

Regional Assiut Vet. Laboratory,  
Director Prof. Dr. S.M. Nashed.

## AEROBIC BACTERIAL FLORA FROM RUMENAL JUICE OF EXPERIMENTALLY IMPACTED SHEEP

(With 4 Tables)

By

SEHAM M. ALY; NAWAL GH. KHALIL and A.A. AMER\*

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البكتريا الهوائية لعصرة الكرش في حالات عسر الهضم  
عند الأغنام

سهام على ، نوال غبريال ، أحمد عامر

تم فحص ٢١ عينة من عصارة الكرش للأغنام تم فحصها بكتريولوجيا من حالات طبيعية وحالات عسر هضم وبعد العلاج بكتلت الميكروبات التي تم عزلها من الحالات الطبيعية هي سيدومونس ، الميكروب العنقودي الهفنيا والميكروب القولوني والستروباكتري. وحالات عسر الهضم قد تم عزل السيدومونس ، الميكروب القولوني - الميكروب العنقودي ، الهفنيا لاكتوباسيس ، ستروباكتري ، بروقدنسيا ، وبعد العلاج تم عزل السيدوموناس ، الميكروب العنقودي ، والميكروب القولوني ، والهفنيا. وتمت مناقشة تأثير البكتريا الهوائية في الحالات الطبيعية وحالات عسر الهضم وبعد العلاج.

### SUMMARY

Ruminal samples of 21 sheep were examined bacteriologically from normal animals, impacted ones and after being treated from impaction. The microorganisms which were isolated were Pseudomonas sp., Staphylococcus sp., Hafnia, E.coli and Citrobacter sp. in normal animals, Pseudomonas sp., E.coli, Staphylococcus, Hafnia, Lacto-bacillus, Citrobacter and Providencia in impacted sheep and Pseudomonas, staphylococcus, E.coli and Hafnia sp. in treated sheep. The aforementioned organisms are arranged in a chronological order. The role of aerobic bacterial flora is discussed.

### INTRODUCTION

Early observations on the bacterial flora of the rumen of sheep indicated fluctuation in the numbers and kinds of bacteria when different rations were fed. However, such evidence was based largely on studies by microscopic methods which were limited in scope because little information was obtained on numbers of different kinds of bacteria present. The recent development of methods for the cultivation of large numbers of bacteria from rumen contents has opened the way for obtaining

\*: Dept. of Vet. Med., Fac. of Vet. Med., Assiut University.

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more accurate informations on the kinds of bacteria present. Several studies were done on the numbers and types of rumenal bacterial anaerobic flora [KOHLER, 1940; JOHNSON, *et al.*, 1944; GALL, *et al.*, 1947, 1949; VAN DERWATH, 1948; BURROUGHS, *et al.*, 1950; GALL and HUHTANEN, 1951 and HUNGATE, *et al.*, 1951].

The literatures of aerobic bacteria flora of normal and impacted rumen of sheep are lacking to the best of our knowledge.

The aim of this study is to show the role of rumenal bacterial flora in normal and impacted sheep and its relation to feeding ration.

**MATERIAL and METHODS****Animals**

Twenty one sheep were used in the present work. These animals were Balady sheep of 3-5 years old. The experimental part of this study was made in collaboration with Department of Medicine, Faculty of Veterinary Medicine, Assiut University while bacterial isolation was fulfilled in Assiut Veterinary Regional Laboratory. Before applying the experiment all animals were proved healthy both by clinical and laboratory methods.

Full details of scheme of experiment is illustrated in table (1).

Table (1): Scheme of the Experiment:

Groups	Pre Experiment		Experimental		After treatment	
	Ration	Amount of ration Kg.	Time of sample	Amount of ration Kg.	Time of sample	Time of samples
1	Wheat Bran	2	Before Experiment	7	12 h'	After one week
2	Conct. mixture	2.5		7.5	24 h'	
3	Crushed Horse Bean	3		7.5	24 h'	
4	Sorghums	3		7.5	12 h'	
5	White Maize	3		7.5	24 h'	
6	Dry-bread	2		7	24 h'	
7	Bran + Conct. mixture	3		7	24 h'	



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### System of feeding:

The sheep were divided into 7 groups, each was composed of two animals. Control animals (one for each group is the third control animal of each of the seven group) were fed the same rations but in normal quantity (Table 1). Experimental animals were fed the same kind of ration taken by control individuals but in excessive quantity, nearly about 7 - 7.5 kgs, after starvation for 36-48 hours. The ration was left to the time which was sufficient to induce impaction syndrome. All animals were put on the same ration for seven days (2-3 K.g) before the experiment. The impacted symptoms appeared after 12-24 hours on all experimental animals after excessive feeding.

The impacted animals were treated with bismuth carbonate, neptin and charcoal to those showing diarrhoea. The ration was changed to tebin for one week to those with no diarrhoeic symptoms.

### Sampling and culture procedures:

Samples of rumen contents were obtained by stomach tube (3/4 in inside diameter) without the aid of vacuum pump, as described by POUNDEN (1952). About 1/2 liter of rumenal content was siphoned in 1000 ml Erlenmyer sterile flask, stoppered and taken immediately to the laboratory as soon as possible under strict aseptic condition.

Three rumenal samples were taken: one before excessive feeding from the third control animal of each of the seven group, the second after the symptoms of impaction and the third after one week from treatment. All the samples were subjected for bacteriological examination. The samples were thoroughly mixed and 20 ml. were taken from each sample in a sterile Maccarteny bottle. The bottles was centrifuged at 3000 RPM for 15 min. From the sediment culture were made on nutrient broth, blood and MacConkey's agar plates. All media were incubated at 37°C for 24-48 h. From the inoculated plates, the isolated colonies were picked up and subjected to further identification based on colonial and cellular morphology and biochemical reactions according to CRUICKSHANK, et al. (1975) and BUCHANAN and GIBBONS (1975).

## RESULTS

Results are tabulated in table 2, 3, 4.

Table (2): Aerobic Bacterial Isolates from control group.

No. of Animal	Kinds of Ration	Isolated Microorganisms
1	wheat Bran	Pseudomonas - Staphylococcus - Hafnia
2	conc. mixture	Pseudomonas - Staphylococcus - E.coli
3	crushed. horse bean	Pseudomonas - Staphylococcus
4	Sorghums	Pseudomonas -Hafnia
5	white Maize	Pseudomonas - Citrobacter - Staphylococcus
6	Dry Bread	Pseudomonas - Staphylococcus
7	Bran and Conc. Mixture	Pseudomonas - E.coli - Staphylococcus

Table (3): Aerobic Bacterial Isolates from Impacted sheep.

No. of Animal	Kinds of Ration	Isolated Microorganisms
2	Wheat Bran.	Lacto + baccilus + pseudomonas
2	Conc. mixture	E.coli + Hafnia + Citrobacter
2	Crushed. horse bean	E.coli + Hafnia + pseudomonas
2	Sorghums	Pseudomonas + E.coli + Anthracoid
2	White maize	Hafnia + E.coli + Pseudomonas
2	Dry bread	Pseudomonas + Staphylococcus + providencia
2	Bran and Conc.. Mixture	E.coli + Pseudomonas + Staphylococcus

Table (4): Aerobic bacterial Isolates from Impacted sheep after treatment.

No. of Animal	Kinds of Ration	Isolated Microorganisms
2	Wheat Bran.	Pseudomonas + Staphylococcus
2	Conc. mixture	Pseudomonas
2	Crushed. horse bean	Pseudomonas + Staphylococcus + E.coli
2	Sorghums	Pseudomonas + Hafnia
2	White maige	Staphylococcus + Hafnia
2	Dry bread	Pseudomonas + Staphylococcus
2	Bran and conc. mixture	Pseudomonas + E.coli



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## DISCUSSION

It has been increasingly realized that more fundamental Knowledge of rumenal microorganisms is needed to obtain more efficient ration and better control of metabolic disorders in ruminants (BRYANT, et al. 1958).

From table (2) it is shown that the frequency of different types of microorganisms isolated from control animals were *Pseudomonas* sp. 100%, followed by *Staphylococcus* sp. 86%, *Hafnia* 29%, *E.coli* 29% and Lastly *Citrobacter* sp. 14%.

From the data of table (3). It is evident that the percentage of *Pseudomonas* sp. decreased in the impacted sheep than in control animal (the percentage reach 86%), and *Staphylococcus* also decreased than in normal one (29%) while *E.coli* increased than in normal ones (71%) and so did *Hafnia* (43%).

Therefore that most of the Gram's negative microorganisms were increased in the rumen of impacted sheep than that of control animals; another Gram-negative organism, *Providencia*, could be detected in the former group but not in the latter.

After treatment of impacted animals the following microorganisms were isolated (*Pseudomonas* sp., *E.coli*, *Staphylococcus* sp. and *Hafnia* sp.) in most rumenal samples. This means that the rumenal flora returns to the normal one as in case of control animals.

The results of this study showed that the majority of the isolated organisms from all samples of animals fed on concentrated ration were gram-negative bacilli. This finding is supported by that of GALL, et al. (1949) which showed that the bacteria isolated from the rumen on all rations presented a rather uniform picture except that, the numbers of fast growing organisms increased with the amount of grain in the ration.

NASSAR, et al. (1986) stated that on dry concentrated ration, the frequency of Gram-negative microorganisms were increased.

Our present finding is also supported by OSBOURN, et al. (1970); JOURNET (1971) and STEWART (1977) who stated that in ruminants maintained on green forage diet and changed to concentrated diet with large amount of starch, the cellulose producing bacterial were decreased and amylase producing species proliferated. So, it is clear that the bacterial flora brings about a complex fermentation process which is essential to the maintenance of normal digestion and nutrition of the animal.

It has been recognized that many bacteria in the rumen cannot be cultivated by the more common bacteriological technique (HASTINGS, 1944), because of their apparently peculiar growth requirement. One of the most problems limiting the progress of the study of these bacteria, has been the lack of development of adequate cultural method.

More studies must be done on the role of anaerobes and protozoa in relation to impaction syndroms.



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