# SALMONELLOSIS IN RABBITS. FIELD AND LABORATORY RESULTS DURING 1999-2011

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## ABSTRACT

In this study, we evaluated the incidence risk of salmonellosis on 394 commercial rabbit farms in Spain (374) and Portugal (21). Data were gathered on 2,269 visits performed by a trained veterinarian, during 2008-2011. The median size of the farms was 740 does (minimum to maximum: 40-9,000 does), and 50 bucks (minimum to maximum: 8-544 bucks). Refrigerated samples obtained from on-farm necropsies of aborted does, kits and runt growing rabbits were analyzed for isolation and typing of *Salmonella* spp. Clinical incidence risk was 4%, the same result as in a precedent retrospective study performed by the same practitioner, on 868 rabbit farms during 1997-2007. From the perspective of the Associations for managing Animal Health in food-producing animals, it was useful to point out the diffusion of infected young breeders and semen from selection breeder's centers and artificial insemination centers, respectively, to production farms.

**Key words**: Animal Welfare, Public Health, diseases prevention, rabbit, salmonellosis.

## INTRODUCTION

Salmonellosis in animals intended for human consumption is of great concern from the Public Health perspective (EC, 2003). Monitoring and surveillance protocols of the rabbit farms include this disease and its risk factors, in particular on farms in the open air. From the perspective of Associations for managing Animal Health in food-producing animals protocols include the comparison of strains between affected farms and its connections with centers of selection, breeder's multiplication or insemination centers, as well as its relationships with slaughterhouses.

The updating of individual and collective control methods is essential, as well as the training of technicians and breeders.

In a preliminary work we have shown the usefulness of vaccination in the control and eradication of *Salmonella enterica* ser. Typhimurium, on a farm evaluated during 5 years (Saco *et al.*, 1997).

The classification of the serotypes identified during the years 1990 - 1999 has been described in detail in a previous work by Peeters *et al.*, 2000

A few years later, we made a retrospective evaluation of the incidence of salmonellosis in 868 farms with a presumptive clinical diagnosis and with laboratory confirmation in 34 farms (4%), during the years 1997-2007 (Rosell *et al.*, 2009). The objectives of this retrospective study were: 1) to estimate the incidence of cases with a presumptive clinical diagnosis in the farms visited during the years 2008-2011; 2) to show the serotypes identified from 1999 to 2011; 3) to assess the existence of recurrences or relapses in affected farms during 1997-2007 and 2008-2011 and 4) to show updated results about

the clinical signs, epidemiology and control methods used recently against salmonellosis in farms of intensive production of rabbits.

## MATERIALS AND METHODS

### Farms and rabbits

Between January 2008 and December 2011, a trained veterinarian visited 394 farms, 373 in Spain and 21 in Portugal. Of the visited farms 374 were complete-cycle farms, with females (273) or females and males (101), 14 were insemination centers and 6 harbored weaned rabbits. 388 farms were for meat production, 2 farms were suppliers for pharmaceutical laboratories, 2 for fur rabbit and 2 for wild rabbit. Median size of the farms was 740 does (rank between 40 and 9,000 does) and 50 bucks (rank between 8 and 544 bucks). Rabbits for meat were from all the genetic types available in Spain and Portugal. The females were inseminated (AI) in 82% of farms at 11 days (67%), 18 days (12%), 25 days (12%) or > 25 days (9%). From the entire visited farm the population of risk was 368,077 rabbits and 10,369 males.

### Visits and protocols

Between January 2008 and December 2011 the veterinarian (Rosell) made 2,269 visits to 394 farms. A working protocol including clinical signs compatible with salmonellosis, the viability rate of kits, fertility rates, stillborn and abortions was used.

In addition, we analyzed kits, growers (mainly runts) and does dead, dying or culled. In suspected cases of salmonellosis refrigerated uterus, spleen, liver or caecal content samples were submitted to the microbiological laboratory. In other farms we took samples of caecotrophs, semen, surfaces of cages, feed, water or litter of the nests. Special attention to the existence of poultry farms in the surroundings or the presence of birds or rodents in the farm were also annotated. Insemination centres were requested for the microbiological results of semen analyses during recent months. Finally, therapeutic measures or medical prophylaxis applied were registered.

### Analyses

Animal or farm samples were collected and homogenized in sterile containers with 50 mL buffered peptone water (BPW, Merck, Germany). After 18 h at 37 °C, 0.1 mL of buffered peptone water broth was inoculated in Rappaport Vassiliadis Soy Peptone broth (RVS, Merck & Sigma) and incubated at 41.5 °C for 24-48 h. After this incubation period, aliquots of RVS broth were inoculated on two different Salmonella-selective agar plates (Xylose-Lysine-Dextrose Agar, Merck, Germany; and Xylose-Lysine-Tergitol 4 Agar, Difco, USA) and incubated at 37 °C for 24 h. Five presumptive *Salmonella* spp. colonies from selected plates were further sub-cultured overnight at 37 °C in Tryptone Soya Agar (TSA, Merck, Germany) before biochemical identification was undertaken using the Vitek system (BioMérieux, France).
*Salmonella* serotypes were determined according to the Kauffman-White classification scheme and using Difco's and Pasteur's specific somatic and flagellar antisera.

## RESULTS AND DISCUSSION

From 868 farms examined during 1997-2007, 34 farms (3.91%) had clinical signs compatible with salmonellosis and were confirmed by laboratory isolation of *Salmonella* spp. From 394 farms examined during 2008-2011, 16 farms (4.06%) had clinical signs compatible with salmonellosis and were confirmed by laboratory isolation of *Salmonella* spp.; this incidence was similar to that obtained during the period 1997-2007. Our data on incidence was similar to the data obtained by Borrelli *et al.* (2011) working on 25 farms, but less than the data reported by Agnoletti *et al.* (1999) on 23 farms and greater than the data obtained by Boucher *et al.* (2001).

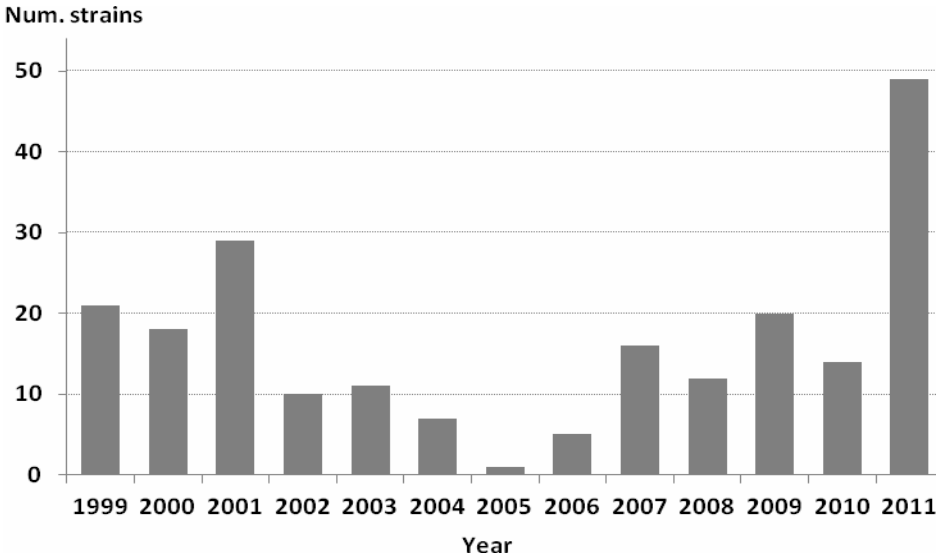


**Figure 1:** Yearly distribution of the number of *Salmonella* strains.

**Table 1**: Serotype distribution of *Salmonella* spp. detected during the periods 1999-2007 and 2008-2011.

| | 1999-2007 | | 2008-2011 | |
|---|---|---|---|---|
| | **Number strains** | **%** | **Number strains** | **%** |
| Enteritidis 9,12:g,m:- | 19 | 16,1% | 24 | 25,3% |
| Typhimurium 1,4,12:i:1,2 | 59 | 50,0% | 57 | 60,0% |
| *Salmonella* spp. 1,4,12:i:- | 9 | 7,6% | 1 | 1,1% |
| *Salmonella* spp. IIIa 48:z4,z23:- | 6 | 5,1% | 3 | 3,2% |
| *Salmonella* spp. IIIa 48:-:- | 1 | 0,8% | 10 | 10,5% |
| *Salmonella* spp. IIIa 48:Z4C... | 6 | 5,1% | ND | - |
| Hadar 6,8:z10:e,n,x | 2 | 1,7% | ND | - |
| Bovismorbificans 6,8:r:1,5 | 1 | 0,8% | ND | - |
| Panama 9,12:l,v:1,5 | 2 | 1,7% | ND | - |
| *Salmonella* spp. 9,12:l,v:- | 1 | 0,8% | ND | - |
| Mikawasima 6,7:y:e,n,z15 | 2 | 1,7% | ND | - |
| Grumpensis 13,23:d:1,7 | 5 | 4,2% | ND | - |
| Bredeney 1,4,12:l,v:1,7 | 1 | 0,8% | ND | - |
| *Salmonella* spp. 13,23:-:- | 1 | 0,8% | ND | - |
| *Salmonella* spp. IIIb 60:k:z53 | 1 | 0,8% | ND | - |
| Othmarschen 6,7:g,m:- | 1 | 0,8% | ND | - |
| Senftenberg 1,3,19:g,s,t:- | 1 | 0,8% | ND | - |
| **Total** | 118 | | 95 | |

ND = not detected.

During years 2008-2011 the hygiene procedures applied included the elimination of sick and suspect animals, disinfection of silos, farm facilities and drinking systems. Animal therapeutic was based on antimicrobial susceptibility tests carried out by CReSA or by other authors (Busani *et al.*, 2004, Gracia *et al.*, 2004), including antimicrobials used by parenteral route (*i.e.* sulfadiazine plus gentamicin or amoxicillin plus colistin), water (enrofloxacin, doxycycline) or feed (sulfadiazine, oxytetracycline, neomycin, apramicina or colistin). The immunoprophylaxis consisted in the use of autobacterins by oral route, applying 2 doses to adults and future breeding stock and one additional dose every six months.

It is interesting to note the higher serotype diversity during the 1999-2007 period and the persistence of the serotypes Enteritidis, Typhimurium and IIIa 48:z4,z23 or -:- throughout the whole analyzed period.

## CONCLUSIONS

The clinical incidence of salmonellosis is low. However, it is necessary to maintain the control in selection farms and centers of artificial insemination, as well as systematic microbiological studies in productive farms.

It seems that *Salmonella* IIIa 48:, *Salmonella* Enteritidis and *Salmonella* Typhimurium are well adapted to rabbit and are involved in almost all salmonellosis outbreaks in rabbitries of Spain.

## REFERENCES

Agnoletti F., Lenarduzzi M., Ricci A., Marotta A. 1999. Isolation of *Salmonella* spp. from Italian commercial rabbitries. *In: Proc.2nd International Conference on rabbit production in hot climates. Adana (Turkey), September 7th-9th, 1998. Testik A., Baselga M. (Eds.) Cahiers Options Méditerranéennes CIHEAM-IAMZ 41 189-193.*

Borrelli L., Fioretti A., Ruggiero V., Santaniello A., Cringoli G., Ricci A., Barco L., Menna L.F., Dipineto L. 2011. *Salmonella* Typhimurium DT104 in Farmed Rabbits. *J. Vet. Med. Sci.* 73, 385–387.

Boucher S., Gracia E., Villa A., Fernández A., Nouaille L., Briffaud M.A., Albizu I., Baselga R. 2001. Pathogens in the reproductive tract of farm rabbits. *Vet Rec. 149, 677-678.*

Busani L., Graziani C., Battisti A., Franco A., Ricci A, Vio D., Digiannatale E., Paterlini F., D'Incau M., Owczarek S., Caprioli A., Luzzi I. 2004.Antibiotic resistance in Salmonella enterica serotypes Typhimurium, Enteritidis and Infantis from human infections, foodstuffs and farm animals in Italy *Epidemiol. Infect.,132, 245–251.*

EC (European Communities) (2003) . Regulation (EC) No 2160/2003 of the European Parliament and of the Council of 17 November 2003, on the control of Salmonella and other specific food-borne zoonotic agents. *Official Journal of the European Union 12.12.2003, L 325/1-15. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:325:0001:0015:EN:PDF, accessed January 14, 2012.*

Gracia E., Baselga R., Fernández A., Albizu I., Villa A. 2004. Etiología de las diarreas en conejos. *Bol. de Cunic. 131, 20-26.*

Peeters J.E., Licois D., Saco M., Badiola J.I., Rosell J.M. 2000. Enfermedades del aparato digestivo: causas infecciosas (*Diseases of the digestive system: infectious causes*). *In: Rosell J.M. (Ed.), Enfermedades del Conejo (Diseases of the Rabbit). Chapter XV, Volume II. Mundi - Prensa Libros S.A., Madrid, Spain, 163-213.*

Rosell JM, de la Fuente LF, Badiola JI, Fernández de Luco D, Casal J, Saco M. 2009. Study of urgent visits to commercial rabbit farms in Spain and Portugal during 1997-2007. *World Rabbit Sci. 17, 127-136.*

Saco M., Badiola Sáiz J.I., Rosell J.M. 1997. Salmonellosis in a commercial rabbitry. A five-year survey from the laboratory and the field perspective. *In: Proc.: Symposium Salmonella and Salmonellosis'97. Ploufragan, France. May, 387-389.*