



# THE ROLE OF WILD ANIMALS AS A RESERVOIR OF ZONOTIC AGENTS : *Clostridium difficile*

## 1. INTRODUCTION

The ecology and epidemiology of wildlife diseases, particularly those that are zoonotic, must become an area of focus for public health programs (Siembieda et al., 2011). Many studies have demonstrated that wild animals are reservoirs of zoonotic pathogens that can represent a risk for the human health, such as viral zoonotic pathogens (West Nile or Influenza A), parasites (*Toxoplasma gondii*) and multidrug-resistant zoonotic bacteria (Vidal et al., 2016). However, there are few studies in wildlife related to emergent pathogens.

*Clostridium difficile* (CD) is considered one of the most important nosocomial agent producing severe disease (diarrhea, colitis, toxic megacolon) and mortality in hospitalized patients. This pathogen is a Gram-positive, anaerobic, spore-forming bacillus, with the capacity of producing toxins that are responsible of these human deaths. In the last years, CD has also been associated with outcomes of diarrhea in the general population (community cases) suggesting the involvement of livestock (pig, beef, poultry) as a reservoirs of CD infection (CDI). However, the role of wild animals in the epidemiology of CDI is scarcely investigated. For this reason, the main objective of this study has been to determine the presence of wildlife as reservoirs of CD toxigenic strains



## 2. OBJECTIVES

- ❖ To assess the prevalence of toxigenic strains of *C. difficile* (*tcdA* and *tcdB*) in wildlife of Catalonia.
- ❖ To determine the presence of other potential zoonotic enteropathogens in wildlife of Catalonia.



Gram stain of *C. difficile*

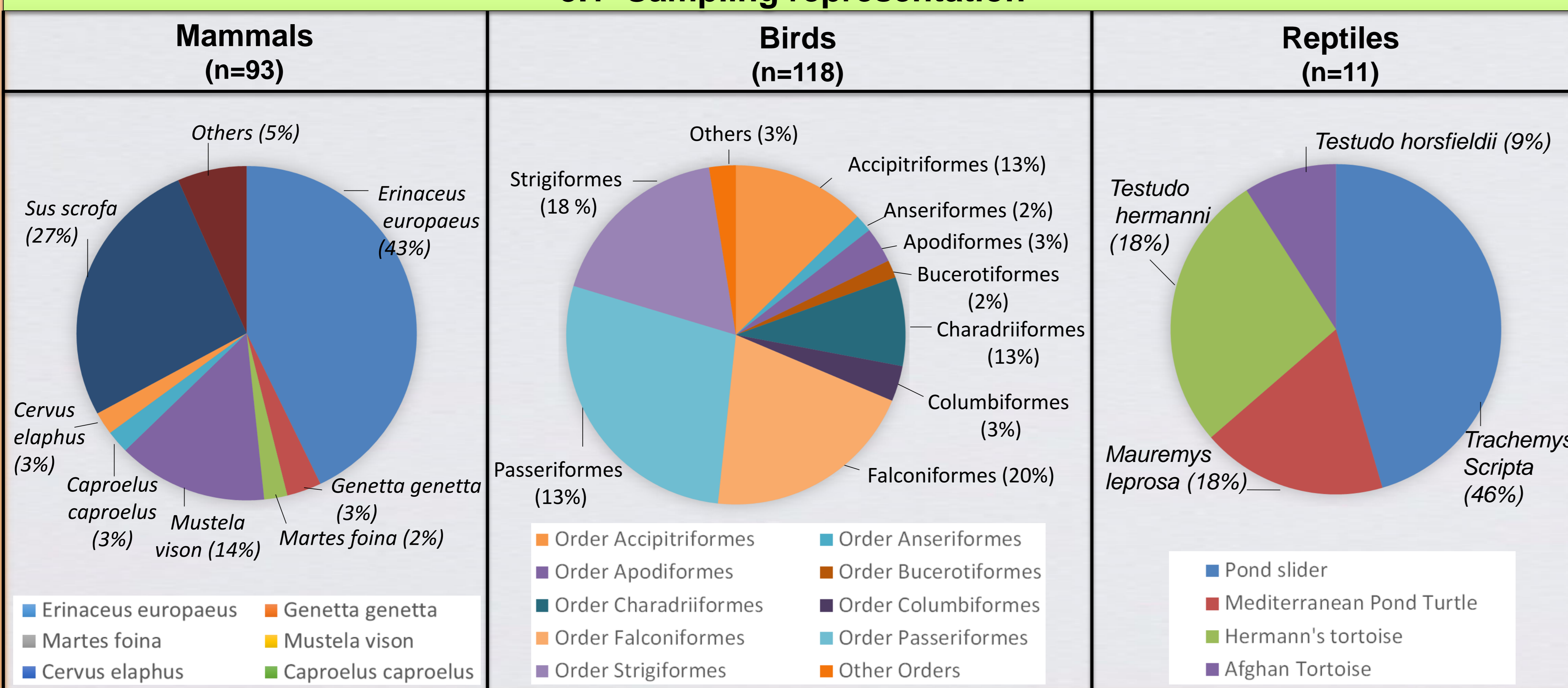


Selective culture of *C. difficile*

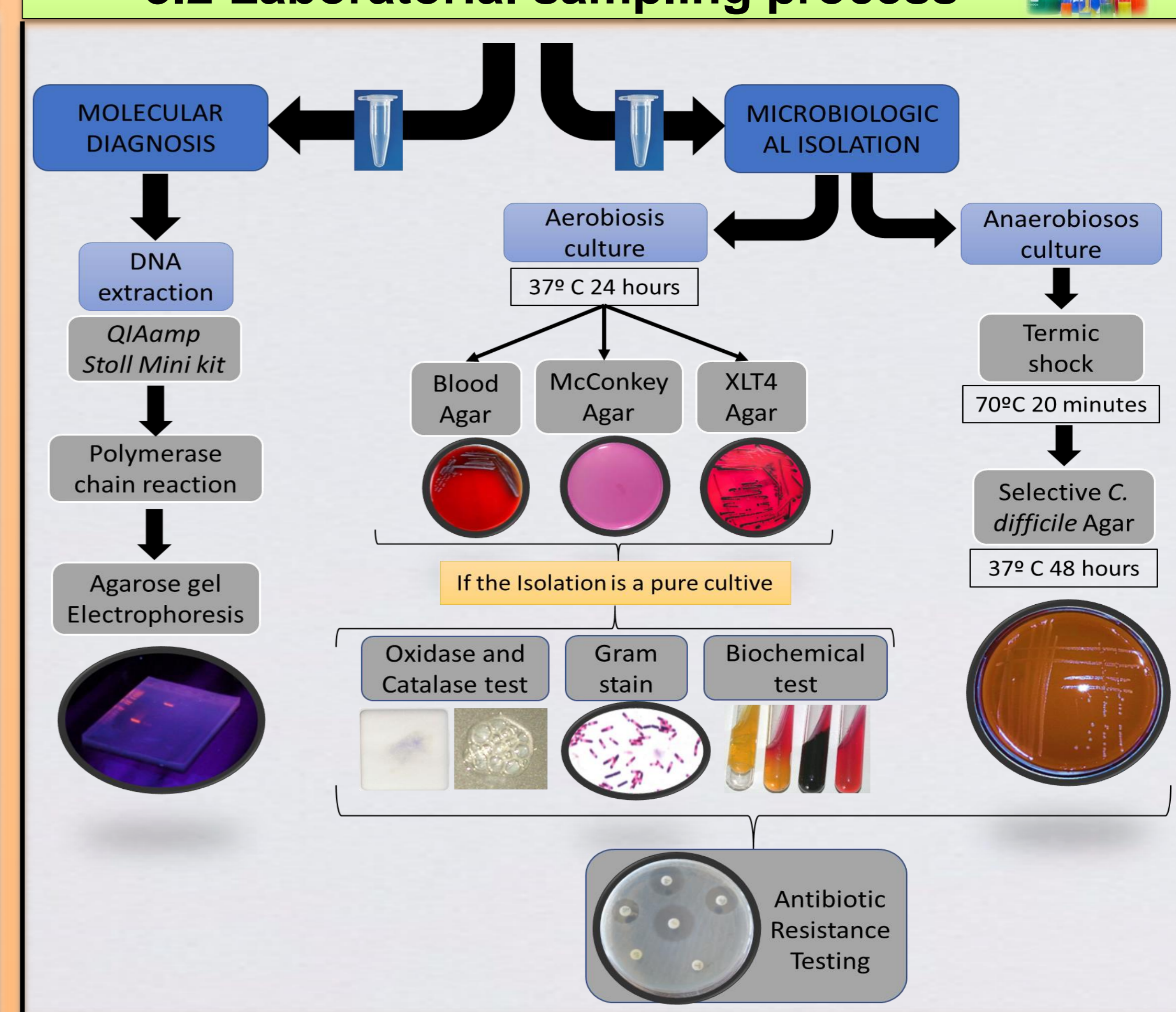
## 3. MATERIAL and METHODS

Wildlife attended at the Wildlife Rehabilitation Center (WRC) of Torreferrusa (Catalonia, Spain) were sampled at the first moment of the admission during the period between October of 2016 and May of 2017. Fecal or cloacal samples were systematically analysed by means of standardized microbiological and molecular techniques as shown in the scheme (3.2)

### 3.1 Sampling representation



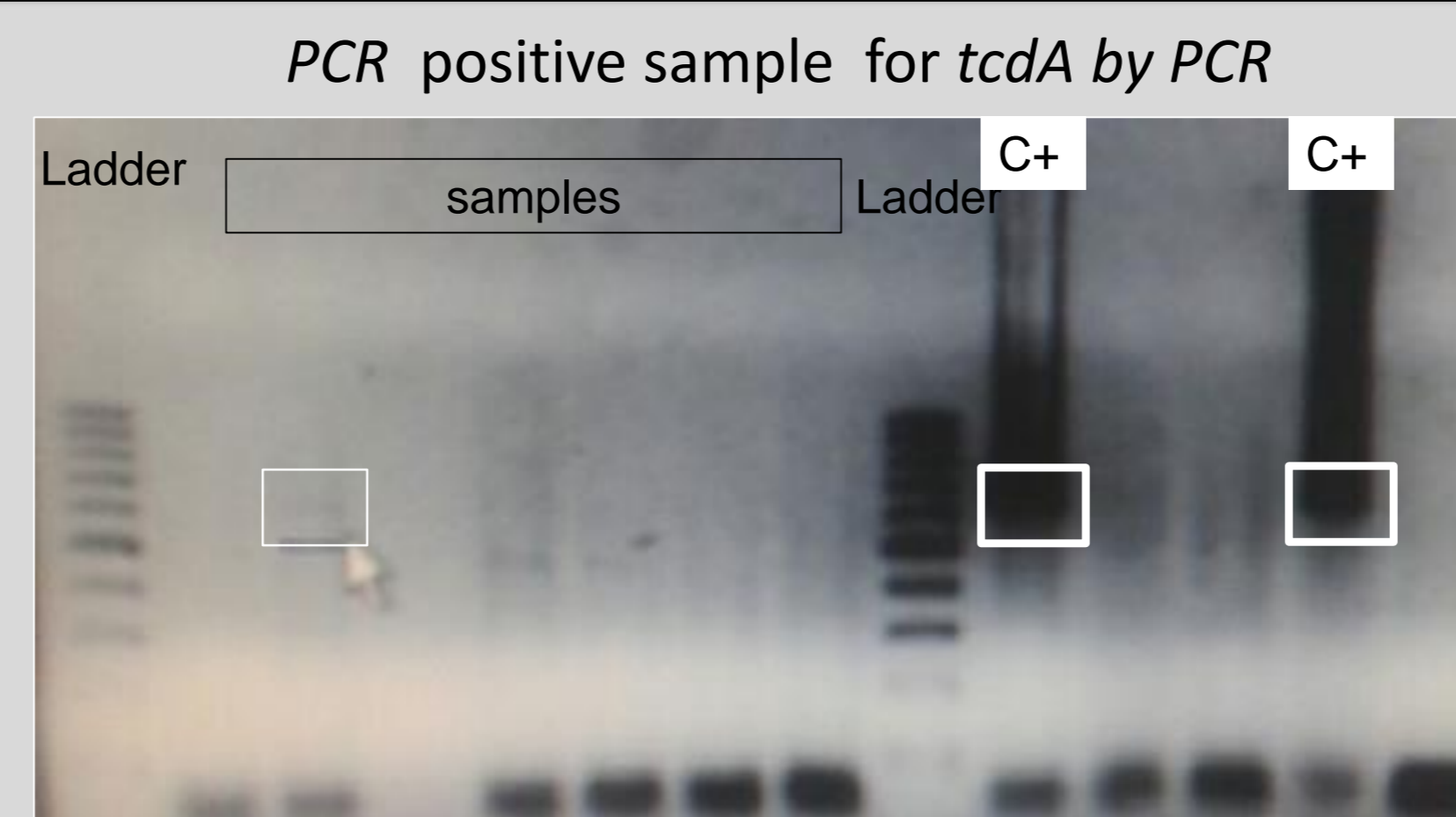
### 3.2 Laboratorial sampling process



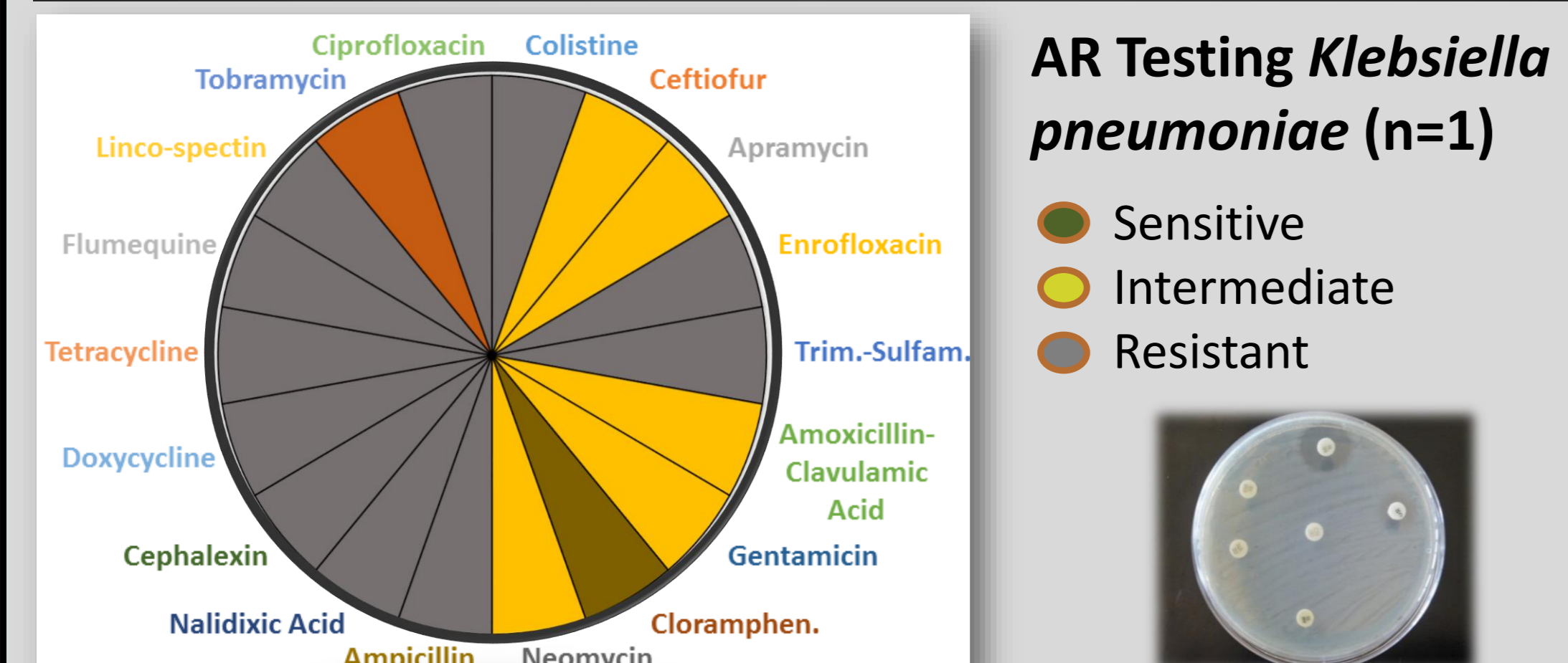
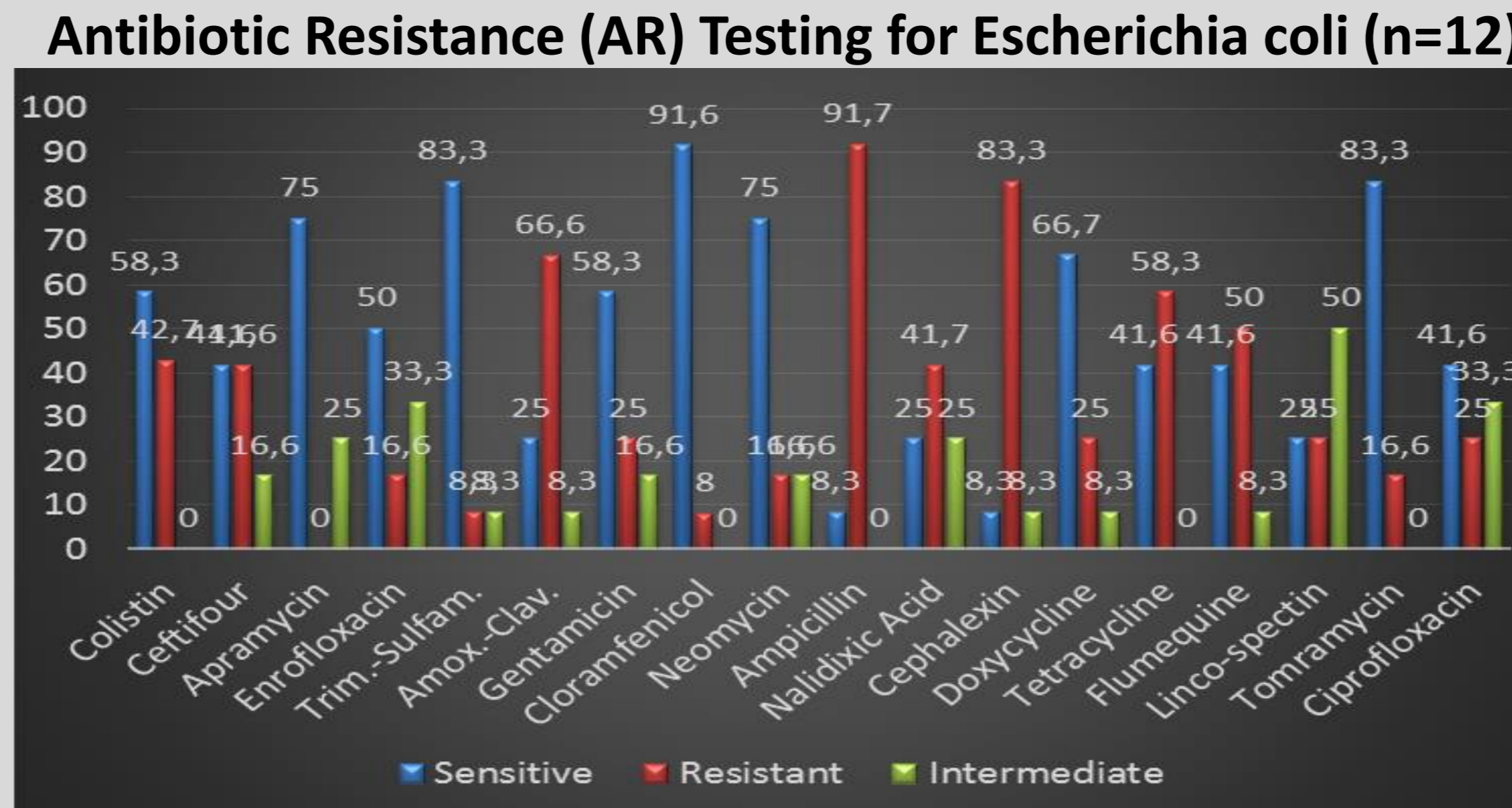
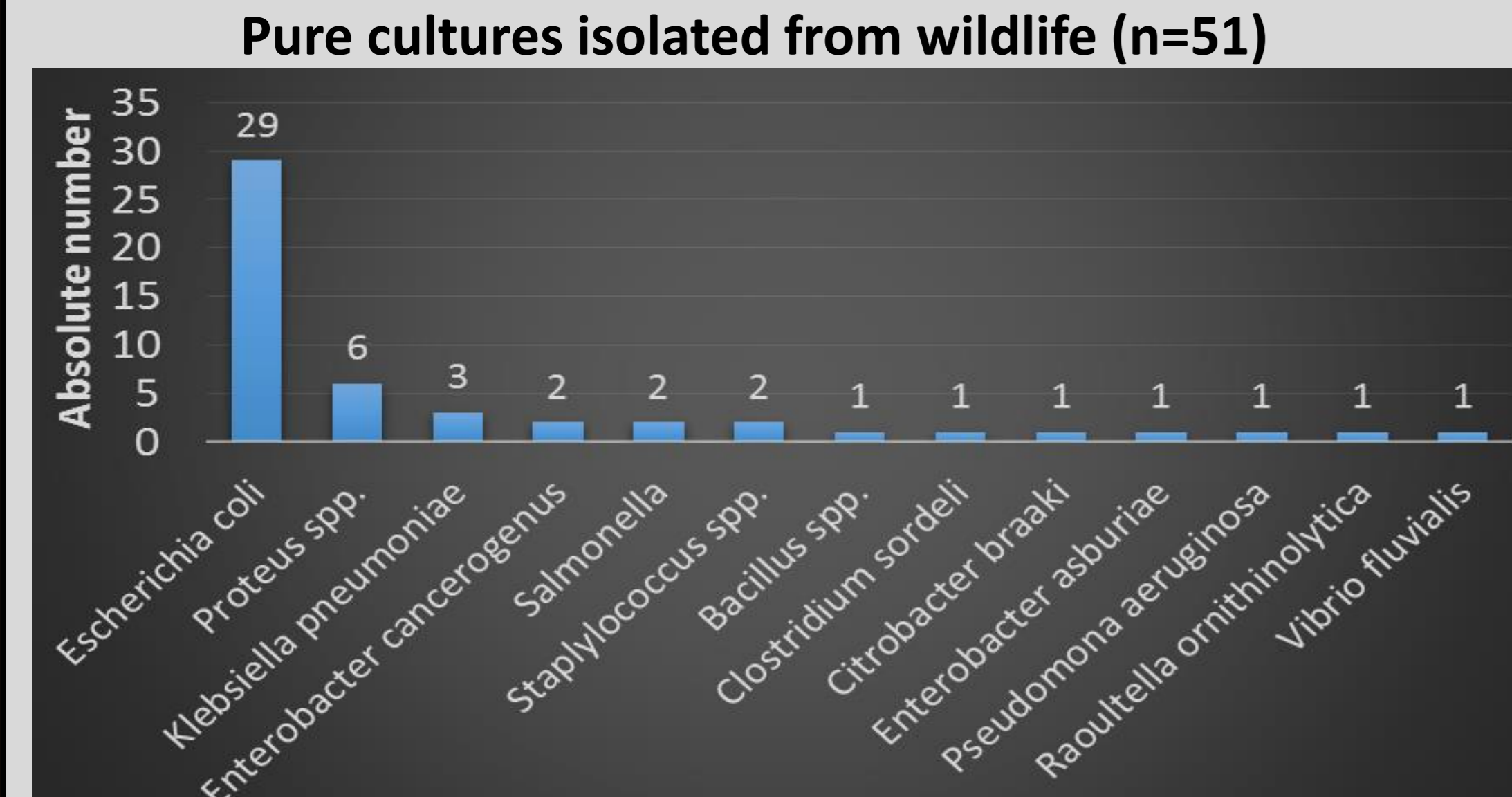
## 4. RESULTS

### 4.1 Prevalence of Clostridium difficile

- A total of 235 samples were analysed by microbiology and 123 by PCR.
- No direct *C. difficile* isolation was obtained in the selective media culture.
- Only 1 out of the 123 PCR-analysed samples was positive for the toxin A gene (*tcdA*). The sample came from an Eurasian Sparrowhawk (*Accipiter nisus*). None of the samples were positive for the toxin B gene (*tcdB*).



### 4.2 Other potential zoonotic bacteria



## 6. CONCLUSIONS

- This is the first description of a wild raptor positive by PCR to *C. difficile* toxigenic A strain in Catalonia.
- The presence of MDR bacteria in no-drug-treated wildlife is a real fact.
- The source of these infections could be the environment pollution secondary to intensive livestock production.
- The presence of these zoonotic bacteria in wild animals should be considered of relevance at the animal-human-ecosystem interface since they can disseminate them through their migratory routes.

## 5. DISCUSSION

The highest prevalence source of *C. difficile* infections have been described in pig and cattle farms. There are few studies reporting CD toxigenic strains in farm pest species such as rodents and shrews (Jardine et al. 2013; Himsforth et al. 2014; Andrés-Lasheras et al. 2017). However, studies from farming-free areas or Nature Parks such as Doñana in Spain (Alderete et al. 2011) or in Slovenia (Bandelj et al. 2011) obtained zero prevalence of CDI in wild animals and in wild passerine birds respectively.

In our study, a cloacal swab from an *Accipiter nisus* was positive for the toxin A gene of CD by PCR. This is the first description of a CDI positive wild raptor in Catalonia. This animal came from the Maresme (Catalonia) and it was hospitalized due to a mild electrocution. The *Accipiter nisus* is one of the smaller size raptors in Spain, which habits in non-urbanized, wooded areas, catching small birds. Catalonia is a region with a high density of porcine farms. Although we cannot determine the origin of this infection, it might be supposed that the *Accipiter nisus* could get infected by CD predated small birds and rodents that were in close contact to pig farms. As regards the microbiological results, the fact that no CD isolation could be seen even after the ethanol treatment for activating the spore germination, could be explained by the low burden load of wildlife as reservoirs of CDI. So, it is important to complement the detection using a much more sensitive method like the PCR.

Finally, the prevalence of multidrug resistant (MDR) bacteria such as *E. coli* or *Klebsiella pneumoniae* in wild animals, was another relevant fact. Most of *E. coli* isolates (75%) were resistant to 3 or more antimicrobials, as previously reported (Pinto et al. 2015; Vidal et al. 2016). The highest percentage of resistance were to Ampicillin (91,6%), Cephalexin (83,3%) and Amoxicillin-Clavulanic acid (66,6%). Moreover, *K. pneumoniae* was only sensitive to 11% antibiotics (Chloramphenicol and Tobramycin). All samples of the study provided from non-treated wild animals suggesting the source of MRD bacteria infection could be the pollution of the environment by the high density of livestock farms and the intensity of antimicrobials treatments.

## 7. Bibliography

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