbits could have been truly nasal negative. Some recent work in this laboratory shows that of 70 necropsied rabbits in which P. multocida was recovered, 98% were nasopharyngeal positive, but only 72% were nasal positive. Moreover, all the rabbits that were nasal positive were also nasopharyngeal positive, but not all of the nasopharyngeal positive rabbits were nasal positive (9).

Additional research in this laboratory has assisted in the development of a serological test which detected 12 nasal negative adult rabbits as having high P. multocida titers. These rabbits had been determined to be nasal negative by obtaining a minimum of three nasal cultures from both nares usingcalcium alginate swabs. Eight of these 12 rabbits were necropsied and the presence of P. multocida in the nasopharyngeal area was confirmed by culture. The remaining four rabbits were sedated and oral cultures taken. Three of the four rabbits were positive for P. multocida (unpublished data). Therefore, it is entirely possible that the three rabbits with positive drinking nipples were truly nasal negative, but perhaps nasopharyngeal positive. Unfortunately, these rabbits are no longer available to test.

A recent publication presented data which suggested that P. multocida proliferates initially on the nasopharyngeal mucosa due to selective adhesins which facilitate the attachment of the P. multocida to squamous epithelial cells of the pharyngeal area (5). This initial colonization of the pharyngeal area may be an important aspect of the pathogenesis of rabbit pasteurellosis. If one considers the possible transmission of P. multocida from contaminated drinking valves to the offspring of a doe, it may be that daily assult of the oropharyngeal area with P. multocida bacteria during drinking periods soon leads to colonization of that area. The number of organisms needed may not be great especially if there is an affinity for these epithelial cells as stated by Glorioso et. al. If this is a mechanism by which young rabbits can be contaminated with P. multocida, it would certainly explain how a rabbit can be nasopharyngeal positive and yet nasal negative.

Contamination of the watering nipples seemed to be independent of the style or type of valve involved. All types were about equally contaminated. It has recently been determined by this laboratory that the P. multocida is viable on the watering nipples for no longer than 36 hours regardless of the environmental conditions or the style of water nipple (unpublished data). As a routine practice, many rabbit managers fill an empty cage with a new rabbit or rabbits on the same day it is emptied to maximize profits. This procedure may lead to infection of the new rabbits, if the watering device is still contaminated. A better procedure would be the disinfection of the drinking nipple and ancillary equipment or to let the cage stand idle for 48 hours.

Table 1. Newborn group: culture information for the working does and their litters.

Doe Information				Newborn Information		
No.	Nasal Condition	Nasal Culture	Water valve Culture	Age(days) Cultured	No. pos./ No. Cultured	Area Cultured
7004	dry	+[1]	+	< 1	4/5	throat
3775	dry	+	-	1	0/5	throat
3702	dry	++	+	4	0/10	throat
5533	discharge	+++	++	4	4/8	throat
3367	discharge	+++	++	5	7/7*	lung/liver

*Entire litter died at 5 days of age, necropsy results revealed lung and liver cultures to be P. multocida (+).

[1]Relative numbers of P. multocida recovered on culture:

+ = scant (1-5 colonies)
++ = moderate (6 - 20 colonies)
+++ = heavy (21 - TNTC-too numerous to count)

Table 2. Necropsy culture results of 2 does which had *P. multocida* (+) litters.

Doe No.	Nasal	Oral-Trachea	Lung	Liver	Vagina	Uterus	Ovary
7004	+[1]	++	-	-	-	-	not cultured
3867	+++	++	-	-	-	-	-

[1]Relative numbers of *P. multocida* recovered on culture:

+ = scant (1 - 5 colonies)
++ = moderate (6 - 20 colonies)
+++ = heavy (21 - TNTC -(too numerous to count)

Table 3. Culture results of 3 week-old litter from nasal positive doe prior to weaning.

Litter No.	Age(days) Necropsied	Nasal	Oral Pharynx & Trachea	Lung	Sinus	Middle Ear Rt.	Middle Ear Lf.
7015-1	21	+	+	-	-	-	-
2	21	-	-	-	-	-	-
3	21	-	-	-	-	-	-
4	21	-	-	-	-	-	-
5	21	+	-	-	+	-	-

Table 4. Culture results of weaned litters from nasal positive does

Litter No.	Age(days) Weaned	Age(days) Cultured Necropsied	Nasal	Oral Pharynx & Trachea	Lung	Sinus	Naso-Pharynx	Middle Ear Rt.	Middle Ear Lf.
47 -1	32	44	-	+	-	-	+	-	-
2	32	44	-	+	-	+	+	-	-
3	32	44	-	-	-	-	-	-	-
4	32	79	-	-	-	-	+	+	+
5	32	79	-	-	-	-	-	+	-
9310- 1	27	39	+	+	-	+	+	+	+
2	27	39	+*						
3	27	48	-	-	-	-	-	-	+
4	27	54	+*						
5	27	54	+*						
43 - 1	27	74	-	-	-	-	-	-	-
2	27	74	-	-	-	-	-	-	-
3	27	74	-	-	-	-	-	-	-
4	27	74	-	-	-	-	-	-	-
7549 -1	27	39	-	-	-	-	-	-	-
2	27	39	-	-	-	-	-	-	-
3	27	74	-	-	-	-	-	-	-
4	27	74	-	-	-	-	-	-	-
5	27	74	-	-	-	-	-	-	-

* These rabbits were saved for serological tests

Table 5. Recovery incidence of P. multocida from eight necropsied pasteurella positive weanling group rabbits.

Area Cultured	No. Positive	Incidence
Nasal	3	37.5%
Sinus	3	37.5%
Middle Ear	4	50.0%
Pharynx (Oral & Nasal)	5	62.5%

Table 6. Prevalence of Pasteurella multocida in the nares of rabbits and on watering nipples within their cage in a commercial rabbitry

Total No. Cultured	No. P. multocida Positive	Incidence
Rabbits 142	73	51.4%
Water Valves142	52	36.6%

Table 7. Incidence of Pasteurella multocida positive watering nipples in relationship to nasal occurance.

No. of rabbits	No. water nipple positive	Incidence
Nasal positive	49	67.1%
Nasal negative	3	4.3%

Table 8. A comparison of three categories of rabbits that are nasal positive for Pasteurella multocida

Nasal condition	No. nasal positive	No. water valve positive	Incidence
Severe Rhinitis[1]	20	18	90.0%
Mild Rhinitis[2]	16	11	68.7%
"Clinically normal"[3]	37	20	54.1%

[1]Nasal discharge [2] moist nares [3] dry nares

REFERENCES

1. Carter G.R., Diagnostic Procedures in Veterinary Microbiology. Springfield: Charles C. Thomas Co. 1973:61-68.

2. Cowie-Whitney J.: Diseases of the Commercial Rabbit. Vet. Res. 1977; 101:299-303.

3. Cheeke P.R., Patton N.M., Templeton G.S.: Rabbit Production, 5th ed. Interstate Printers & Publishers, Danville, Illinois, 1982:47-49.

4. DiGiacomo R.F., Garlinghouse Jr., L.E., Van Hoosier G.L.: Natural History of Infection with Pasteurella multocida in Rabbits. J. Amer. Vet. Med. Assoc. 1983; 183:1172-1175.

5. Glorioso J.C., Jones G.W., Rush H.G., Pentler L.J., Darif C.A., Coward J.E.: Adhesion of Type A Pasteurella multocida to rabbit pharyngeal cells and its possible role in rabbit respiratory tract infections. Infect. Immun. 1982; 35:1103-1109.

6. Flatt R.E., DeYoung D.W., Hogle R.M. : Suppurative otitis media in the rabbit: prevalence, pathology, and microbiology. Lab. Anim. Sci. 1977; 27:343-347.

7. Hagen K.W.: Enzootic pasteurellosis in domestic rabbits. I. Pathology and bacteriology. J. Amer. Vet. Med. Assoc. 1958; 133:77-80.

8. Holmes H.T., Patton N.M., Cheeke P.R. : The incidence of vaginal and nasal Pasteurella multocida in a commercial rabbitry. J. Appl. Rabbit Res. 1983; 6:95-96.

9. Holmes H.T., Patton N.M., Cheeke P.R. : The anatomical distribution and incidence of Pasteurella multocida in rabbits. J. Appl. Rabbit Res. (in press).

10. Hoskins H. P.: Snuffles of rabbits; its etiology and treatment. J. Amer. Vet. Med. Assoc. 1920; 57:317-321.

11. Lelkes L.B. : New insight into that common rabbit disease, Pasteurella multocida. 1982; Rabbits 5:7-8.

12. Lu Y. S., Ringler D., Park J. : Characterization of Pasteurella multocida isolates from the nares of healthy rabbits and rabbits with pneumonia. Lab. Anim. Sci. 1978; 28:691-697.

13. Watson W. T., Goldsboro J. A., Williams F. P., Sueur R.: Experimental respiratory infection with Pasteurella multocida and Bordetella bronchiseptica in rabbits. Lab Anim. Sci. 1975; 25:459-464.

14. Webster L. T.: The epidemiology of a rabbit respiratory infection. II. Clinical,pathological, and bacteriological study of snuffles. J. Exp. Med. 1924; 39:843-856.

15. Webster L. T.: The epidemiology of a rabbit respiratory infection. III. Nasal flora of laboratory rabbits. J. Exp. Med. 1924; 39:857-877.

16. Weisbroth S. H. and Scher S.: The establishment of a specific-pathogen-free rabbit breeding colony. II. Monitoring for disease and health statistics. Lab. Anim. Sc. 1969; 19:795-799.

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SUMMARY

Experiment 1: Cultures from five litters of rabbits consisting of 35 offspring (1 - 5 days of age) revealed Pasteurella multocida in three of the litters with a total incidence of 42.8%. In another group of five litters consisting of 24 rabbits (3 - 11 weeks of age) P. multocida was isolated from three of the litters with an incidence of 45.8%. Both groups were progeny from P. multocida nasal positive does.

Experiment 2: Watering valve and nasal cultures were obtained from 142 working does in a commercial rabbitry. The incidence of nasal Pasteurella multocida was 51.4%, whereas the incidence of pasteurella recovered from the watering nipples was 36.6%. However, 67.1% of the 73 nasal positive rabbits had P. multocida positive watering valves. Rabbits exhibiting an acute rhinitis with a nasal discharge had an incidence of 90% positive watering nipples.

RESUMEN

En cultivos hechos a cinco camadas con un total de 35 gazapos (1-5 días de edad), tres camadas resultaron positivas a Pasteurella multocida, siendo el total de incidencia del 42.8%. En otro grupo de cinco camadas con un total de 24 gazapos (3-11 semanas de edad), se aisló P. multocida en tres de las camadas, una incidencia del 45.8%. Ambos grupos eran de hembras positivas a P. multocida nasal.

Cultivos de las válvulas de los bebederos, y cultivos nasales se obtuvieron de 142 hembras en producción en un establecimiento comercial. La incidencia de Pasteurella multocida nasal fué de 51.4%, en tanto que la incidencia de pasteurella recuperada de las válvulas de los bebederos fué de 36.6%. Sin embargo, 67.1% de las 73 conejas positivas nasales tenían bebederos con válvulas positivas a P. multocida. Los conejos que exhibían rinitis aguda con descarga nasal, tuvieron una incidencia del 90% de bebederos con válvulas positivas a P. multocida.