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THE TUNISIAN TRADITIONAL RABBIT BREEDING SYSTEM *VERSUS* THE COMMERCIAL SYSTEM: AN EPIDEMIOLOGICAL PERSPECTIVE

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Abstract: Rabbit breeding is practiced in many hot climate countries and contributes in terms of agricultural activities to rural development. In Tunisia two different rabbit breeding systems can be identified - the "traditional" (an integrated free range and underground system) and the "commercial" (employing sheds and wire net cages) practice. 24 Tunisian rabbitries (10 traditional and 14 commercial) were included in an epidemiological study aimed at comparing the prevalence of pathogenic bacteria responsible for upper respiratory and digestive diseases in the two breeding systems. A total of 128 adult rabbits (mean age of 9.4 months) were tested using deep nasal and rectal swabs. Symptoms of nasal discharge and/or diarrhea were recorded. 281 bacterial strains were isolated in total, 138 in commercial and 143 in traditional farms. The bacteria most frequently isolated (61.2%) were Gram-positive strains, which included *Streptococcus* sp. (22.8%), coagulase negative *Staphylococcus* sp. (17.8%) and coagulase-positive *Staphylococcus* (17.4%) strains. Among the Gram-negative isolated bacteria, *Escherichia coli* was the most frequent (12.5%), followed by *Proteus* sp. (5.7%). The two *Salmonella* strains isolated were *Salmonella bongori* (found in the commercial system), and *Salmonella typhimurium* definitive phage-type (DT) 104 (found in the traditional farming system). The absence of *Pasteurella multocida* in the list of isolated bacteria may be directly correlated to the method of conserving the samples (storage at freezing temperature). The coagulase-positive *Staphylococcus* strains were frequently isolated (73.5%) from healthy rabbits, but seldom from rabbits with rhinitis (18.4%) or diarrhea (8.2%). *Staphylococcus aureus* strains were recovered at a higher rate in commercial farms (21%) in comparison with traditional (11.9%) farms. However, this difference was not statistically significant. Four *Staphylococcus aureus* strains, all belonging to commercial farms, proved positive when tested for enterotoxin production. The antimicrobial susceptibility of isolated *Staphylococcus aureus* strains was also investigated. Most of the antibiotics tested were very effective: the highest level of susceptibility was observed with enrofloxacin (100%). The low performance of Tunisian traditional rabbit rearing has been linked to the high mortality rate, which may possibly be due to the increased presence of pathogens at the rabbit flock level. However, this was not confirmed by the results of our study due to the fact that the bacterial contamination seems to be comparable in both the traditional and the commercial breeding systems.

Key Words: rabbit, breeding system, bacterial diseases, *Staphylococcus aureus*, Tunisia.

INTRODUCTION

Rabbit breeding is practiced in many hot climate countries and, in terms of agricultural activities it contributes to rural development (Lebas *et al*., 1986) through the improved availability of an animal protein

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source in addition to the extra income generated by sale of the animals (Lukefahr and Cheeke, 1991). In Tunisia, until the late 80's, backyard rabbit breeding was exclusively a rural occupation, producing meat for self consumption and district commercialization (Bergaoui and Kriaa, 2001). However, in the last 20 years rabbit breeding in Tunisia has developed and continues to do so with the introduction of new technology and Government support. International organizations, such as the FAO with initiatives such as "International Observatory on Rabbit breeding in Mediterranean Countries" and the "International Centre for Advanced Mediterranean Agronomic Studies" (CIHEAM), have also endorsed some projects to improve Tunisian rabbit production.

Currently, two different rabbit breeding systems can be identified in this country (Kennou, 1990):

Traditional system

This is a backyard breeding practice normally integrating free range and underground systems. The free range system is the simplest, since it imitates the rabbit's natural living conditions. Feed and water are regularly supplied and rabbits are kept under control by some form of periphery fence. During the hot season grazing is combined with food leftovers, fresh forage, hay, barley and maize bran. Water is generally supplied via containers. The underground shelter imitates the living conditions of wild rabbits in that it consists of underground "burrows" dug out from the soil. This system provides a satisfactory environment for the rabbits, especially if they are free to move in and out according to the outdoor temperature (Kennou and Lebas, 1990).

Commercial system

This system usually employs sheds and wire net cages, reproducing the commercial-scale rabbit production that is largely diffused worldwide. Feeding is provided by commercial fodders (pellets); generally bucks and does are fed a measured amount, while growing rabbits are fed *ad libitum*. Water is supplied using wells or taps. Under hot climate conditions some problems should be considered, such as the expense of importing buildings and/or cages, and the fact that environmental control is more difficult to achieve than in temperate countries. As a consequence, productivity frequently fails to reach the level required for successful economic results (Finzi and Amici, 1991).

Many studies have been performed to compare free range and commercial systems on the basis of zootechnical parameters in order to determine if alternative methods of rabbit production could increase productivity in less developed, hot climate countries (Lebas *et al*., 1986; Nguyen Quang Suc *et al*., 1996). While some advantages are commonly recognized for free range and underground breeding systems —such as low costs, simple management, efficiency in environmental thermoregulation— the health conditions of rabbits in these traditional farms remain questionable (Finzi, 1986; El-Raffa, 2004). The objective of this study is to evaluate the prevalence of the prime pathogenic bacteria responsible for upper respiratory and digestive diseases in both traditional and commercial Tunisian rabbit farms, and detect the hygienic status of the rabbits maintained under the two breeding systems. The antimicrobial susceptibility of the *Staphylococcus aureus* strains isolated during the survey was also investigated, given that highly virulent strains can persist at the rabbit flock level due to the ineffectiveness of antibacterial treatments.

MATERIALS AND METHODS

Selection of animals and clinical observations

24 Tunisian rabbit farms were included in the study on the basis of the data provided by the Ministère de l'Agriculture et des Ressources Hydrauliques, Office d'Elevage et Pâturage (2006). Thirteen farms qualified as "traditional" (rural small-scale, colony-housing of rabbits with free range and/or underground

systems) and 11 as "commercial" (medium-scale, in hutches with wire net cages) (Kennou, 1990; Bergaoui and Kriaa, 2001). A sample ranging from 5 to 7 subjects was tested for each farm, in accordance with the size of the rabbitry. A total of 128 adult rabbits, 22 male and 106 female, were included in the study. All rabbits were crossbreeds from Tunisian local and exotic breeds (New Zealand Whites, Burgundy Fawn, Checkered Giant) and had to be over 2 months of age. The presence of nasal discharge and/or sneezing were occasionally recorded. Rhinitis was considered as the appearance of a mucopurulent discharge at the external nares (DiGiacomo *et al*., 1991), and was graded on a scale ranging of 1 (absent), 2 (mild) or 3 (severe). Clinical signs of diarrhea were also recorded. Intestinal disorders were graded on a scale of 1 (clean perineal region), 2 (stool tracks) or 3 (perineal region soiled by mucus and feces).

Collection and identification of bacterial strains

A deep nasal swab (from both nares) and a rectal swab were taken from all rabbits. A total of 130 and 126 samples were obtained in the conventional and traditional farms, respectively. Following collection, the bacterial strains were stored in cryobanks at -20°C until delivery to the Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Val d'Aosta (Sezione di Novara). Cryopreserved nasal and rectal samples were recovered by incubation for 16-24 h in a Buffered Peptone Water (BPW) and plated onto Columbia blood agar (CBA) and rabbit plasma fibrinogen (RPF) medium. Bacteria growing on CBA were identified using colony morphology, Gram staining characteristics (Gram-reaction and microscopic morphology) and oxidase (Gram- negative bacilli) or catalase (Gram-positive cocci) tests (Carter, 1979).

Bacteria growing on RPF medium were observed for the detection of coagulase positive *Staphylococci*. *Staphylococcus aureus* was identified using colony morphology, haemolytic patterns on CBA, Gram staining characteristics, catalase, coagulase production and a positive result with the API Staph biochemical identification system (Biomérieux®SA, Marcy-L'étoile, France). Enterotoxin production was tested using the Transiaplate Staphylococcal Enterotoxin Test (ST0796; Diffchamb®, Lyon, France). Cryopreserved rectal samples were recovered by CBA in an RPF medium and in a Rappaport Vassiliadis soya peptone broth (RVS) for 24 hours. Bacteria grown on RVS were transferred both to Xylose-Lysine-Deoxycholate Agar (XLD) and to Brilliant Green Agar (BGA) in order to identify *Salmonella* sp. and *Escherichia* sp. The detection of *Enterobacteriaceae* was confirmed using the reaction in Triple Sugar Iron Agar slants (TSI), according to the standard diagnostic procedures (Carter, 1979). *Enterobacteriaceae* species were recognized using the biochemical API 20E identification system (Biomérieux®SA, Marcy-L'étoile, France). All isolated *Salmonella* strains underwent serotyping (Kauffmann–White method) and the *S. typhimurium* was sent to the Istituto Zooprofilattico Sperimentale delle Venezie (Sezione di Padova) for phagetyping.

Antimicrobial susceptibility testing

Each *Staphylococcus aureus* strain was subjected to susceptibility testing. A standardized disk diffusion antimicrobial susceptibility test was performed according to the Kirby-Bauer method. Seven antimicrobial agents were tested: enrofloxacin (ENR), gentamicin (GM), oxytetracycline (OT), trimethoprim-sulfamethoxazole (SXT), spiramycine (SPR), flumequine (FLU) and colistine (COL). The zone of inhibition of each disk was measured, and each bacterium was classified as "Sensitive", "Intermediate" or "Resistant" in accordance with the norms of National Committee for Clinical Laboratory Standards (NCCLS, 2002).

Statistical analysis

Differences in frequency of bacterial isolation findings between traditional and conventional farms were tested for significance ($\alpha<0.05$) by sample proportion comparison with normal approximation; whereas contingency tables tests was performed by chi-square analysis or Fisher's exact test using SAS (2002).

Table 1: Number of cases and percentage (%) of clinical signs of upper respiratory tract disease.

Rhinitis [a]	Commercial farms	Traditional farms	Total
Grade 1	47 (72.3%)	52 (82.5%)	99 (77.3%)
Grade 2	11 (16.9%)	8 (12.7%)	19 (14.9%)
Grade 3	7 (10.8%)	3 (4.8%)	10 (7.8%)

[a] Grade 1: absent; Grade 2: mild; Grade 3: severe.

RESULTS

Description of the sampled population

The age of the animals tested ranged from a minimum of 4 to a maximum of 24 months. The mean age was 9.4 months (11.5 and 7.3 months in commercial and traditional farms respectively). When considering the whole population the prevalence of nasal discharge was higher (22.7%) than the prevalence of diarrhea (13.3%), but the difference was not statistically significant .

Detected upper respiratory tract diseases are reported in Table 1. Nasal discharge was observed in 22.7% of the total rabbit population studied. The prevalence of both mild and severe rhinitis was higher (27.7%) in rabbits from the commercial breeding system when compared to those from traditional farms (17.5%). This difference was not statistically significant.

Clinical signs imputable to intestinal disorders are reported in Table 2. 13.3% of the observed rabbits showed signs of diarrhea. The common prevalence of diarrhea graded on scales 2 and 3 was similar for both commercial (10.7%) and traditional (15.9%) farms.

Microbiological results

Isolation and identification of bacteria

281 bacterial strains were isolated from the 256 swabs, 138 in commercial and 143 in traditional farms. All bacteria isolated during this study are presented in Table 3. A considerable amount of opportunistic pathogens was isolated, these being *Streptococcus* sp., coagulase negative *Staphylococcus* sp., *Enterobacter* sp., *Pseudomonas* sp. and *Klebsiella pneumoniae*. Among the 64 swabs contaminated by *Streptococcus* sp., 28.1% was recovered from rabbits with nasal discharge and 15.6% from rabbits with intestinal disease. A similar rate was found for the coagulase-negative *Staphylococcus* strains (32% associated with nasal discharge and 16% with diarrhea). The bacteria most frequently isolated were Gram-positive strains (61.2%), which included *Streptococcus* sp. (22.8%), coagulase-negative *Staphylococcus* (17.8%) and coagulase-positive *Staphylococcus* (17.4%) strains. Among the Gram negative bacteria, *Escherichia coli* was most frequently isolated (12.5%), followed by *Proteus* sp. (5.7%). 71.4% of *Escherichia* strains were obtained from asymptomatic subjects, 20% from subjects with rhinitis, and 8.6% from diarrheic rabbits. In the commercial system 5.8% (8 of 138) of the swabs carried *Escherichia coli*, which differed

Table 2: Number of cases and percentage (%) of clinical signs of intestinal disease.

Diarrhea [a]	Conventional farms	Traditional farms	Total
Grade 1	58 (89.2%)	53 (84.1%)	111(86.7%)
Grade 2	5(7.7%)	8 (12.7%)	13 (10.2%)
Grade 3	2 (3%)	2 (3.2%)	4(3.1%)

[a] Grade 1: absent; Grade 2: mild; Grade 3: severe.

Table 3: Bacterial isolation findings: number of isolates and prevalence (%).

Bacterial strains	Total	Commercial farms	Traditional farms	Nasal swabs	Fecal swabs
Enterobacteriaceae:	61(21.7)	24(17.4)	37(25.9)	10(6.8)	51(37.8)
Escherichia coli	35(12.5)	8(5.8)	27(18.9)	3(2.1)	32(23.7)
Enterobacter sp.	6(2.1)	3(2.2)	3(2.1)	5(3.4)	1(0.7)
Klebsiella pn.	1(0.4)	0(0)	1(0.7)	1(0.7)	0(0)
Salmonella Typh.	1(0.4)	0(0)	1(0.7)	0(0)	1(0.7)
Salmonella sp.	1(0.4)	1(0.7)	0(0)	0(0)	1(0.7)
Proteus sp.	16(5.7)	11(8)	5(3.5)	1(0.7)	15(11.1)
Providencia st.	1(0.4)	1(0.7)	0(0)	0(0)	1(0.7)
Pseudomonas sp.	1(0.4)	0(0)	1(0.7)	1(0.7)	0(0)
Pseudomonas aer.	2(0.7)	2(1.4)	0(0)	1(0.7)	1(0.7)
Other Gram- bacteria	12(4.3)	8(5.8)	4(2.8)	5(3.4)	7(5.2)
Streptococcus sp.	64 (22.8)	29(21)	35(24.5)	16(11)	48(35.6)
Staph. coagulase -	50(17.8)	23(16.7)	27(18.9)	38(26)	12(8.9)
Staphylococcus aureus	46(16.4)	29(21)	17(11.9)	45(30.8)	1(0.7)
Bacillus sp.	9(3.2)	4(2.9)	5(3.5)	7(4.8)	2(1.5)
Polymicr. Culture	33(11.7)	16(11.6)	17(11.9)	22(15.1)	11(8.1)

significantly ($P<0.001$) from the 18.9% (27 of 143) found among swabs collected in the traditional system. The two *Salmonella* strains isolated were *Salmonella bongori* (found in a commercial system) and *Salmonella typhimurium* definitive phagetype (DT) 104 (in a traditional farm). They were both recovered from rabbits without any clinical signs of disease. A high percentage of coagulase-positive *Staphylococcus* strains (73.5%) were isolated from healthy rabbits, and a lower percentage from rabbits with rhinitis (18.4%) and diarrhea (8.2%). All but one *Staphylococcus aureus* and most ($P<0.001$) coagulase-negative *Staphylococcus* sp. were found in nasal samples. *Staphylococcus aureus* strains were recovered at a higher rate in commercial farms (21%) compared to traditional farms (11.9%), but this difference was not statistically significant. Four *Staphylococcus aureus* strains from the 46 isolated, which were recovered in commercial farms, tested positive for enterotoxin production. The 33 swabs with no identified polymicrobial cultures belonged either to healthy rabbits (78.8%) or to rabbits suffering from snuffles (12.1%) or from diarrhea (9.1%).

Antimicrobial susceptibility

Antibiotic susceptibility results are presented in Table 4. The majority of the antimicrobial agents were very effective against *Staphylococcus aureus* strains. The highest level of susceptibility was observed with ENR (100%), followed by SXT (97.8%), FLU and GM (95.7%), SPR and OT (93.5%).

DISCUSSION

Based upon the characteristics of rabbits documented decades ago in the classic paper by Owen (1976), scientists have for years been advocating the great potential for rabbit production in less developed, hot climate countries. However, certain obstacles have hampered the transfer of sophisticated technologies

Table 4: Percentage of antibiotic susceptibility results and between brackets total number of strains isolated.

	Staphylococcus aureus (46)		
Antibiotic[1]	Sensitive	Intermediate	Resistant
COL	8.7	6.5	84.8
ENR	100	0	0
FLU	95.7	0	4.3
SPR	93.5	2.2	4.3
SXT	97.8	2.2	0
OT	93.5	0	6.5
GM	95.7	0	4.3

[1]COL, colistine; ENR, enrofloxacin; FLU, flumequine; SPR, spiramycine; SXT, trimethoprim-sulfamethoxazole; OT, oxytetracycline; GM, gentamicin.

from industrialized regions. Firstly, infrastructures were often inadequate to support the needs of complex production systems, and secondly environmental conditions of rabbitries were more difficult to control than in temperate climates (Finzi, 1986). In the last two decades, a variety of traditional housing systems has been tested to determine if alternative systems of rabbit production could improve rabbit breeding in rural regions of hot climates countries, overcoming the negative effects of high temperatures. For example, a traditional rabbit housing system characterized by underground cells was settled in West Noubaria (Egypt) as a sideline enterprise of an FAO consultancy. This development obtained good standards of animal welfare and satisfactory productive results (Finzi, 1986). Another similar system has been recommended for rabbit rearing in the Saharan region (Finzi and Amici, 1991). In tropical Vietnam, a study by Nguyen Quang Suc *et al*. (1996) reported favorable productivity performances linked to an underground shelter system of housing. A similar free-range system was reported by Lukefahr (1998) from Uganda, where farmers allowed their rabbits to graze during the day and coralled the flock into small huts for the night as a safeguard against predators and thieves. The advantages that associate free range with underground breeding systems are: low costs, simple management, efficiency in environmental thermoregulation and adaptability to different situations. On the other hand, the hygienic conditions of these traditional rabbits farms are questionable, given that under such hot climate conditions, where rabbits are more prone to suffering from diseases, healthcare becomes essential (El-Raffa, 2004). Some authors reported an improvement in hygienic conditions in the free range breeding system as a consequence of the high microbial dispersion into the air and the absence of direct contact between animals (Finzi, 1986). In contrast, other scientists stressed that housing rabbits on the ground may heighten the likelihood of disease outbreaks as a result of the animals being more directly exposed to infectious agents (Lukefahr and Cheeke, 1991).

Infectious upper respiratory tract disease, more generally called ‘‘snuffles’’, is considered to be one of the most common diseases observed in rabbits (Langan *et al*., 2000). Clinical signs include nasal discharge and sneezing. The agent most commonly implicated in these symptoms is *Pasteurella multocida* (Langan *et al*., 2000, Rougier *et al*., 2006). However, other pathogens are also cited, such as *Bordetella bronchiseptica* and *Staphylococcus* sp., although apparently this is not an exhaustive list. Here we confirmed a tendency towards an elevated occurrence of nasal discharge (mild and severe rhinitis) in rabbits from the commercial system compared to those from the traditional breeding system. However, this difference was not statistically significant. The absence of *B. bronchiseptica* and *P. multocida* in our list of isolated bacteria may be directly linked to the methods adopted for collecting and identifying the bacteria. Both *Pasteurella* and *Bordetella* grow on blood agar, but their isolation from field specimens

is often complicated by the overgrowth of other organisms. Subsequently, selective media are generally required for cultures (Lariviere *et al*., 1993). Storage in cryobanks at freezing temperature could also have rendered the preservation of these sensitive bacteria difficult. Compared to apparently healthy rabbits, we observed a greater isolation rate of *Streptococcus* sp. and coagulase-negative *Staphylococcus* strains in snuffling subjects. In contrast, 73.5% of *Staphylococcus aureus* was detected in rabbits without any clinical signs of disease. In effect, two types of *Staphylococcus aureus* strains are identifiable, which have a low and a high virulence and cause two different infection patterns. The low virulence strains, which are likely to be involved in our study, are responsible for clinical signs in only a small number of rabbits belonging to a flock, whereas the high virulence strains can cause an epidemic spread of disease (Hermans *et al*., 2003). *Staphylococcus aureus* was recovered at a higher rate in commercial farms as opposed to traditional farms, but this difference was not statistically significant. We discovered a relatively low level of antimicrobial resistance, which concurred with previous data for the low virulence strains (Vancraeynest *et al*., 2004). None of the isolated *Staphylococcus aureus* strains were resistant to enrofloxacin, and 45 out of 46 strains were sensitive to trimethoprim-sulfamethoxazole. As was to be expected, colistine showed no real activity (8.7%).

Digestive disorders are another common health problem in rabbit farms and are responsible for significant morbidity and mortality. The etiology of intestinal diseases still remains difficult to establish due to the fact that the causes can often be multiple and the clinical signs and intestinal lesions comparable. Furthermore one of the clinical signs, —diarrhea— is largely dominant. Among the microbial causes of the intestinal pathology, enteropathogenic *Escherichia coli* (EPEC) is predominant. Sometimes other bacteria, such as *Salmonella enterica*, *Clostridium spiroforme*, *Klebsiella* sp. and, exceptionally, *Clostridium piliforme*, are involved (Bennegadi *et al*., 2000). We found strains of *S. typhimurium* (DT) 104 on a rural farm. This bacterium is particularly feared due to its multiple antimicrobial resistances (to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracyclines) and its worldwide diffusion (Threlfall, 2000). During the last few decades, it has been recognized as a major hazard for humans in most developed countries, the main source of infection being contaminated food of animal origin (Baggesen *et al*., 2000). In our study the prevalence of diarrhea was comparable in conventional (10.7%) and traditional (15.9%) farms, even if the presence of *Escherichia coli* in traditional farms (18.9% of the 143 strains detected) was higher when compared to commercial farms (5.8% of the 138 strains detected). In traditional farms, the rate of diarrhea was higher than the rate of respiratory diseases, probably due to the features of this breeding system housing rabbits on the ground. The prevalence of bacteria involved in intestinal disorders in the entire sampled population concurs with the literature (DiGiacomo *et al*., 1991), in that we found a high prevalence of *Escherichia coli* (12% of the whole population), followed by *Enterobacter* (2.1%) and *Salmonella* (0.8%).

CONCLUSIONS

Tunisian traditional rabbit rearing has quite a low performance level which is linked to a high mortality rate (Bergaoui, 1992). It was assumed that this could be explained by the increased presence of pathogens at the rabbit flock level, but this may not necessarily be the only cause as the diversity of bacteria appears to be comparable in both traditional and conventional breeding systems.

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