

***Neisseria cuniculi* in ruminants: epidemiological aspects**

D. ELAD, S. SHLOMOVITZ, M. BERNSTEIN AND J. BASSAN

*Department of Bacteriology, The Kimron Veterinary Institute, P.O. Box 12,
50250 Beit-Dagan, Israel*

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SUMMARY

Neisseria cuniculi was isolated, between March 1987 and March 1989, from 38 cases of respiratory disease in small and large ruminants. In all but five cases *N. cuniculi* was cultured together with other potential respiratory pathogens. A survey was conducted to assess the prevalence of *N. cuniculi* in the pharyngeal region of Merino and Awassi purebred sheep, Awassi/East-Friesian and Merino/Romanov crossbred sheep and one exotic cross breed (goat/ibex).

N. cuniculi was isolated from 80–88% of the animals under 1 month of age. Among older animals, the microorganism was isolated from 20·5% of the pure bred animals and 79·3% of the crossbred ones. This difference was significant ($P < 0\cdot001$) by the χ^2 test. The prevalence of *N. cuniculi* in the second age group coincides with the susceptibility of the breeds to respiratory pathology.

This, we believe, is the first report of *N. cuniculi* involved in multiple cases of respiratory pathology and of a survey assessing the prevalence of this microorganism in small ruminants.

INTRODUCTION

Bacteria of the genus *Neisseria* are Gram-negative aerobic cocci (except for one species: *N. elongata*), which usually occur in pairs with flattened adjacent sides.

N. cuniculi, the taxonomy of which is still uncertain, has been classified as a 'false neisseria' [1]. It has been isolated from healthy rabbits [2] and fur seals [3]. Our first isolation of *N. cuniculi* was from a pneumonic lamb in March 1987 [4]. During the following 2 years this microorganism was isolated from cases of pneumonia in small and large ruminants. The prevalence of *N. cuniculi* in pneumonic sheep during this period was second only to the *Pasteurella* species, roughly equalling that of another pathogen, *Actinomyces* (*Corynebacterium*) *pyogenes* (Table 1). These findings promoted us to conduct a survey to assess the prevalence of *N. cuniculi* in the upper respiratory tract of small ruminants, the results of which are presented here.

MATERIALS AND METHODS

Post-mortem examination of organs was performed as previously described [4].

Kibbutz-type farms, with similar intensive management systems and a high standard of husbandry, but rearing different breeds of sheep were chosen for the survey. Purebred Awassi and Merino sheep, crossbred Assaf (Awassi/East-

Table 1. *Pathogenic and potentially pathogenic bacteria isolated from pneumonic sheep between March 1987 and March 1989*

Microorganism	No. of isolations
<i>P. haemolytica</i>	407
<i>P. multocida</i>	173
<i>N. cuniculi</i>	29
<i>A. pyogenes</i>	28
<i>Corynebacterium pseudotuberculosis</i>	6
Other <i>Corynebacteria</i>	5

Friesian) and Merino/Romanov sheep and a cross between goat and ibex (*Capra ibex nubiana*) named Yaez were sampled. As, on the type of farm surveyed, Merino sheep are kept for cross breeding only, no young livestock of this breed was available for sampling. Healthy animals of all ages were swabbed. All the specimens were taken between September and December 1988.

Preliminary examinations indicated that although *N. cuniculi* may be isolated from the external nares, it is more common in the pharynx of small ruminants. This region was, therefore, chosen to assess the prevalence of the microorganism in various breeds of the latter. Cotton-tipped swabs were used to take samples. The swabs were rinsed in sterile saline, which was inoculated onto 5% sheep blood agar, nutrient agar and McConkey agar, all of which were then incubated overnight at 37 °C.

From each Petri plate with suspect *N. cuniculi* colonies, three were reisolated and identified by microscopical morphology and biochemical characteristics as previously described [4].

Identification and biotyping of *P. haemolytica* and identification of *P. multocida* and *A. pyogenes*, were made by standard methods [5]. *Moraxella ovis* was identified as previously described [6].

The χ^2 test was used to analyze the different prevalence of *N. cuniculi* in purebred and crossbred sheep aged 1 month or older.

RESULTS

N. cuniculi was isolated from pneumonic lungs of sheep, calves, Yaez crosses and Camerooni sheep (*Ovis aries camerooni*), the latter held in a zoo. Nasal swabs from Camerooni sheep suffering from rhinitis also yielded the microorganism. *N. cuniculi* was isolated from more than one pneumonic animal from only two farms: two Yaez kids from one farm and seven lambs sent to our institute from a farm that suffered high mortality losses during the autumn and the winter of 1988. In all the other cases *N. cuniculi* was isolated from one pneumonic animal only, even if several cases from the same farm were submitted to our laboratory for bacteriological examination. Isolation of *N. cuniculi* showed no seasonal variation. The pathological and bacteriological findings in the specimens from which *N. cuniculi* was isolated are summarized in Table 2. The bacteria listed in Table 2 were the only ones isolated from the pneumonic lungs; other bacteria, possibly saprophytes, were absent. The two Assaf sheep from which *N. cuniculi*, *P. haemolytica*, *P. multocida* and *A. pyogenes* were isolated were 2 years old and suffered from pulmonary adenomatosis (pathological and histopathological

Table 2. *Bacteriological and pathological findings in animals from which N. cuniculi was isolated*

Species	Breed	Number	Organ	Pathology	Other pathogen
Sheep	Assaf	2	Lung	Pneumonia	None
Sheep	Assaf	12	Lung	Pneumonia	<i>P. haemolytica</i>
Sheep	Assaf	4	Lung	Pneumonia	<i>P. multocida</i>
Sheep	Assaf	4	Lung	Pneumonia	<i>P. haemolytica</i> <i>P. multocida</i>
Sheep	Assaf	2	Lung	Pneumonia	<i>P. multocida</i> <i>P. haemolytica</i> <i>A. pyogenes</i>
Sheep	Merino	1	Lung	Pneumonia	None
Sheep	Merino	1	Lung	Pneumonia	<i>P. haemolytica</i> <i>P. multocida</i>
Sheep	Camerooni	1	Nostril	Rhinitis	<i>P. haemolytica</i> <i>P. multocida</i>
Sheep	Camerooni	2	Nostril	Rhinitis	<i>P. multocida</i> <i>M. ovis</i>
Sheep	Camerooni	1	Nostril	Rhinitis	<i>P. haemolytica</i> <i>M. ovis</i>
Sheep	Camerooni	1	Lung	Pneumonia	<i>P. haemolytica</i>
Cattle	Israeli Holstein	2	Lung	Pneumonia	None
Cattle	Israeli Holstein	1	Lung	Pneumonia	<i>P. haemolytica</i> <i>A. pyogenes</i>
Cattle	Israeli Holstein	2	Lung	Pneumonia	<i>P. multocida</i>
Yaez		1	Lung	Pneumonia	<i>P. multocida</i>
Yaez		1	Lung	Pneumonia	<i>P. haemolytica</i>

Table 3. *Number and age of animals from which N. cuniculi was isolated in relation to a pathological process*

Species	Number	Age
Sheep	5	2-4 years
Sheep	6	4-6 months
Sheep	14	2-4 months
Sheep	1	1-2 months
Camerooni	4	Adults
Camerooni	1	5 months
Cattle	5	10 days-6 months
Yaez	2	4-6 months

diagnosis). The number and age of the animals from which *N. cuniculi* was isolated in relation to a pathological process (pneumonia or rhinitis) are summarized in Table 3.

All the isolates of *P. haemolytica* belonged to biotype A.

The results of the survey are reported in Table 4. *N. cuniculi* was isolated from the pharynx of 80-88.8% of animals under 1 month of age regardless of breed. In several cases, *N. cuniculi* was the only microorganism isolated from lambs or kids, all less than 1 week old. In animals one month or older a clear difference between pure breeds and cross breeds was found. While the percentage of animals carrying *N. cuniculi* remained high in the latter (70-83.3%) it steeply declined in the former (18.7-22.2%). This difference was found to be statistically highly significant: $P < 0.001$.

Table 4. *Incidence of N. cuniculi isolated during the survey from Yaez crosses and different breeds of sheep in relation to age*

Breed	Younger than 1 month			1 month and older		
	Sampled	Positive	Percent	Sampled	Positive	Percent
Awassi	12	10	83.3	18	4	22.2
Merino	NE	NE	NE	16	3	18.7
Assaf	7	6	85.7	10	8	80.0
Merino/Romanov	5	4	80.0	13	10	76.9
Yaez	9	8	88.8	6	5	83.3
Total pure breeds	12	10	83.3	34	7	20.5
Total cross breeds	21	18	85.7	29	23	79.3

NE: Not examined.

DISCUSSION

It is clear from the results of our survey that *N. cuniculi* is part of the normal bacterial flora of the upper respiratory tract of the small ruminant breeds examined up to about 1 month of age. The prevalence of *N. cuniculi* in animals aged 1 month or older differs significantly between the pure breeds and the cross breeds. The prevalence of the microorganism in the upper respiratory tract of the majority of the purebred animals is probably reduced, relative to other bacteria, to a number undetectable by the methods used in our survey. In the crossbred animals, on the other hand, *N. cuniculi* appears to remain more prominent.

The most obvious question arising from these findings concerns the clinical importance of *N. cuniculi*. Although clearly not a primary pathogen, two facts are noteworthy: (1) All the animals from which we isolated *N. cuniculi* in association with a pathological process were older than 1 month, the age at which the prevalence of the microorganism is reduced in purebred animals. (2) The breeds in which the prevalence of *N. cuniculi* remains high after the age of 1 month are identical to the ones known to be relatively susceptible to respiratory problems whereas the pure breeds examined, Awassi and Merino, tend to be more resistant [7, 8].

The conclusion is that sheep younger than 1 month are probably not susceptible to infection with *N. cuniculi*. The fact that the breeds which are relatively resistant to respiratory disease coincide with the ones in whose upper respiratory tract the number of *N. cuniculi* seems to be greatly reduced after the age of about 1 month might indicate that the mechanisms which reduce the numbers of this microorganism could be responsible for the reduction of the numbers of other potential pathogens which normally reside in the same tract.

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