# CHARACTERIZATION OF RABBIT FARMS IN TUNISIA AND RETROSPECTIVE EPIDEMIOLOGICAL STUDIES ON *RHDV-2*

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## ABSTRACT

The Tunisian rabbit breeding structure has changed this last decade passing from a traditional breeding to a more modern structure allowing the industrialization of rabbit farming to thrive. However, rabbit industry faces several health problems leading to an important morbidity and mortality. Among these, Rabbit Haemorrhagic Disease (RHD) is one of the pathogen causing the highest damage in the Tunisian rabbit farms. In this context, we carried out an epidemiological study consisting in a retrospective survey involving 60 industrial farms located in 16 governorates and 166 traditional farms. The objectives were to characterize the Tunisian rabbit farms and to estimate the prevalence of RHD by molecular analyses in suspected farms in order to detect possible risk factors of the appearance and the spread of the disease.

**KEYWORDS:** RHDV2, Tunisia, Rabbit farms, Retrospective survey

## INTRODUCTION

In Tunisia, rabbit villages kept under smallholder-low input systems are considered highly valuable, but in recent decades, industrial systems have been gradually multiplied. Indeed, in the last 20 years, rabbit breeding in Tunisia has been developed and continues to do so with the introduction of the new technology, and the government and national organizations support. Currently, two different rabbit breeding systems can be identified in Tunisia: (i) Industrial rabbit farms which are characterized by the installation of modern breeding structure using breeds of more successful exotic origins and in fenced hutches and (ii) traditional rabbit rearing which has a low performance level linked to a high mortality rate (Bergaoui, 1992). However, both traditional and conventional breeding systems are currently facing several constraints such as the low skills of the breeders and the poor quality of food and habitat. This could be explained by the increased presence of pathogens likely to generate several animal health problems. Among these pathogens, Rabbit hemorrhagic disease (RHD) is a real menace for the Tunisian rabbit farms. RHD is a notifiable and highly contagious disease caused by Rabbit hemorrhagic disease virus (RHDV), member of *Caliciviridae* family and *Lagovirus* genus. Recently, single species of *Lagovirus*, called "Lagovirus europaeus", has been proposed, and all RHDV strains have been allocated to the *Lagovirus europaeus* genogroup I/GI (Le Pendu*et al*., 2017).

In Tunisia, the appearance of GI.1 was first suspected in the South during 1989 and confirmed in 1992. It rapidly spread all over the country causing 80 to 90 per cent of mortality in small rural and intensive farms and therefore, was responsible for great economic losses in the rabbit industry (Bouslama*et al.*, 1996). In 2010, a new pathogenic *Lagovirus* (RHDV2), affecting European rabbits and related to butdistant from RHDV, was identified in France and was designated as *Lagoviruseuropaeus/*GI.2. Nowadays, GI.2 spread throughout the world and is replacing older circulating strains such GI.1 in many countries (Le Gall-Reculé*et al*., 2011, 2013). In Tunisia, GI.2 was noticedfor the first time in 2015, in Kairouan, and was then followed by outbreaks in industrial rabbit farms in different governorates causing considerable economic losses (Chakroun*et al*., 2015).

In order to get precise data helping to estimate the prevalence of this new genotype and to characterize Tunisian rabbit farms, a cross-sectional retrospective survey on industrial and traditional farms was carried out in 2019. This survey is a first in its kind and itcarried out in rabbit farms in Tunisia.

## MATERIAL AND METHODS

### Areas, Period and Population of Study

The survey was lead between October 2018 and February 2019. It concerned 60 rabbit industrial farms located in 6 governorates, and 166 rabbit traditional farms (Figure 1). Concerning the industrial sector, the survey was exhaustive, covering all rabbit breeders. For traditional ones, and for the lack of a list of breeders, only 10% of them were sampled through the use of the "snowball" method.

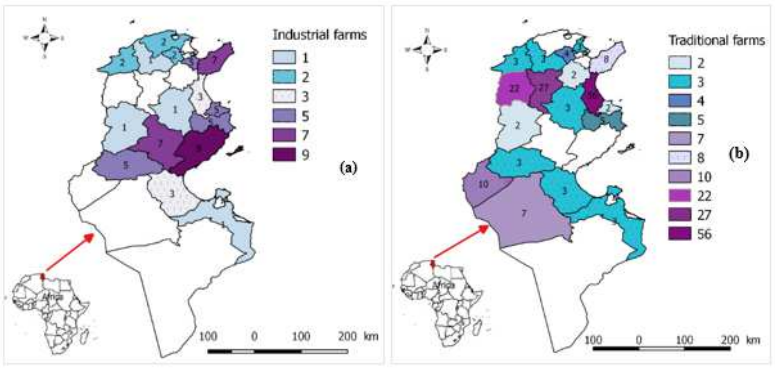


**Figure 1:** Distribution of industrial (a) and traditional (b) farms by governorate

### Data Collection and Analyses

To collect survey data, two questionnaires were elaborated respectively for industrial and traditional farms. Data included two sections: Section 1 dealing with general information (locality names, survey date and contact details of the investigator) and Section 2 is related to specific information (typology, management, biosecurity and history of RHD). Data were collected and analyzed with Access and descriptive statistics were reported in this study using R software (3.4.3) and Arc Gis software (10.4).

### Molecular Analyses

Based on history of RHD circulation, 16 out of the 60 industrial farms were sampled for further analyses in order to confirm or infirm the presence of RHDV. A total of 73 liver samples were collected (5 per farm)from apparently healthy animals of 16 industrial rabbit farms located in 7 governorates. Viral RNA was extracted from ~30mg of liver homogenized in MEM medium (Dibco) using the Purelink viral RNA/DNA Mini Kit (Invitrogen), according to the provided protocol. Confirmation of the presence of GI.2 was performed by qRT-PCR using Superscript III Platinium One-step qRT-PCR kit (Invitrogen) and Genesig standard kit providing primers targeting portion of the major structural proteinVP60 and probes following the manufacturer's instructions.

## RESULTS AND DISCUSSION

### Farms Characterization

The number of industrial breeding was decreased from 260 in 2015 (TPRA, 2015), to 60 in our study. This could be explain by, first, the rise of the feed prices, second, the weak appreciation of rabbit meat by the Tunisian consumer, and finally, the increased presence of pathogens at the rabbit flock level (Ouertani*et al*., 2016).The last factor is likely to be the most evident. Traditional farms, numbering 166, were widespread in our country similarly as in Egypt (Mostafa*et al*., 2020), Algeria (Saidj*et al*., 2013) and Morocco (Jaouzi*et al*., 2006).

In all industrial farms concerned by the survey, rabbits were reared in cages.Industrial livestock buildings contain rows of bare wire cages arranged either according to the one-storey system, "Flat Deck" (38%),or stacked on two levels (57%) or by using the two systems, platform and double platform (5%). However, according to an Algerian study, the majority of traditional breeding is done on the ground (70.4%) while only 19% of farms use traditional cages and 10.6% of them are carried out in barrels (Saidj*et al*., 2013). In Tunisia, according to this study, the ground breeding represents only 26% of traditional farms.

The Californian White (47%) and the New Zealand (39%) strains were the most common breeds identified in our study. This could be explained by the rabbit genetic improvement program in Tunisia, which concerned these two strains intended for the production of rabbit meat. In Kenya (Serem *et al*., 2013) and Nigeria (Mailafia *et al.*, 2010), these strains were also considered to be the most popular breeds for meat production due to their good growth characteristics and high meat/bone ratio.

**Reproduction**

Artificial insemination was practiced by 61% of Tunisian breeders, while in 2016, it concerned 77% of breeders (Ouertani*et al*., 2016). In 2013, an Algerian study showed that all breeders practice natural mating (Saidj*et al*., 2013). Likewise, in Côte d'Ivoire, artificial insemination is not implemented even in the intensive breeding system (Kimse*et al*., 2017). The reproductive rhythms were between 0 and 20 days. Sperm is collected either by the breeder or by the technician, from their own breeding bucks (53%). It can also be donethrough an exchange between males from other farms(41.6%). In the Tunisian rabbit industrial farms, the average longevity of a rabbit doe is equal to 17 kindlings every 2 years.While, in Abidjan, the number of litters for local breeds is equal to 4 per year with an average of 6 kits per birth (Kimse*et al*., 2017). This difference could be due to the mode of reproduction in Abidjan district, which is mostly extensive with local breeds. In fact, the poor performance linked to the genetic strain of "local breeds" generates low prolificacy (Lebas, 2004).

**Biosecurity**

To evaluate the health state of industrial farms, we relied on the assessment of the farm's application of some biosecurity indicators. Our results show some diversity in the implementation of biosecurity and decontamination measures. Surveyed farms were generally equipped with a footbath (38/60) and most of them seems to practice a partial (15/60) or complete (35/60) decontamination (disinfection of equipment, surfaces and cages). Concrete floor is the most used in industrial farms since it is easy to clean,predator-proof, long lasting and easy to maintain (Figure 2).However, some flaws in biosecurity measures were noticed. In fact, some industrial farms revealed the presence of pests that could be a virus reservoir. Indeed, it has been demonstrated that wildlife, birds, rodents and even domestic pets represent a reservoir of RHD through their dejections (Rocha, 2017). Outfit changing for each category is also an important factor for limiting virus dissemination within the building and it is not implemented in the majority of farms. We also noticed that same employees work in different farm categories. The lack of "rotoluves" and disinfection of means of transport but also the use of a common equipment in fattening and maternity were also observed. All these features may cause passive transmission of the virus. In fact, it was demonstrated that contaminated material and human activities play a role in the spread of the disease (Cooke and Fenner, 2002).

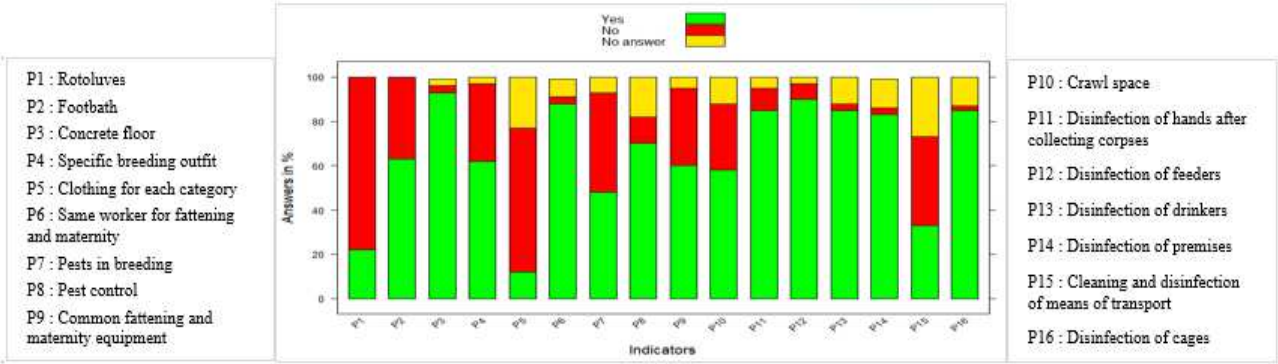


**Figure 2:** Distribution of industrial farms based on the biosecurity indicators

**RHD's Prevalence Estimation**

To estimate the prevalence of the disease, the breeders were asked about the RHDV's passage in the industrial herd. According to the questionnaire in industrial farms, only 27% (16/60) of breeders suspected the passage of the virus. The mainly clinical signs observed were epistaxis (27%), sudden death (25%) and cyanosis of the mucous membranes (15%). The death rate exceeded 75% in suspected infected herds. Among the 16 suspected industrial farms, 13 reported that rabbits, mainly mothers, were vaccinated against RHDV. Based on the questionnaire's answers and the observed

clinical signs, suspected animal's livers were sampled for molecular analysis. Fourout of 16 suspected industrial rabbits farms were confirmed to be positive to RHDV2 (GI.2) (25%, IC 95% 0.04-0.46), and all were located at the governorate of Monastir. Positive animals were aged around 70 days. No circulation of GI.1 were detected in these sampled farms. The low prevalence of RHD in surveyed farms (6%)could be explained by the fact that our epidemiological study concerned healthy animals originating from suspected farms, and that no outbreaks of the disease were observed during the survey. A more exhaustive sampling should be carried out to estimate more precisely GI.2 prevalence. Furthermore, no major factor in the risk of infection has been highlighted in these farms.

## CONCLUSION

This study allowed us to have an update about Tunisian industrial rabbit farms. It is therefore clear that this sector requires special attention to promote it and the government efforts for commercialization. The retrospective survey has characterized rabbit farms in Tunisia, in one hand, and on the other hand, it has explored the presence of RHD at a given period. Large industrial farms were located mainly on the coasts. According to the results of molecular analyzes, four among 16 industrial suspected farms were positive for the new variant. Significant variability in biosecurity, cleaning and disinfection practices on farms, as well as a lack of awareness among most farmers of the importance of vaccination as a medical prophylaxis, would modulate the risk of the disease occurrence. Therefore, a detailed study of biosecurity practices, and especially their observance, should be carried out to assess their impact on the farms contamination risk.

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