Proceedings 4th World Rabbit Congress, 10-14 October 1988, Budapest Hungary, Vol. 2, 137-148

ESTABLISHMENT OF A SPECIFIED PATHOGEN FREE BREEDING COLONY (SPF) WITHOUT HYSTERECTOMY AND HAND-REARING PROCEDURES

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INTRODUCTION

Research concerning rabbit pathology necessitated the establishment of a SPF breeding colony in our Institute. The first SPF rabbits were obtained by aseptic hysterectomy followed by hand-rearing (Schellenberg 1976). This type of technique used by several researchers since the first successful experiments by Wostman and Pleasants (1959) presents several difficulties, advantages and risks.

The difficulties primarily concern the hand-rearing of neonates (composition of milk formula, aspiration into lungs) (Stepankova et al 1972). The advantages of this method are that it eradicates all parasites except perhaps Encephalitozoon cuniculi (Kruijt 1985). The disadvantages stem from the micro-flora which establishes itself. Apart from cases where the doe's own milk is used, from the beginning the microflora is non-specific to the rabbit (Gouet et al 1976, Rioux et al 1976). In any case, all the researchers quoted above, and many others (Dabard et al 1976) have shown that in the absence of early existence of microflora, there was a high rate of mortality due to excessive development of caecum (megacoecum). The only research team not to indicate this development is Scher et al (1969). This micro-flora must be host-specific (holoxenic) to obtain animals which will later reveal a physiological and pathological state subject to interpretation by the users. This implantation is generally carried out with the caecal content of an adult presumed to be healthy (Boot et al 1985). This point presents the greatest risks.

In fact, the latter technique which we adopted in 1969 turned out to be effective, since during the following eight-year period, no specific pathological problem appeared in the breeding colony.

On the one hand, the appearance of a fungus (Aspergillus clavatus) which developed on the droppings, highly allergenic for humans, and on the other the necessity of replacing our breed descended from Fauve de Bourgogne by a breed closer to the ones which exist in commercial rabbitries led us to replace our stock completely and create a SPF breed.

The hysterectomy method was not used as it presents the uncertainties mentioned above. This method is very costly, necessitating more personnel due to hand-feeding, and takes a long time to set up, particularly when, from the start, there has to be sufficient breeding animals to avoid inbreeding. The method described in our work is based entirely on well-known medical and hygienic prophylaxy, and on a certain number of hypotheses established regarding the implantation of a holoxenic micro-flora capable of forming a barrier to undesirable germs.

Material and Method

The general procedures improving the sanitary status of a breed are based on two complementary methods : the elimination of certain pathogenic agents through specific or global prophylactic methods and the repetition of these methods on three generations which rapidly succeeded each other and which were reared in separate rooms.

I. Breeding units and general hygiene measures

- Original breeding colony *

This is an experimental breeding colony for selection. The conditions of hygiene are stricter than those usually enforced in conventional rabbitries. For several years there has been no external animal brought in and the diet is supplemented with Robenidine.

- Barrier breeding system** * I.N.R.A. centre de recherches de Toulouse ** I.N.R.A. centre de recherches de Tours - Nouzilly

Separate from the experimental zone, we have three breeding rooms. Each one contains 76 breeding cages and an equal number of rearing cages. There is no means of communication between these rooms and each has specific personnel. Before the animals are introduced into the rooms all unfixed material is autoclaved (cages, nesting boxes, feedboxes, watering valves, water pipes). The room is disinfected with pressurized steam, the internal temperature maintained at 40°C for a 12-hour period to kill any oocysts present. The final disinfection is carried out with gazeous formol during a 24-hour period. Once the animals are inside, no other non-autoclaved material is allowed into the room. Only one person has access to the room and has to respect strict rules of hygiene : he must go through several lock-chamber with clothing and shoes specific to each stage. One-piece overalls, gloves and a mask are obligatory in the breeding room. Ventilation is carried out through superpressure of air filtered to 10 um. Diet is not autoclaved but the bags are treated with formol. There is no additive to the diet apart from an anticoccidian.

II. Succession of generations (Tab. 1)

The original colony is made up of the INRA 1077 breed*, which comes from a breed of New-Zealand White.

The first generation introduced into our protected zone was made up of 40 four-week old does and 40 4-week old males. The only precautions taken in the original colony was a treatment of the females with tetracycline 2 days before weaning, which took place at 21 days ; this same treatment was administered to young rabbits on the day that they were transferred to the Institute (600 km). On arrival, two groups were formed. 40 0 and 10 0 (generation 1) were placed in a breeding room (A) and the 30 remaining males, of which there was at least one element of each litter introduced

into the breeding cell, were placed in the experimental zone. These males were submitted to different tests in order to identify the pathogenic agents of which they would have been carriers.

The first generation was put into reproduction at 17 weeks. The first litter of each female (generation 2 = 60 0 and 22 0), weaned at 21 days, was placed immediately into another breeding room (B) and after having been submitted to the same prophylactic measures as generation 1. This second generation gave birth to the third generation in the same conditions (76 0 and 300 0), weaned at 21 days and on the same day placed in a third breeding room (C). This third generation represents the final stage of our experiment. The following generations will no longer stem from the first litters, in view of the zootechnical disadvantages that this represents. To limit the increase of inbreeding on the one hand, and on the other to diminish genetic variability, we apply a coupling plan adapted to small reproductive groups (Matheron, Chevalet 1976).

III. **Methods of elimination of pathogenic agents** (Tab 1)

- **Elimination** of coccidia (Eimeria sp). This was obtained through the permanent use of diet with anticoccidian supplement.

Considering the lack of effectiveness of certain anticoccidians on certain species (Peeters et al 1979, Coudert, Provot 1988) and the possible acquisition of chemical resistance (Peeters et al 1987), several anticoccidians were used in rotation every 2 to 3 weeks : Toltrazuril 35 ppm, Salinomycine 20 ppm, Lasalocide 90 ppm, Robenidine 66 ppm).

- Elimination of Oxyuris (Passalurus ambiguus). Fenbendazole 50 ppm in the diet was used three times during a 2-week period.

- **Elimination** of mange (Sarcoptes and Psoropted). Ivermectine 200 ug/kg in intra muscular form was administered 3 times to the first generation.

- Elimination of pasteurella (P. multocida). Considering the frequency of contaminated breeding does (Coudert et al 1986), we felt there was reason to consider that all the original females were potentialy contaminated. Basing our conclusion on our own observation and other works such as Holmes (Holmes et al 1983, 1984a, 1984b), we considered that the neonates could be contaminated particularly during three specific periods : at birth, on emergence from the nesting box, especially when in contact with the watering valve, and finally at the time of weaning, which could produce stress in the young favouring the colonization of Pasteurella.

Based on these hypotheses, a medical and hygiene prophylaxis was established. The gestating females were treated on the 24th and 28th day of gestation and 3 days before weaning, with terramycine "long acting" (40 mg/kg by IM). This product was chosen because the rabbit tolerates it particularly well (Schroder 1982) and also because of its long-lasting action (Cringoli and Paparella 1986) which reduces the number of manipulations (Mc Elray 1987). The same treatment was used on the young rabbits at the age of 21 days (weaning) and 24 days (20 mg/kg by IM).

The hygiene prophylaxis was based on a maximal separation between the young rabbit and its environment (mother, watering values, cage...) ; the nesting box was hence closed and the mother could only feed the young 5 minutes a day. Additionally, weaning was carried out at an early stage, 21 days. To reinforce these preventive measures, the young rabbits were vaccinated with an autovaccine made up from the only strain of pasteurella

isolated in the original breeding group, which is of type A3, and a strain of Bordetella bronchiseptica of the same origin. The vaccine was administered at 5, 9 and 16 weeks with the last booster given a few days before the animal was first mated.

- Global prophylaxis of infectious enteropathy. To try and obtain a favourable intestinal flora, we based our work on the standard observation that foods rich in protein were a risk factor (diahorrea, enterotoxaemia) and also that food rich in cellulose was a secure factor.

The extent and nature of caecal micro-flora which establishes itself in the young rabbit (Christ and Victor 1975 ; Gouet and Fonty 1979) plays a major role in triggering off digestive problems. Mathes (1969) was one of the first researchers to try and analyze this, and work by Salse and Raynaud (1985) shows more precisely that in their experimental conditions the early implantation of a dominant cellulalytic micro-flora results in a general improvement.

Taking into consideration these latter observations, the young rabbits from the first generation which never had access to the mother's feed (nesting boxes closed until weaning at 21 days) were given a diet rich in cellulose, inside the nesting box from the 17th day until 3 days after weaning from the 25th day onwards they were given standard diet. The first diet specially manufactured for this case contained 18 % cellulose and 13 % protein (Lebas, personal communication) and the second is a commercial feed : 16 % cellulose and 16 % protein.

IV. Methods of detection of pathogenic agents

- Coccidium : at the time of weaning at least two animals from each of the first litters are transferred to experimental rooms and fed with a coccidiostatic-free diet. The coproscopic tests are carried out several times before the animals are killed at 10 weeks of age. All successive litters are controlled in the breeding room after a 2-week break in the administration of the supplement. The coproscopy is carried out after floating in a solution of magnesium sulphate (d=1,14).

- Oxyuris : a coproscopic test is performed on future breeders aged 11 and 15 weeks.

- Mange : all the breeders are clinically examined and samples taken from the outer ear are analyzed under the microscope.

- Pasteurella : the P.m. were detected through direct bacteriological techniques or after enrichment. The samples were taken from the turbinates and in adults from the middle ear. A sample member of each litter descended from the parent group was examined after being killed at 8 weeks. An autopsy and tests were carried out on all the females from this same first generation when their first litter was weaned. After this stage (generations 2 and 3), all the unfit females were examined bacteriologically, as well as a certain number of their unvaccinated offspring which remain as control subjects in the cell until they are 90 days old, before undergoing tests ; 59 bacteriological tests were thus carried out.

- Clostridium and E. coli : in each generation, several samples of 10 to 20 young rabbits were treated either with clindamycine (15 mg/kg/day per os for 3 days) or with ampicillin (30 mg/kg by IM for 2 days). In all species, the first antibiotic favours multiplication of Clostridia and is particularly

effective in rabbits (Katz et al 1978, Fischetti et al 1986). Following the work by Milhaud et al (1976) the second product has been recognized as "toxic" for the rabbit as it systematically brings on colibacillarian diahorrea. These two antibiotics are frequently used in our laboratory in studies of experimental diahorrea (Licois 1980). The two germs specially tested for were Clostridium spiroforme and E. coli 0103, which in France at least, is suspected to be the main cause of mortality due to enteritis after weaning.

Results

Pathogenic agents

- Coccidium and mange : these two families of parasites were never detected, including in the group of animals in the first generation which came directly form the original breeding colony.

- Oxyuris : the animals from the first generation were contaminated. All the tests on the second and third generation proved negative.

- Pasteurella : two animals from the first generation proved to be carriers. All their brothers and sisters (generation 1) and offspring (second generation recently weaned) were eliminated. After this all the bacteriological tests carried out on the animals from rooms B and C proved negative. It may be remarked at this point that all the animals tested are carriers of Bordetela bronchiseptica.

- Clostridium spiroforme and E. coli 0103 : these two germs were identified in several animals from the group of 30 males taken as control subjects on arrival from the original breeding colony. After this all the tests carried out on animals from the second and third generation were negative.

Assessment of production and morbidity

The general results are shown in Fig. 2. To analyze this data and to compare it with the same breed in standard breeding conditions, it is necessary to take into account three important points :

- the females had a very short reproductive life (max 3 to 4 litters) hence the impact of the first litter is important.

- these breeding does are all members of their mother's first litter

- the rythm of reproduction is sometimes deliberately lengthened to regroup the mating in order to provide the users with large groups of contemporany animals.

Finally, we would like to point out that the state of health of the females which is noted at each birth revested no mastitis, no coryza and no wry neck ; 3 females showed signs of pododermatitis and were eliminated.

Discussion

The elimination of parasites is not surprising but was one of the main objectives. The elimination of Pasteurella was not an easy task, especially considering that we chose to begin with a large number of breeders. The absence

of control groups does no enable us know whether one of the four techniques used simultaneously was unnecessary or not. The large number of healthy carriers and the fact that from the second generation onwards the animals were pasteurella-free encourages us to believe that each stage was necessary : the antibiotic treatment before parturition to limit the risks of contamination in utero, maximal separation from the mother (nesting boxes closed and early weaning), antibiotic protection during the peri-weaning stage (which always entails stress), and finally the vaccination after weaning.

As far as the other germs are concerned the techniques used to detect the presence of E coli O103 and/or C spiroforme are not sufficiently reliable to assert that our breeding colony is absolutely safe from these two pathogenic germs. Nevertheless, the diversity and number of vain attempts carried out to reveal their existence allow us to remain optimistic. It is difficult to specify with certainty why they were (doubtless) eliminated. We believe that the early administration of a diet likely to favour the development of an autochthonous holoxenic cellulolytic micro-flora can play an important role in its effect as a "barrier".

This hypothesis is strengthened by the fact that it is precisely at weaning that the Clostridia appear in the intestine (Gouet and Fonty 1979). We may also remark that without taking any particular precautions Christ-Victor (1973) found no Clostridium in the group of animals reared in wire cages and fed with a commercial granular diet, whose composition she unfortunately does not mention in her work.

Finally, it should be noted that Borrielo and Carman (1983) who were the first to identify C spiroforme in the rabbit consider that this germ is not normally present in the rabbit (Carman and Borrielo 1984). Moreover, it is also suggested (Carman and Evan 1984) that the Clostridium would not develop if adequate microflora were present. It is also possible that the injection of terramycine on the 21st day contributed to the control of this germ in view of its important action on Clostridia. As far as E. coli O103 is concerned, apart from the fact that the E. coli are spontaneously few in number in the rabbit, only the "barrier" effect of the micro-flora which established itself can be evoked. We did not verify the "cellulolytic" nature of this micro-flora, but the almost total absence of mortality after weaning (30 deaths out of 3,200 young rabbits weaned in a 18-month period) indicates its appropriateness to the rabbit. It is also important to note that the experimental results obtained with these SPF animals are sometimes different from those obtained previously with conventional animals (for example, the pathogenic effect of E. intestinalis caused at the same time a high mortality rate and heavy diahorrea (Coudert 1976) ; with the current animals and the same strain of E. intestinalis, the mortality rate remains unchanged but diahorrea has become rare or inexistant. This remark is not generally applicable since the extent and frequency of diahorrea due to E flavescens are not modified compared to previous observations (Coudert 1977).

The results concerning morbidity and production of the 136 females placed in experimental conditions and the 488 litters produced are equally satisfying. The only symptoms observed in fewer than 10 females are asthenia or paresis at the end of gestation or at the beginning of lactation (these females and their litter were eliminated). The mortality of pregnant or suckling does presents the same characteristics as these observed previously in the same strain : a perfectly healthy animal, sudden death without prodromal symptom, especially at the end of the gestation period (Coudert et al, 1984, Lebas and Coudert 1986, Coudert and Brun 1988). It would appear

that mortality is proportionally higher on the last day of gestation (11 females out of 16) than in our conventional colonies. However, more data is necessary to be able to study this aspect of the pathology of breeding does, as other factors vary. The average number of young born per litter is lower than the regular average of the breed. This is partly due to the proportion of first litters in our sample group and probably also to a maternal effect stemming from the fact that all the breeding does come from on first litter. These same observations also explain the fact that the stillbirth rate is slightly higher than the norm. On the other hand, the mortality between birth and weaning is 40% lower than that in the best breeding colonies (Henaff et al 1987). The respective weights at weaning (4 weeks) and at 11 weeks are approximately 10 % lower than those obtained elsewhere, which stems from the low-protein diet we deliberately used.

Conclusion

The eradication of parasites and pasteurella seems to us to be an attainable aim even in breeding conditions which are less sophisticated than the ones we used, provided that the hygiene requirements are respected. The elimination of pathogenic Clostridia and E coli is more uncertain, due to the complete absence of prophylactic medical methods ; however it is certain that the micro-flora which establishes itself just before weaning at 4 weeks plays a determining role.

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Tab 1 : Summary of different prophylactic methods used to improve the sanitary statute of a rabbit live-stock

UNITS	AGE	PROPHYLACTIC METHODS
Original breeding colony	Does	. Tetracycline 2 days before weaning . Robenidine supplemented feed
Barrier breeding system	Suckling	. Precocious weaning . Tetracycline
Room A 1st generation	24 days	. Tetracyclin . Vaccination : P multocida + 1st booster . Sequence of 3 coccidiostatic drugs (toltrazuril, Salinomycine, lasalocide) . Anthelmintic treatment : 3 x 2 weeks with Fenbendazole . Mange : Ivermectin at 30 and 50 days
	100 days	. Robenidine supplemented feed
	115 days	. 2st booster (P multocida)
	120 days	. Mating
	First parturition	. 7 and 2 days before parturition : tetracycline . 2 weeks after parturition : Ivermectine
	Suckling 17 days	. Nest-box open 5 mn per day . Feed with high-fiber level given in nest-box
Room B 2nd generation	21 days	. Precocious weaning : Tetracycline . Tetracycline . Vaccination : P multocida at 5,9 and 16 weeks
	120 days	. Mating
	First parturition	. 7 and 2 days before parturition : tetracycline
	Suckling 17 days	. Nest-box open 5 mn per day . Nest-box open from 9 to 16 o'clock : feed with high-fiber level in mother's feedbox and cleaning of watering valves 3 times per day
Room C 3rd generation	21 days	. Precocious weaning : Tetracycline . Tetracycline - Standart medicated feed . Vaccination : P multocida at 5, 9 and 16 weeks
	120 days	. Mating . Standart breeding methods ; the only remaining medical prophylaxis is the use of a robenidine medicated feed . Each breeding room is devoided cleaned and fumigated after 12 months

Tab. 2 - Global result of production and morbidity of SPF rabbit breeding does

	1rst generation	2nd generation	Total 1rst and 2nd generations
Number of breeding does	60	76	136
Number of pregnant does	234	254	488
Number of eliminated pregnant does	0	1	1
Number of dead pregnant does	6	9	15
Total number of litters at parturition (Pa)	228	244	472
Number of living litters at parturition (Pb)	220	234	454
Total new-born rabbits/pa	8.47	8.77	8.63
Still-born rabbits/Pa (%)	9.7	12.7	11.5
Living new-born rabbits/Pb	7.89	7.98	7.94
Mortality of sucklings (%)	12.3	12.8	12.5
Number of eliminated suckling does	0	1(+2)*	
Number of dead suckling does	2	2	4
Number of weanlings/Pb	6.91	6.93	6.92
Average weight at weaning (g)	499	519	511
Mortality after weaning (%)	1	1	1
Average weight of 77 days old rabbits	-	2132	-

* 2 dead does one day after weaning

Proceedings 4th World Rabbit Congress, 10-14 October 1988, Budapest Hungary, Vol. 2, 137-148

ABSTRACT

ESTABLISHMENT OF A S.P.F. BREEDING COLONY WITHOUT HYSTERECTOMY AND HAND-REARING PROCEDURES.
P. COUDERT, D. LICOIS, J. BESNARD.

A S.P.F. rabbit breeding colony was obtained from a group of 50 animals reared untill weaning in a conventional rabbitry. The usual method, hand-rearing following an aseptic hysterectomy, was not chosen and the reasons are discussed ; especially the necessity of implantation of a holoxenic microflora before weaning is always problematical. The aim was at least the eradication of six specific pathogen agents : coccidia, mange, passalurus, pasteurella, clostridium and E. coli 0103. These objectives were reached in a barrier breeding system after two generations succeeding one another rapidly. The methods of eradication were mostly based on medical prophylaxis for the parasites and pasteurella, and on hygienic prophylaxis for pasteurella, clostridium and pathogenic E. coli. The theorical and practical aspects of this successful procedure are discussed.

RESUME

OBTENTION D'UN ELEVAGE DE LAPINS EXEMPTS D'AGENTS PATHOGENES SPECIFIQUES (SPF) SANS HYSTERECTOMIE ET SANS ALLAITEMENT ARTIFICIEL.
P. COUDERT, D. LICOIS, J. BESNARD.

Un cheptel de lapins SPF a été obtenu à partir d'un groupe de 50 reproducteurs élevés jusqu'au sevrage dans un élevage conventionnel. La méthode traditionnelle d'obtention de lapins SPF par hystérectomie aseptique suivie d'allaitement artificiel n'a pas été retenue pour des raisons qui sont discutées et notamment à cause de la nécessité d'introduire une flore holoxénique. L'objectif minimal était l'éradication de six agents pathogènes : coccidie, gale, oxyure, pasteurelle, clostridium et colibacille 0103. Il a été atteint en élevage protégé sur deux générations qui se sont rapidement succédées. Les méthodes d'éradication utilisées sont essentiellement basées sur la prophylaxie médicale pour les parasites et les pasteurelles et sur la prophylaxie hygiénique pour les pasteurelles, les clostridiums et le colibacille 0103. Les bases de cette prophylaxie hygiénique et les raisons de leur succès sont discutées.