

# Gut microbiota of Barn Swallow (*Hirundo rustica*) nestlings in Northeast Algeria

Soumia HADDAD<sup>1</sup>, El-Yamine GUERGUEB<sup>1</sup>, Yassine NOUIDJEM<sup>3\*</sup>,  
Meriem ROUAIGUIA<sup>2</sup> & Moussa HOUHAMDI<sup>2</sup>



Received: December 26, 2023 – Revised: April 08, 2024 – Accepted: April 10, 2024

Haddad, S., Guergueb, El-Y., Noudjem, Y., Rouaiguia, M. & Houhamdi, M. 2024. Gut microbiota of Barn Swallow (*Hirundo rustica*) nestlings in Northeast Algeria. – Ornis Hungarica 32(1): 154–161. DOI: 10.2478/orhu-2024-0011

**Abstract** The intestinal microbiota plays an important role in the digestion of food provided by parents to their hatchlings. Non-pathogenic bacteria in the digestive tract can be significantly beneficial to the host species, while pathogenic bacteria can reduce hatchling survival and lead to a decline in the number of new generation. Microbiological analysis of cloacal microflora of hatchlings revealed a highly diverse microbial load present from hatching until fledging (at 15 days) in Barn Swallow (*Hirundo rustica*). The intestinal microflora of 15-day-olds was the most diverse, and two groups are dependent on age: CPG and Lactobacilli are mostly present in hatchlings of the second and third ages (10 and 15 days). Our research was conducted to identify certain bacterial species, such as *E. coli*, *Salmonella*, *Pseudomonas*, *Lactobacillus*, *Streptococcus*, and *Staphylococcus*. The presence of most species was related to age, while the presence of *Salmonella* was accidental.

Keywords: Northeastern Algeria, Barn Swallow, cloacal microflora, hatchlings

**Összefoglalás** A bél mikrobiótája fontos szerepet játszik a szülők által a fiókák számára kínált táplálék megemésztésében. A nem kórokozó baktériumok az emésztőrendszerben jelentős előnyöket nyújthatnak a gazdafajnak, míg a kórokozó baktériumok csökkenthetik a fióka túlélését, és az új generáció egyedszámának csökkenéséhez vezethetnek. Füstí fecske (*Hirundo rustica*) fiókák kloákális mikroflórájának mikrobiológiai elemzése egy rendkívül változatos mikrobiális terhelést mutatott ki a kikeléstől a kirepülésig (15 napig). A 15 napos fiókák bélflórája volt a legváltozatosabb, és két csoport függött az életkortól. A CPG és a *Lactobacillus* főként a második és harmadik életkor kategóriájú fiókákban (10 és 15 naposak) találhatóak. Kutatásunk célja az volt, hogy azonosítsuk bizonyos baktériumokat, mint például az *E. coli*, a *Salmonella*, a *Pseudomonas*, a *Lactobacillus*, a *Streptococcus* és a *Staphylococcus*. A legtöbb faj jelenléte összefüggött az életkorral, míg a *Salmonella* jelenléte véletlenszerű volt.

Kulcsszavak: Északkelet-Algéria, fecskefajok, kloákális mikroflóra, fiókák

<sup>1</sup> Laboratoire Valorisation et Conservation des Ecosystèmes Arides (LVCEA), Faculté des Sciences de la Nature et de la Vie et Sciences de la Terre, Université de Ghardaïa, 47000 Ghardaïa, Algeria

<sup>2</sup> Laboratoire Biologie, Eau et Environnement (LBEE), Faculté SNV-STU, Université 8 Mai 1945 Guelma, 401, 24000 Guelma, Algeria

<sup>3</sup> Faculty of Science, University of M'sila P.O. box Ichebilta, 28000 M'sila, Algeria

\* corresponding author, e-mail: yacine.noudjem@univ-msila.dz

## **Introduction**

Environmental factors, like body mass at fledging (Linden *et al.* 1992), physical conditions, as well as immune competence, are good predictors of individual survival (Møller 2004). The growth of bird species depends on external bioresources, which are fragmented into simple, more easily degradable molecules through the digestive processes (Hooper *et al.* 2002).

In addition to various physical and chemical processes in the digestive tract involved in digestion, a diverse and host-assembled microbiota is of paramount importance for optimal food digestion and, consequently, the nutritional status of hatchlings (Hooper *et al.* 2002). However, pathogenic bacteria can compromise the investment in growth and reduce the likelihood of survival or even kill their hosts (Nuttall 1997, Potti *et al.* 2002).

The migration of certain birds affects the composition of the gastrointestinal tract microbiota, which has a strong environmental component (Lucas & Heeb 2005). Microbial sampling of the cloaca is often considered a good approach to studying the diversity of these microorganisms in the *in vivo* digestive system, as feces and urine are evacuated at this level (Mead 1997), and microbial cells attached to the intestinal wall make the end of the digestive tract rich and diversified in microbial complexes (Mead 1997).

In this context, we conducted a study aimed to characterize the cultivable cloacal microbiota of Barn Swallow (*Hirundo rustica*) hatchlings nesting in the city of Guelma (Northeast Algeria), through microbial isolation, enumeration and identification.

## **Material and Methods**

### **Study area**

Our study area was located in Guelma province, Northeast Algeria (36°27'40 "N; 7°26'18" E) at 60 km from the extreme North Algeria at an altitude of 279 m above sea level (Kafi *et al.* 2015), covering an area of 3686.84 km<sup>2</sup>, located halfway between the north, the High Plateaus, and the south of the country (Haddad *et al.* 2015). The average annual temperature is 18.7 °C (winter 4.6 °C, summer 36.3 °C) and the mean annual rainfall in the area is 590 mm (Bensouilah & Barrientos 2021).

### **Sampling method**

We studied the cloacal microflora of nestling Barn Swallow (*Hirundo rustica*) nesting in the province of Guelma. Five nests were randomly chosen in three cities (Guelma city, Bouchegouf city and Hammam Debagh city). Guelma city is the capital of the province and it is an urban area, but the others are rural cities with three nestlings each. Samples were collected from hatchlings aged five, ten, and fifteen days. To avoid confusion between chick ages, age was determined using a scale used by McGinn and Clark (1978) (Table 1).

Cloacal samples were collected by swabbing (swab of 5 mm long and 2 mm wide) directly from the cloaca of the nestlings. Then, while observing aseptic conditions, only the cotton

Table 1. Determination of hatchlings age (McGinn & Clark 1978)  
1. táblázat A fiókák korának meghatározása (McGinn & Clark 1978)

Age (day)	Determination criterion
<b>First days</b>	Not all eggs have hatched, the hatchlings are blind; the egg tooth is still present on their beaks
<b>4-5 days</b>	The eyes are partly open. Primary and secondary feathers begin to grow
<b>5-6 days</b>	The primary feathers of the tail begin to grow
<b>6 days</b>	The growth of the tarsus slows down. The chicks can be ringed
<b>9 days</b>	The tail feathers have grown
<b>10 days</b>	The growth of the tarsus is almost complete
<b>13 days</b>	Feathers have grown almost all over the body
<b>16 days</b>	Last possible date for ringing
<b>18 days</b>	Capable of flying
<b>20-22 day</b>	The hatchlings leave the nest

of the swab was introduced into 10 ml of appropriate nutrient broth diluted to  $10^{-1}$  (Kyle & Kyle 1993, Lombardo *et al.* 1996).

Clostridia were searched for in the selective VF (Meat-Liver) medium after a heat shock was applied to the samples (at 80 °C for 10 min and then directly transferred to 4°C for 10 min). The resistant forms of these microorganisms, which grow in anaerobiosis at  $37 \pm 1$  °C for 24 h and/or 48 h, give typical colonies that reduce sodium sulphite.

The samples were transported to the laboratory in a cooler ( $\approx 4$  °C) within a maximum time of 30 minutes (Kyle & Kyle 1993, Lombardo *et al.* 1996). After appropriate dilutions, a volume of 10 ml of the diluted solution was filtered through 45  $\mu$ m diameter nitrocellulose membranes. These membranes were then placed on selective media (Table 2).

For the examination of bacteria, the samples were incubated for 24 h at 37 °C and yeasts for 72 h at 25 °C. It should be noted that the search for lactobacilli was carried out under anaerobic conditions. After cultivation, the microorganisms were counted and then well isolated and identified based on their enzymatic arsenal and biochemical profiles.

Table 2. Detection of microorganisms in the cloaca of Barn Swallow hatchlings  
2. táblázat Mikroorganizmusok kimutatása a füstti fecske fiókák bélcsatornájában

Microorganisms sought	Media used	Dilution
<b>Aerobic bacteria</b>	TSA (Trypticase soy agar)	$10^{-4}$
<b>Gram-positive cocci</b>	Columbia	$10^{-3}$
<b>Staphylococci</b>	Chapman	$10^{-2}$
<b>Gram-negative bacilli</b>	Mac Conkey	$10^{-3}$
<b>Coliforms</b>	TTC tergitol 7	$10^{-3}$
<b>Lactobacilli</b>	MRS	$10^{-4}$
<b>Clostridium</b>	ML (Meat liver agar)	$10^{-2}$
<b>Yeasts</b>	SAB (Sabouraud agar)	$10^{-2}$

## Data analysis

For presenting the results, the number of microorganisms was multiplied by the dilution factor, and then transformed into log with base 10  $(1+n)$  CFU (colony-forming units) per swab, where “n” is the number of colonies on each filter. This allows us to properly present high numbers of bacteria and also to report negative samples as log with base 10  $(1+0) = 0$ . The results of microbial enumeration were expressed as a percentage of occurrence in the analysed samples and as mean  $\pm$  standard deviation.

Each nestling was considered as an independent variable in the statistical analysis. The Shannon index, the equitability index, and the species richness were calculated based on the age of the nestlings, and the average of microbial counts for each age group [5 days, 10 days, and 15 days] was calculated before the calculation of the indices. The relationships between the dependent and explanatory variables were assessed using analysis of variance (ANOVA one way).

First, one-way ANOVA was performed to analyse the variation in the number of microorganisms counted as a dependent variable among nestlings. The second analysis aimed to determine whether there was a variation in the number of microorganism species across different ages (5, 10, and 15 days).

## Result

Our investigation confirms a fairly significant microbial diversity both quantitatively and qualitatively in the cloaca of Barn Swallow hatchling breeding in the city of Guelma. The one-way ANOVA test showed a highly significant difference in the number of microorganisms counted ( $F= 4.3504$ ,  $df = 10$ ,  $P < 0.001$ ) between nestlings. Therefore, all nestlings possess a more or less individualized microbial flora, they all exhibit unique differences. Practically, all searched microbial genera were isolated and identified in more than 60% of the explored samples, with the complete absence of Clostridium (Anaerobic Sulphate-Reducing bacteria “ASR”) in all samples (Figure 1), from nestling hatching to fledging (Table 3).

Gram-positive cocci were the most abundant family, mainly composed of faecal Streptococci (group D) with an average of



Figure 1. Absence of Sulphate-Reducing Anaerobic bacteria “ASR”

1. ábra Szulfátredukáló anaerob baktériumok hiánya „ASR”

Table 3. Main microorganisms isolated from the cloaca of Barn Swallow (*Hirundo rustica*) hatchlings

3. táblázat A füstifecske fiókák (*Hirundo rustica*) kloákájából izolált fő mikroorganizmusok

Microorganisms detected	Percentages of positive samples	Mean ± standard deviation	Most appeared species
<b>Aerobic bacteria</b>	80%	4.21±1.84	<i>Pseudomonas</i> ssp.
<b><i>Lactobacillus</i> spp.</b>	66.67%	3.94±3.11	<i>Lactobacillus</i> ssp.
<b>Gram-negative bacteria</b>	80%	4.39±2.08	<i>E coli</i> spp.
<b>Gram-positive bacteria</b>	73.33%	5.24±1.19	<i>Streptococcus</i> ssp.
<b><i>Staphylococcus</i> spp.</b>	93.33%	3.60±1.21	<i>Staphylococcus aureus</i>
<b>Yeasts and fungi</b>	66.66%	2.83±2.29	/
<b><i>Clostridium</i> spp.</b>	0%	0±0	/

Table 4. Estimated ecological indices for the three age groups (5, 10, 15 days)

4. táblázat Becsült ökológiai mutatók a három korcsoportra (5, 10, 15 nap)

Age group of chicks	Specific richness of microorganisms	Shannon index	Equitability index
<b>5 days</b>	8	2.815	0.938
<b>10 days</b>	9	2.901	0.915
<b>15 days</b>	10	3.134	0.944

5.24 (log (n+1) CFU/ml) and a percentage of more than 73% of positive samples, including staphylococci. Gram-negative bacilli followed with an average of 4.21 (log (n+1) CFU/ml) and an occurrence rate of 80%, belonging to the Enterobacteriaceae family, of which coliforms represented the predominant part, namely *E. coli* (the most frequent species) and some samples are positive for *Salmonella* ssp.

*Lactobacilli*, considered the main protective barrier of intestinal mucosa, were identified in 67% of the samples, as were yeasts, which were present at the same frequency. In each age group, the cloacal flora was very diversified and balanced at the same time (Table 4).

The Shannon index shows a balanced flora in the cloaca of the three age groups, with microbial genera present at very similar frequencies. The most diverse and balanced group is the 15-day-old group, indicating intestinal maturity just before fledging. One-way ANOVA showed that two variables are dependent on age:

1. CGP (Gram-positive cocci) ( $F_{1,10} = 4.400$ ,  $P = 0.035$ )

2. *Lactobacilli* (log(X+1) transformed for processing purposes) ( $F_{1,10} = 6.671$ ,  $P = 0.011$ )

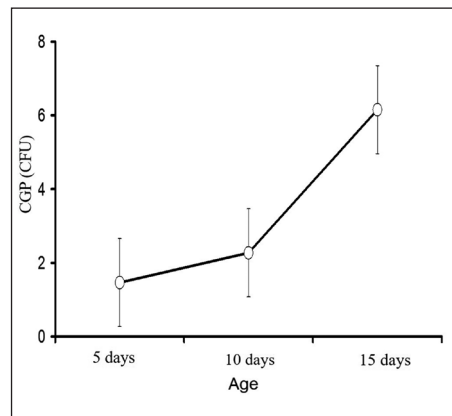


Figure 2. Establishment of Gram-positive cocci (CGP) CFU in the cloaca according to the age

2. ábra A Gram-pozitív cocci (CGP) CFU kialakulása a kloákában az életkor függvényében

For CGP, the post-hoc test (Student-Newman-Keuls) showed that 15-day-old hatchlings were the most affected (Figure 2), while for lactobacilli, it was mostly hatchlings of the second and third ages (10 and 15 days) (Figure 3).

## Discussion

In newly hatched chicks, the sudden transition to solid exogenous food is accompanied by allometric growth specific to each portion of the intestine and physiological changes reflecting the maturation of chick digestive capabilities (Quillien & Vidal 2003).

The sequence of microbial establishment in the digestive ecosystem ends when a state of equilibrium is established (Savage 1977). In healthy adults, equilibrium is stable unless the environment and diet are altered (Savage 1977, 1987). As with the colonization sequence, the general characteristics of the adult's ecosystem are similar across animal species, and at equilibrium, the microbial species observed in most, if not all, animal species studied are the same. However, each bird's intestinal microbiota is characterized by numerous distinct details. Consequently, the equilibrium state of an adult's intestinal microbial ecosystem is unique.

In the present study, the intestinal microflora of 15-day-old hatchlings reached a specific equilibrium, with the isolated microorganisms, mainly lactobacilli, achieving stability for 10 days after birth, and the presence of other taxa being variable. However, the presence of Gram-positive cocci and conqueror was particularly noticeable at 15 days of age, which may promote a balance between different microbial genera, including Gram-negative bacilli.

The indigenous microbial flora prevents the implantation of pathogenic microbial flora. This phenomenon, called the "barrier effect", is established before the complete maturity of the digestive tube immune system. Thus, lactobacilli exclude coliforms in gnotobiotic animals (Fuller 1977). However, in this study, the effect was much less significant, as was the case in conventional poultry (Watkins & Kratzer 1983), probably due to the presence of other microorganisms that prevent or reduce the action of lactobacilli.

The intestinal microbial flora plays an important role in the digestion of food provided by parents to their hatchlings. Therefore, this flora has a strong environmental component and thus varies between individuals of the same species (Lucas & Heeb 2005). This microflora is also linked to the particular nutritional status (Glünder 1997, Engberg *et al.* 2004) and phenotypic quality of individuals (Moreno *et al.* 2003).

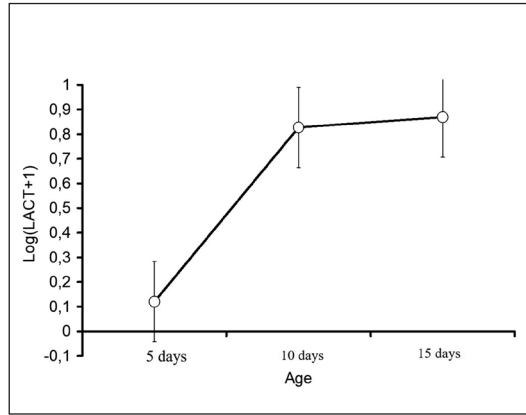


Figure 3. The colonization of lactobacilli in the cloaca according to the age

3. ábra A lactobacillusok kolonizációja a kloákában az életkor szerint

## Conclusion

The study of cloacal microflora in hatchlings showed great stability from the first days of life and good defence against pathogenic bacteria. The results obtained from the identification of cloacal simples showed the presence of six bacterial species: *Lactobacillus* spp., faecal Streptococci (group D) and *Staphylococcus* spp. as gram positive cocci. *E. coli* and *Salmonella* as gram negative bacilli, and *Pseudomonas* as an aerobic bacilli. The intestinal microflora of 15-day-old was the more diverse, while some species appeared from the age of 10 days such as *Pseudomonas*, *Staphylococcus* and also affected the 15-day-old category. As for *Salmonella*, its appearance was not related to the age of the individuals we found it with *E. coli*, *Streptococcus* and *Lactobacillus* in the first group age of 5 days. It can be said that the appearance and/or disappearance of *Salmonella* is occasional but decrease with age up to 15 days. The diversity of these germs originates from factors that are both extrinsic (environment) and intrinsic (individuals).

## References

- Bensouilah, T. & Barrientos, R. 2021. Greenfinches nesting in algerian orchards delayed their breeding and produced less fledglings in the driest of two years. – Journal of Arid Environments 184(September 2020): 104312. DOI: 10.1016/j.jaridenv.2020.104312
- Engberg, R. M., Hedemann, M. S., Steinfeldt, S. & Jensen, B. B. 2004. Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. – Poultry Science 83(6): 925–938. DOI: 10.1093/ps/83.6.925
- Fuller, R. 1977. The importance of lactobacilli in maintaining normal microbial balance in the crop. – British Poultry Science 18(1): 85–94. DOI: 10.1080/00071667708416332
- Glünder, G. 1997. Influence of diet on the occurrence of some bacteria in the intestinal flora of wild and pet birds. – Uebersichten Zur Tierernaehrung (Germany) 25(2): 203–204.
- Haddad, S., Hanane, S. & Houhamdi, M. 2015. La reproduction de l'Hirondelle rustique (*Hirundo rustica*) dans un milieu urbain nord-africain: quel impact des conditions climatiques et de l'application des insecticides? [Reproduction of the Barn Swallow (*Hirundo rustica*) in an urban environment in North Africa: what is the impact of climatic conditions and insecticide application?]. – Revue d'Écologie 70(3): 280–290. (in French with English Summary)
- Hooper, L. V., Midwedt, T. & Gordon, J. I. 2002. How host-microbial interactions shape the nutrient environment of the mammalian intestine. – Annual Review of Nutrition 22: 283–307. DOI: 10.1146/annurev.nutr.22.011602.092259
- Kafi, F., Hanane, K., Zeraoul, A., Brahmia, H. & Houhamdi, M. H. 2015. Les facteurs déterminant le succès de la reproduction des tourterelles des bois (*Streptopelia turtur*) dans un milieu agricole nord-africain [Factors determining the reproductive success of Turtle Doves (*Streptopelia turtur*) in a North African agricultural environment]. – Revue d'Écologie 70(3): 271–279. (in French with English Summary)
- Kyle, P. D. & Kyle, G. Z. 1993. An evaluation of the role of microbial flora in the salivary transfer technique for hand-rearing Chimney Swifts. – Wildlife Rehabilitation 8: 65–71.
- Linden, M., Gustafsson, L. & Part, T. 1992. Selection on fledging mass in the Collared Flycatcher and the Great Tit. – Ecology 73(1): 336–343. DOI: 10.2307/1938745
- Lombardo, M. P., Thorpe, P. A., Cichewicz, R., Henshaw, M., Millard, C., Steen, C. & Zeller, T. K. 1996. Communities of cloacal bacteria in Tree Swallow families. – Condor 98(1): 167–172. DOI: 10.2307/1369521
- Lucas, F. S. & Heeb, P. 2005. Environmental factors shape cloacal bacterial assemblages in Great Tit *Parus major* and Blue Tit *P. caeruleus* nestlings. – Journal of Avian Biology 36(6): 510–516. DOI: 10.1111/j.0908-8857.2005.03479.x
- McGinn, D. B. & Clark, H. 1978. Some measurements of swallow breeding biology in lowland Scotland. – Bird Study 25(2): 109–118. DOI: 10.1080/00063657809476582

- Mead, G. C. 1997. Bacteria in the gastrointestinal tract of birds. – In: Mackie, R. I., White, B. A. & Isaacson, R. E. (eds.) *Gastrointestinal Microbiology*. – New York: Chapman and Hall
- Møller, A. P. 2004. Protandry, sexual selection and climate change. – *Global Change Biology* 10(12): 2028–2035. DOI: 10.1111/j.1365-2486.2004.00874.x
- Moreno, J., Briones, V., Merino, S., Ballesteros, C., Sanz, J. J. & Tomás, G. 2003. Beneficial effects of cloacal bacteria on growth and fledging size in nestling Pied Flycatchers (*Ficedula hypoleuca*) in Spain. – *Auk* 120(3): 784–790. DOI: 10.1093/auk/120.3.784
- Nuttall, P. A. 1997. Viruses, bacteria, and fungi of birds. – *Host-Parasite Evolution: General Principles and Avian Models*, pp. 271–302.
- Potti, J., Moreno, J., Yorio, P., Briones, V., García-Borboroglu, P., Villar, S. & Ballesteros, C. 2002. Bacteria divert resources from growth for magellanic penguin chicks. – *Ecology Letters* 5(6): 709–714. DOI: 10.1046/j.1461-0248.2002.00375.x
- Quillien, J. F. & Vidal, M. 2003. Flair-flow 4: Bringing European food research to the end-users. – *Trends in Food Science and Technology* 14(1–2): 32–42. DOI: 10.1016/S0924-2244(02)00241-8
- Savage, D. C. 1977. Microbial ecology of the gastrointestinal tract. – *Annual Review of Microbiology* 31(70): 107–133. DOI: 10.1146/annurev.mi.31.100177.000543
- Savage, D. C. 1987. Microorganisms associated with epithelial surfaces and stability of the indigenous gastrointestinal microflora. – *Food / Nahrung* 31(5–6): 383–395. DOI: 10.1002/food.19870310511
- Watkins, B. A. & Kratzer, F. H. 1983. Effect of oral dosing of *Lactobacillus* strains on gut colonization and liver biotin in broiler chicks. – *Poultry Science* 62(10): 2088–2094. DOI: 10.3382/ps.0622088

