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Original article

Psychobiological consequences of two different weaning methods in sheep

Pierre Orgeur[a*], Serge Bernard[b], Murielle Naciri[c], Raymond Nowak[a], Benoist Schaal[a], Frédéric Lévy[a]

[a] Laboratoire de comportement animal, Inra/CNRS, URA 1291, 37380 Nouzilly, France
[b] Laboratoire de pathologie infectieuse et immunologie, Inra, 37380 Nouzilly, France
[c] Laboratoire de pathologie aviaire et parasitologie, Inra, 37380 Nouzilly, France

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Abstract — Weaning is associated with a break in the mother–infant contact. In sheep, under natural conditions, the rupture of the social bond is progressive. In contrast, weaning imposed by breeders may result in psychobiological disturbances. The aim of this experiment was to measure the behavioural, hormonal and immune consequences of two types of sudden weaning: total separation (TS) and partial separation (PS), in 42 Ile-de-France ewes and their 60 lambs. TS animals (lambs and ewes) vocalized less frequently and for a shorter period of time than did the PS animals. Neither the plasmatic cortisol level in lambs nor the humoral response of dams and young were modified by the two weaning methods. In contrast, the excretion of coccidial oocysts increased significantly after weaning in TS lambs only and the growth rate of TS females was lower than that of the other lambs. These results suggest that these methods of rupture of mother–young social contact have only limited negative consequences on both partners in sheep, contrary to primates and rodents. © Inra/Elsevier, Paris.

sheep / weaning / psychobiology / welfare

Résumé — **Conséquences psychobiologiques de deux méthodes de sevrage chez les ovins.** Le sevrage correspond à la rupture du lien mère–jeune et se caractérise par un passage d'une alimentation lactée à une alimentation solide. Dans l'espèce ovine, en conditions naturelles, cette rupture est progressive. En revanche, une séparation imposée par l'éleveur pourrait engendrer des perturbations psychobiologiques plus ou moins importantes selon la méthode de séparation. L'objectif de cette étude est de mesurer les conséquences de deux types de sevrage soudain: une séparation totale (ST), et une séparation partielle (SP). L'étude a porté sur 42 brebis et 60 agneaux de race Île-de-France. Les animaux ST vocalisent moins intensément et moins longtemps que les SP. Ni le rythme de sécrétion du cortisol plasmatique des agneaux, ni le pouvoir immunitaire des mères et des jeunes ne sont

* Correspondence and reprints
E-mail: orgeur@tours.inra.fr

modifiés par l'une ou l'autre des méthodes de séparation. En revanche, seuls les agneaux ST excrètent davantage d'oocystes coccidiens après séparation qu'avant et la croissance des femelles de ce même groupe est plus faible que celle des autres agneaux. Ces résultats suggèrent que chez les ovins, la rupture du lien d'attachement mère–jeune, lorsque les agneaux sont âgés de 3 mois, n'a que peu de conséquences négatives chez les deux partenaires, contrairement aux primates et aux rongeurs.

ovins / sevrage / psychobiologie / bien-être

1. INTRODUCTION

In sheep a selective and reciprocal ewe–lamb bond is rapidly established at birth and persists even beyond the lactation period under natural conditions [10]. In contrast, in commercial flocks forced weaning as imposed by breeders results in a sudden mother–young separation. As a result of this practice, lambs experience an abrupt change in diet in terms of composition, quantity of energy and protein intake and social interactive processes of eating as the milk-based diet is replaced by more solid feed intake. In a previous study the consequences of artificial weaning in this species were assessed [18]. Results showed that sudden weaning at 3 months of age induces transient behavioural disturbances, whereas a progressive weaning affects the sensitivity of lambs to parasitic infestation.

In both types of weaning, mothers and young were separated by an open fence and could therefore communicate. However, weaning performed by breeders is sometimes associated with a complete separation of the two partners and such practices may therefore induce more profound behavioural and/or physiological disturbances. For instance, plasma cortisol levels, a presumed indicator of stress, show a greater increase when the partners are totally separated than when they can maintain visual and auditory contact. This has been documented in Rhesus monkeys [14], guinea pigs [8], rats [11, 19] and sows [23]. On the other hand, behavioural parameters such as vocalizations are much more pronounced under partial separation for Rhesus monkeys [14], guinea pigs [8] and cattle [13].

We therefore evaluated the behavioural consequences of these two types of sudden weaning at 3 months in sheep. Ewes and lambs were either completely denied the opportunity to communicate (total separation) or allowed to visually and vocally communicate (partial separation).

Parasitism and immunological effects are of greater agronomic interest, and may result in poor welfare. In addition, some authors have reported that the immune system of sheep can be affected by mother–young separation [2] and that sensitivity to coccidiosis is enhanced in weaned lambs [18]. We therefore also measured the response to a foreign antigen in both ewes and lambs and lambs' excretion of coccidial oocysts.

We hypothesized that complete and partial mother–young separation may have different consequences for the welfare of sheep.

2. MATERIALS AND METHODS

2.1. Experimental animals

Forty-two multiparous Ile-de-France ewes and their 60 lambs were used in this experiment. All ewes had lambed at least twice before. Lambs were born between 1 and 7 March 1996. Lambing was induced by an i.m. injection of 16 mg dexamethasone on day 144 of gestation. Ewes were fed hay and food pellets with water available ad libitum.

Before weaning, mothers and lambs of each subgroup were kept together in an indoor pen measuring 30 m^2, of which 12 m^2 were exclusively reserved for the lambs. These two pens were separated by a single open fence which permitted ewes and lambs to see, hear and smell each other. Lambs had free access to the smaller area, where special food pellets (14 % protein) were available ad libitum for lambs from day 15, and penned in it at weaning in the partial separation subgroups.

2.2. Weaning procedures

Ewes and their lambs were allocated to one of two groups: 1) totally separated (TS): mothers and lambs were moved into two different buildings more than 50 m apart; 2) partially separated (PS): mothers and lambs were housed in the same building but separated by two open fences with a space of 1.5 m between them. Weaning occurred when lambs were 90–96 days old.

Each group was divided into two subgroups (TS1 and TS2, and PS1 and PS2), the compositions of which are given in *table I*. Behavioural data were recorded in subgroups TS1 and PS1 and physiological data were collected in subgroups TS2 and PS2. These subgroups were necessary since behavioural and physiological data could not be recorded on the same animals without obvious confounding effects.

2.2.1. Dependent variables

2.2.1.1. Behavioural measures in lambs and ewes

The behaviour of the ewes and their lambs was recorded in subgroups TS1 and PS1: 8 days before weaning (D–8); the day of weaning (D0); and on each of the following 3 days (D1, D2, D3).

Animals were observed for six consecutive hours daily, during the diurnal period (between 9 a.m. and 4 p.m.), by a person situated outside the sheep pen. Behavioural data recorded for ewes and lambs were: 1) the total number of vocalizations per 5-min period, to estimate the level of social perturbation. The vocalizations were divided into two categories: high pitched bleats (HPB: vocalizations of high intensity emitted with open mouth) and low pitched bleats (LPB: vocalizations of low intensity emitted with closed mouth); 2) the number of animals resting or eating every 5 min.

2.2.1.2. Hormonal measures in lambs

Blood samples were collected from TS2 and PS2 lambs by jugular veinipunctures during two 48-h periods: 1 week before weaning (D–7/8); the day of weaning and the day after (D0/1).

Blood was collected at 2-h intervals in heparin-coated vacutainers for 48 consecutive hours. Previous experiments showed that the beginning of blood sampling induced an increase in cortisol secretion [4]. To prevent this increase, two blank samples were drawn 30 and 15 min before the real sampling period began. Samples were immediately centrifuged and plasma was stored at –20 °C until assayed. Plasma cortisol was radioimmunoassayed in duplicate according to the following procedure: after 1 h of incubation of a mixture of plasma (50 μL), glycine buffer (100 μL), rabbit anticortisol antibody solution (100 μL) and tritiated cortisol solution (250 μL), a second antibody solution made of sheep anti-rabbit gammaglobulin (50 μL) was added. A sec-

Table I. Distribution of animals in experimental groups.

	TS						PS					
	1			2			1			2		
Mothers	10			11			11			10		
Lambs	M	F	Tot.	M	F	Tot.	M	F	Tot.	M	F	Tot.
Singles	3	2	5	4	4	8	2	5	7	3	2	5
Twins	4	6	10	3	4	7	5	3	8	3	7	10
Total	7	8	15	7	8	15	7	8	15	6	9	15

M = male; F = female.

ond incubation for 15 h was followed by two washings with polyethylene glycol (2 mL), each followed by centrifugation and elimination of the supernatant. Ethanol (100 μL) and scintillation liquid (2 mL) were then added, before measuring the level of radioactivity by a beta liquid scintillation counter [18].

2.2.1.3. Immune response in lambs and ewes

Ewes and lambs were vaccinated on the day of weaning, with a vaccinal strain of *Salmonella abortusovis* (SAO Rv6 strain, [12]). Ewes were given a subcutaneous injection of 10^7 live bacteria in the neck, while lambs received 10^6 live bacteria. *Salmonella abortusovis* is a pathogen specific to sheep, which induces abortion and death of new-born lambs. A live attenuated vaccine SAO Rv6 strain has been developed against this disease. This live attenuated vaccine SAO Rv6 strain was used to assess the immune response of the animals. The doses injected, 1 % of the normal quantity, were chosen to allow detection of small variations in the antibody response.

Blood samples were collected by jugular veinipuncture and kinetics of antibody responses against LPS (lipopolysaccharid from the external bacteria membrane) or bacterial SAO antigens were compared between groups (PS and TS) within the 5-week period following weaning (D6, D12, D18, D35). Sera were collected after blood coagulation and frozen (–20 °C) until antibody analysis. ELISA techniques for detecting Salmonella antibody responses have been described previously [3]. Anti-LPS IgM antibodies were quantified using an indirect enzyme-linked immunosorbent assay (ELISA). ELISA tests were performed using 96-well microtiter plates (Nunc-Immunoplates Maxisorp, Nunc 4-42 404). Microplates were coated with 0.5 μL of SAO homologous LPS (*S. abortusequi*, Sigma L 5886) in 50 μL of 0.05 M carbonate buffer pH 9.6 and incubated overnight at 4 °C. After a rapid wash, non-specific binding was blocked by incubation plates for 30 min at room temperature of 100 μL of 5 % skimmed bovine milk in PBS. After three washes in PBS–0.05 % Tween 20, 100 μL of serum sample dilution (1/2 serial dilution in PBS–0.05 % Tween (5 % milk) were incubated for 90 min at 37 °C. After three more washes, the wells of the plate were incubated with specific mouse anti-sheep IgM (Dr Beh, CSIRO, Australia), 1/10 000 diluted in PBS–0.05 % Tween–5 % milk for 90 min at 37 °C. After three more washes, antibodies were revealed using peroxidase-labelled goat anti-mouse Ig (H and L chains, Bio Rad), 1/5 000 dilution, for 90 min at 37 °C. The peroxidase activity was detected with 2.2-azino-bis-(3ethyl-benzothiazoine-6-sulfonic acid), (ABTS Boehringer Mannheim)/H_2O_2 substrate solution and read at 415 min after 1 h incubation at 37 °C.

Anti-Salmonella IgG antibodies were quantified in the same conditions, except that microplates were coated with 10 μg of CWF (cell wall fraction of SAO Rv6 strain [1] in 50 μL of distilled water, dried over night at 37 °C, and fixed with 100 μL of 80 % acetone. IgG antibodies were detected with specific mouse anti-sheep IgG (Dr Beh, CSIRO, Australia).

Antibody titre of each sample was calculated according to a logistic transformation of optical density of the ELISA reaction [18]. Dilution of serum giving Logit (Do) = 0 (inflection point of the logistic curve) was taken as antibody titre.

2.2.1.4. Coccidial oocyst output in lambs

Rectal faecal samples were collected at D–1, D9 and D26 post-separation to quantify the number of oocysts per gram of faeces according to the method described in Orgeur et al. [18].

2.2.1.5. Growth of lambs

All lambs were weighed at birth and at 2, 5, 11, 14, 16 and 18 weeks of age.

2.2.2. Statistical analysis

Non-parametric tests were applied to the behavioural and oocyst output data because they were not normally distributed. For each period of observation the comparison between treatments (TS1 and PS1) for ewes and lambs was performed using the Mann-Witney U-test. For each treatment, comparisons between the two types of bleats and between different periods were made with Wilcoxon's test. Lamb growth rate was studied using an ANOVA for repeated measures. These statistical analyses were conducted using SYSTAT statistical software (Systat 6.0: Evanston, USA). Plasma cortisol data were analysed with analysis of variance (Stat. General MANOVA) using STATISTICA for Windows 4.5 statsoft. The significance threshold was set at $P = 0.05$ for all variables.

The immune response was estimated by optical densities plotted against the two-fold dilution of each sample which gave logistic curves, transformed in linear function after conversion in Logits

$$(\text{Logit OD} = \text{Log}(100 * (\text{OD}/\text{OD Max})/(100 - 100 * (\text{OD}/\text{OD Max}))$$

where OD Max is the maximum optical density reached in the plateau phase of the curve). Dilution of serum giving Logit = 0 (inflexion point of the logistic curve) was taken as the titre of antibody in the serum samples. A multivariate repeated measure MANOVA assessed the effects of period of treatment (before and after weaning), type of treatment (TS and PS) and sampling time on cortisol level as the dependent variable. Finally, *t*-tests for dependent samples comparing each sampling time was also used.

3. RESULTS

3.1. Behavioural measures of ewes and lambs

Overall, the frequency of HPB increased after weaning in both subgroups and for both partners (*figures 1* and *2*). The difference was significant between D–8 and D0, D1 and D2 ($P < 0.001$). By D3, differences in HPB were no longer observed in the TS condition, either in mothers or in lambs, but the vocal activity was still higher in both partners in the PS condition (mothers: $P < 0.01$, lambs: $P < 0.001$). The comparison between TS and PS animals did not show any difference in the number of HPB emitted at D–8, neither in mothers nor in lambs. On the other hand, during the 4 days following weaning HPB was significantly higher in the PS than in the TS condition ($P < 0.001$) for both social partners. Regardless of the separation procedure, lambs vocalized significantly more than mothers on the day of weaning (D0). The reverse was noted on D–8 and D2 in the TS condition, and on D–8, D1, D2 and D3 in the PS condition.

The frequency of LPB was always low relative to that of HPB. Nevertheless, it was often significantly higher after weaning than before, particularly in lambs of both subgroups (*table II*). This frequency was significantly higher in PS ewes than in TS ewes ($P < 0.01$) on D1. The same pattern was shown by lambs on each day of observation,

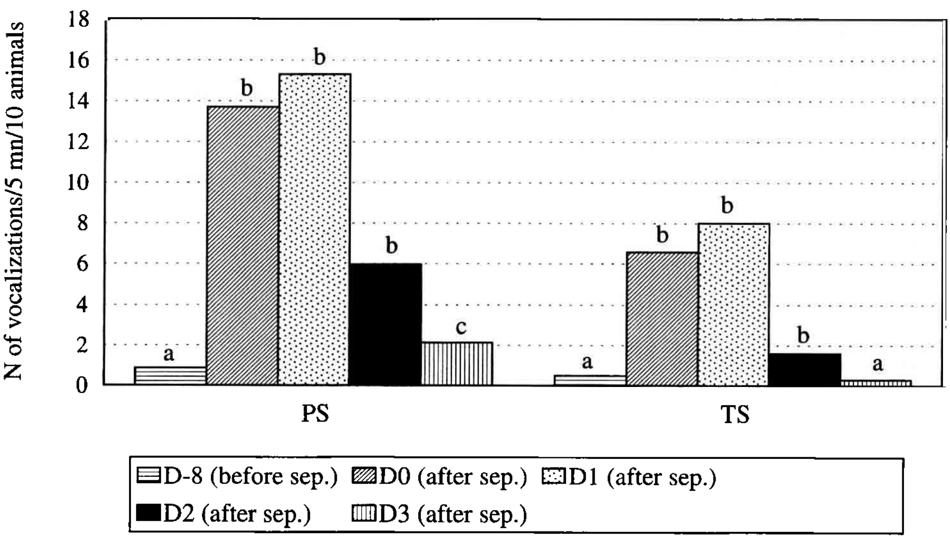

Figure 1. Frequency of high pitched bleats (HPB) emitted by ewes before and after weaning in partial (PS) and total separation groups (TS): 8 days before separation (D–8); the day of separation (D0); the day after (D1), 2 (D2) and 3 days after separation (D3). Results within each day with different letters (a, b, c) are significantly different ($P < 0.001$).

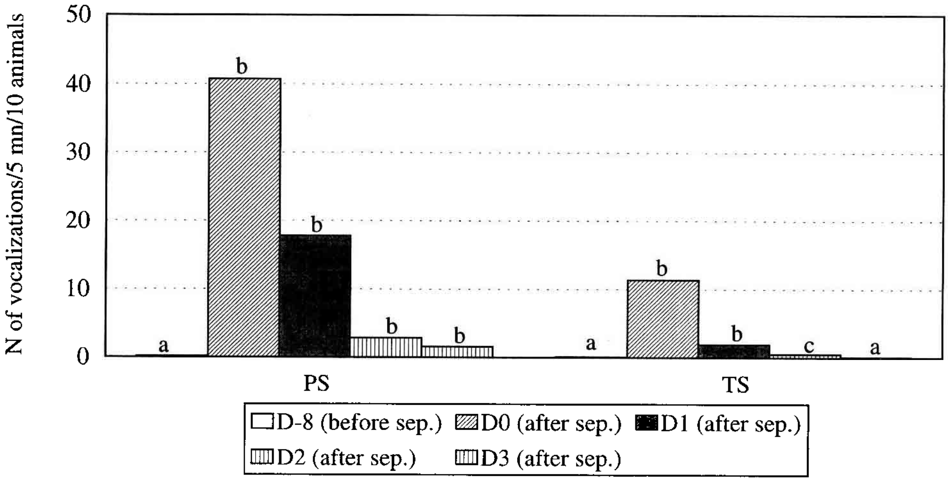

Figure 2. Frequency of high pitched bleats (HPB) emitted by lambs before and after weaning in partial (PS) and total separation groups (TS): 8 days before separation (D–8); the day of separation (D0); the day after (D1); 2 (D2) and 3 days after separation (D3). Results within each day with different letters (a, b, c) are significantly different ($P < 0.001$).

Table II. Mean number (± SD) of low pitched bleats emitted by 10 animals/5 min of observation, before (D–8) and after weaning (D0, D1, D2 and D3).

			D–8	D0	D1	D2	D3
Mothers	partial separation	mean	0.16	0.25	0.83	0.18	0.28
		± SD	0.51	0.53	1.02	0.50	0.58
	total separation	mean	0.12	0.39	0.40	0.46	0.14
		± SD	0.50	0.62	1.27	1.16	0.45
Lambs	partial separation	mean	0.12	0.02	2.89	0.77	0.26
		± SD	0.52	0.12	2.17	1.14	0.47
	total separation	mean	0.01	0.20	0.50	0.08	0.21
		± SD	0.08	0.47	1.45	0.32	0.75

even before weaning (respectively, $P < 0.001$ on D0, D1 and D2 and $P < 0.05$ on D–8 and D3). Such vocalizations were more frequently observed in TS mothers than in their lambs on D0 and D2 ($P < 0.05$, $P < 0.01$, respectively) and in PS mothers on D0 ($P < 0.001$). The reverse was observed on D1 and D2 in PS animals ($P < 0.001$).

The mean number of animals resting was not affected by the weaning procedure nor by weaning itself. Scores were always higher in lambs than in mothers, even on D–8. The mean number of mothers eating was significantly higher in the TS than in the PS condition on D–8 and D0 ($P < 0.01$ and $P < 0.001$). The reverse was true on D1 ($P < 0.01$), D2 ($P < 0.001$) and D3 ($P < 0.01$). In lambs, the number of animals eating was always significantly higher in the TS than the PS group, except on D1 (D–8: $P < 0.05$, D0 and D3: $P < 0.001$, D2: $P < 0.01$). The mean number of animals eating was always significantly higher for ewes than for lambs, in both subgroups, including D–8, and was not affected by weaning (*table III*).

3.2. Hormonal measures in lambs

The analysis yielded significant main effects ($P < 0.001$) of period of treatment and sampling time on cortisol level, indicating that cortisol level was higher before than after weaning (15.62 versus 12.79 ng/mL of plasma), whatever the type of separation. Moreover, this level differed significantly between samples without a circadian rhythm. In addition, significant interaction effects were observed between the type of separation and sampling time factors and the period of treatment and sampling time factors ($P < 0.001$ in both cases). In other words, regardless of the period of treatment TS lambs displayed higher levels of cortisol than did PS lambs (15.05 versus 13.36 ng/mL of plasma). The analysis of the three factors reveals a significant interaction between them ($P < 0.05$).

Finally, the comparison of each sampling time showed that before weaning the cortisol level was significantly higher in TS than in PS lambs in only two cases (*figure 3*), while after weaning it was significantly higher in TS than in PS lambs in seven cases (*figure 3*). A similar analysis comparing the sampling times in both TS and PS lambs reveals that the cortisol level was significantly higher before than after weaning in ten and seven cases, respectively, while the reverse was found in one case for each type of separation.

Table III. Mean number (± SD) of animals resting or eating.

				D-8	D0	D1	D2	D3
Mothers	resting	PS	mean	2.28	2.20	3.51	1.77	2.96
			± SD	2.56	2.44	2.91	1.85	2.68
		TS	mean	2.24	0.36	4.04	3.19	3.14
			± SD	2.63	0.98	3.51	3.04	3.08
	eating	PS	mean	5.72	5.76	5.43	7.32	6.51
			± SD	1.78	1.84	3.02	1.69	2.58
		TS	mean	6.74	7.39	3.96	5.68	5.25
			± SD	2.88	2.55	2.75	2.65	2.89
Lambs	resting	PS	mean	4.61	4.57	5.46	5.70	5.30
			± SD	2.73	1.62	1.18	1.40	1.62
		TS	mean	4.27	4.31	5.47	4.81	5.13
			± SD	2.74	2.64	2.80	2.64	2.18
	eating	PS	mean	3.07	1.17	2.64	1.59	1.56
			± SD	1.59	1.01	1.18	1.30	1.22
		TS	mean	4.08	2.67	2.42	2.42	2.87
			± SD	2.58	1.73	1.90	1.66	1.68

PS = partial separation; TS = total separation.

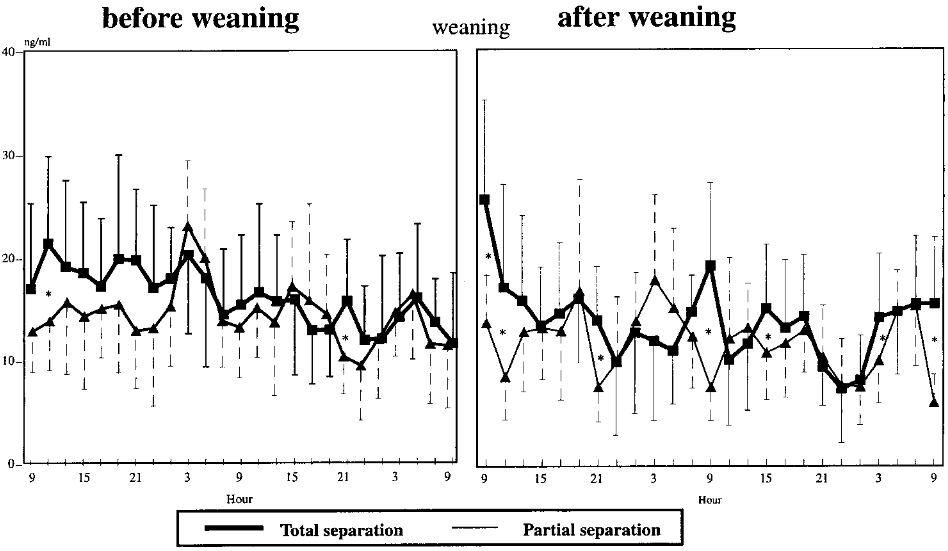

Figure 3. Plasma cortisol concentrations in lambs (mean ± SD), before and after weaning in total (TS) and partial separation groups (PS). * Statistical differences between TS and PS ($P < 0.05$).

3.3. Immune response in lambs and ewes

Antibody titres were always higher in ewes than in lambs. Anti-LPS IgM antibodies appeared on day 6, with a peak between day 6 and day 12. Anti-SAO IgG antibodies, revealed with the cell wall fraction of SAO Rv6 strain, were maximum on day 12. Statistically, in lambs or ewes, no difference was found in kinetics of IgM or IgG antibodies whatever the time after weaning, or whatever the weaning method (TS or PS) (*figure 4a–d*).

3.4. Coccidial oocyst output in lambs

TS lambs excreted significantly more coccidial oocysts 9 and 26 days after weaning than the day before weaning (D–1) ($P < 0.05$). This was not the case for PS lambs. However, the total number of coccidial oocysts did not differ significantly between the two weaning methods, whatever the day of analysis (*figure 5*).

3.5. Growth measures

The procedure of weaning affected growth rate only in female lambs but not in males. After weaning, body weight of TS female lambs was significantly lower than PS female lambs 3 weeks after weaning (31.8 versus 36.7 kg, $P < 0.05$), 5 weeks after weaning (34.6 versus 40.7 kg, $P < 0.01$) and 7 weeks after weaning (36.2 versus 42.8 kg, $P < 0.01$).

4. DISCUSSION

As several authors have shown in various species including Rhesus monkeys [14], horses [15], guinea pigs [9] and cattle [13], partial separation by an open fence permitting vocal and visual communication between lambs and ewes elicited a higher vocalization rate than total separation. Moreover, this increased vocal activity, which was observed in both separation conditions after weaning, decreased more rapidly when

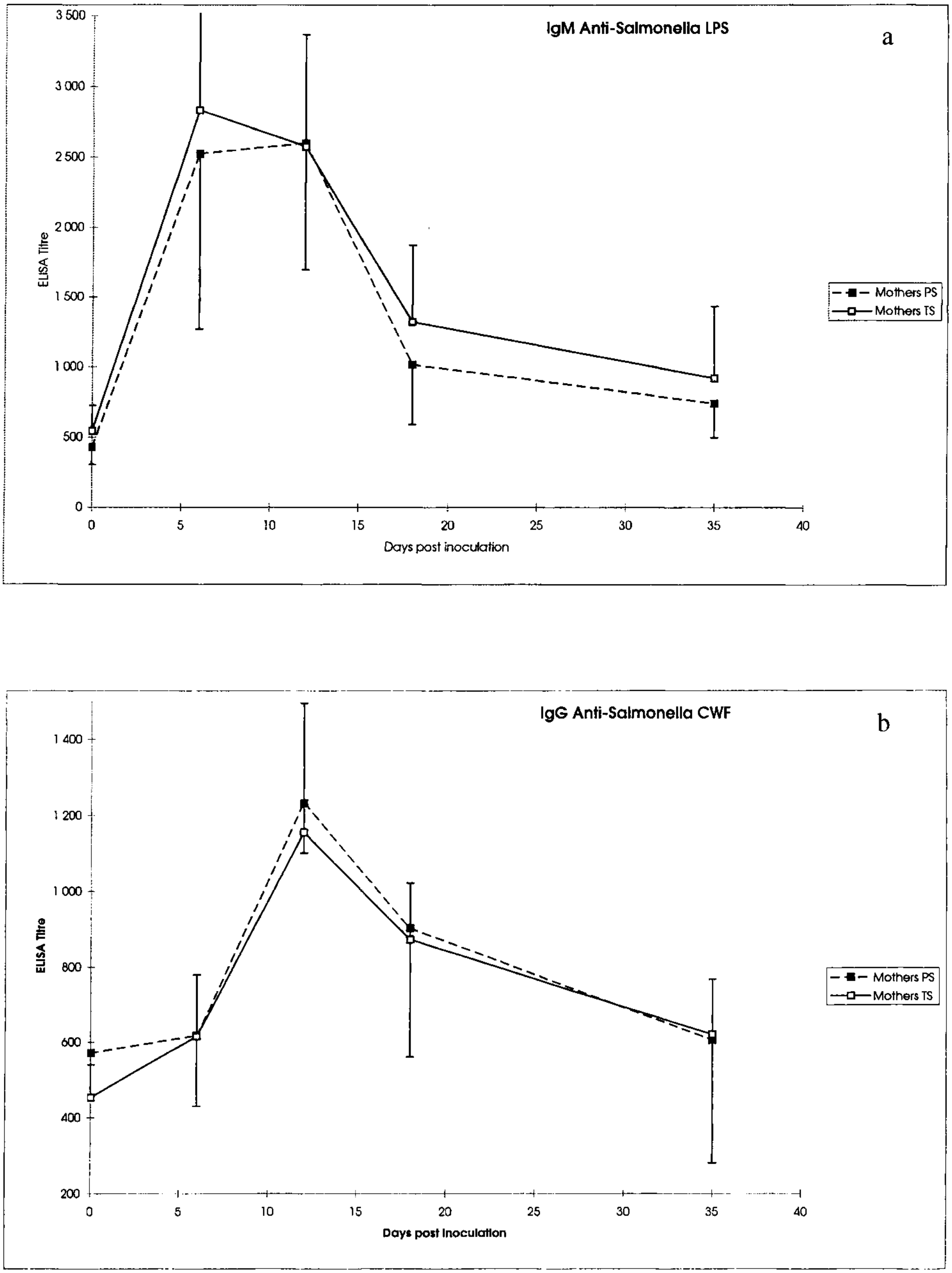

Figure 4. Antibody titres in PS and TS groups of ewes and lambs, after vaccination with *Salmonella abortusovis*. a: anti-Salmonella LPS antibodies in ewes; b: anti-Salmonella CWF antibodies in ewes; c: anti Salmonella LPS antibodies in lambs; d: anti-Salmonella CWF antibodies in lambs.

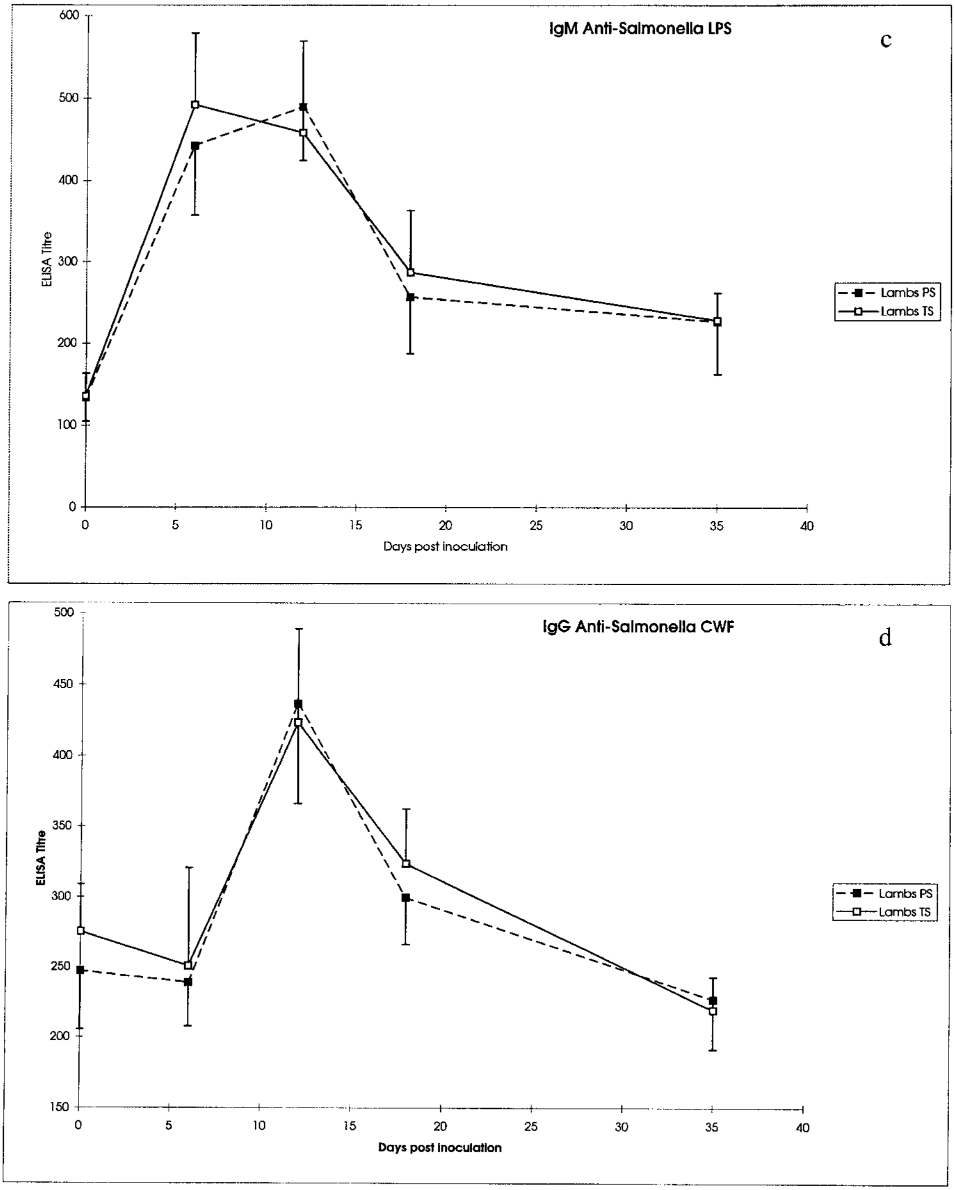

Figure 4. Continued.

communication between mother and young was not possible. Thus, in TS ewes–lambs, the level of vocalizations 4 days after weaning was not different from the preweaning period. As previously shown [18], lambs submitted to either weaning method vocalized more frequently than their mothers on the day of weaning. This result may indicate that lambs were more distressed at the beginning of the separation than their moth-

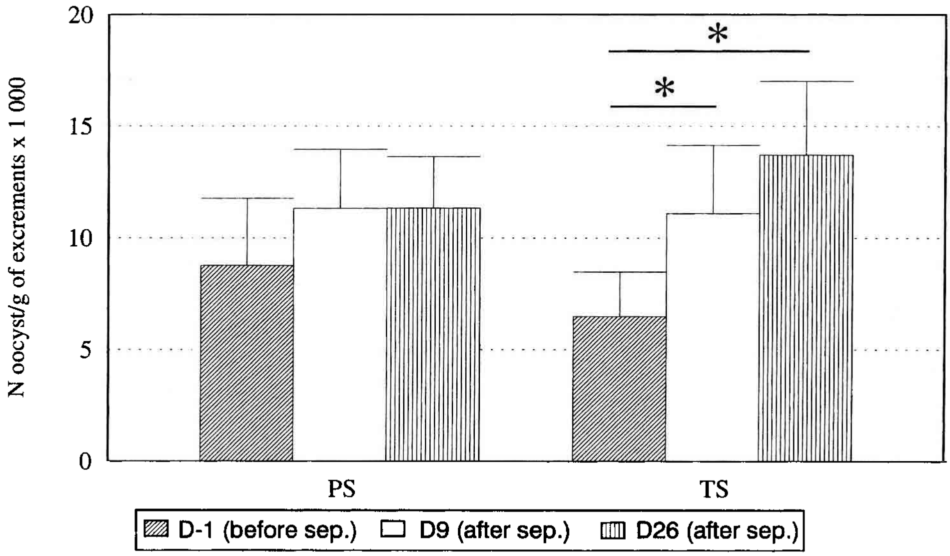

Figure 5. Coccidial oocyst output in PS and TS groups lambs (± SE). The day before separation (D–1); 9 (D9) and 26 (D26) days after separation. *Statistical differences ($P < 0.05$).

ers. One possible explanation is that the ewes (all multiparous) had already experienced several separations during previous lactations and therefore may have become accustomed to it. Moreover, a difference in calling rates between adult and young animals has been already observed. Shillito-Walser et al.'s study [22] on vocal activity of ewes and lambs in the field (before weaning) revealed that ewes are more vocal than their lambs during the early period of lactation, while the reverse is observed towards the last third of lactation. Interestingly, in the present work, the vocalization rates developed differently in mothers and lambs: on the days after weaning, mothers were more vocal than their young. This suggests either a better adaptation by the lambs to weaning or that ewes called their lambs to be suckled.

On the other hand, the low pitched bleats increased mainly in lambs after weaning. Even though their emission rate was considerably lower than the high pitched bleats, it was higher in the partial than in total separation group on the day of weaning. The fact that this type of bleat is particularly observed when ewes and lambs are close to each other may explain this result. Unlike high pitched bleats, the rate of low pitched bleats was greater in mothers than in lambs on the day of weaning.

There was no effect of weaning on the rate of animals' resting or eating regardless of the weaning method. As already shown in this species [18] the vocalization rate seems to be the best behavioural indicant of social perturbation, such as breaking of the mother–young bond.

In some cases, the plasma cortisol level was higher in TS than in PS lambs. Before weaning this was the case for only two sampling times, while after weaning similar differences were found at seven sampling times. These results are in agreement with those obtained in other species where animals totally separated showed a higher level of cortisol than when they can communicate (Levine et al. [14]: Rhesus monkeys;

Hennessy et al. [9]: sows). Although the plasma cortisol level did not increase at any time after weaning, paradoxically it decreased significantly whatever the type of separation. Moreover, in both TS and PS lambs this level was higher before than after weaning, in ten and seven cases, respectively, while the reverse was observed only in one case for each type of separation. Our data suggest that lambs became used to human manipulations during blood sampling, which was conducted 1 week before weaning. This is in agreement with this finding that increased cortisol level induced by shearing is attenuated by previous human manipulations [6]. These results are in agreement with data recorded from sheep by Napolitano et al. [17], showing that separation at 15 or 28 days of age did not induce an increase in cortisol level unlike separation at 2 days of age. On the other hand, they are in opposition with those of Mears and Brown [16] who found that plasma cortisol levels increase in lambs after weaning when they are 7 weeks old. This increase appears at the first hour as well as 24 h after weaning. However, in this last experiment, weaning was associated with weighing, tagging with plastic ear tags and vaccination, which could partly account for it.

The fact that only TS lambs excreted more coccidial oocysts after, than before weaning, suggests that this social experience leads to increased sensitivity to this parasite. Yvoré et al. [24] and Gregory et al. [5] have shown that this sensitivity may be greater at a younger age. These results have been confirmed by Orgeur et al. [18] indicating that lambs daily and progressively separated between 3 weeks and 3 months excreted more coccidial oocysts than lambs suddenly weaned at 3 months. However, no clinical sign of coccidiosis characterized by diarrhoea and oocyst output was observed in each group. This may be explained by the treatment with 20 mg/kg of body weight of Baycox® 2.5 % (8 mL/10 kg) dispensed to all lambs 14 days before weaning because of diarrhoea due to coccidiosis. This effective treatment rapidly controlled the disease and animals excreted few oocysts 1 day before weaning. Thus, lambs were resistant to coccidiosis.

The difference in the excretion of parasites between preweaning and postweaning periods in the TS group was an indication of modified immune response during weaning. Except for the level of serum antibodies, the kinetics observed were comparable to those generally obtained after *Salmonella abortusovis* immunization. Anti-Salmonella LPS were observed briefly 1 week after immunization. Previous results showed that they are mainly IgM antibodies, while anti-Salmonella CWF are IgG1 (pers. obs.). In these conditions it was not possible to see any differences in the IgM or IgG antibody immune responses between the two methods of weaning. Lambs and ewes from PS or TS groups had the same kinetics of antibody production. These results are in agreement with previous findings, which suggests a slight interaction between the weaning method and the systemic antibody immune response [2, 18]. A clear-cut effect of weaning on the anti-KLH (keyhole limpet haemocyanin) antibody response was only seen when lambs were removed from the mother 2 days after birth. No significant differences were found between controls and animals separated at 15 or 28 days after birth [17]. Although the titre of antibodies was reported to be lower when the antigens were injected 3 days after the stress, compared to an injection on the same day [7], the timing of injections could be a key element for the induction of immune responses. Utilization of a live strain of bacteria was chosen because of its similarity to a natural infection. These results did not permit us to assess whether the influence of nutrition shift is greater than that of stress induction as Pollock et al. [20] and Shaw et al. [21] have shown.

Our data showed that TS increases parasitism infestation, which could have a consequence in growth rate. Indeed, TS female lambs showed both a lower body weight and a greater coccidial oocyst output than PS female lambs. However, if the plasma cortisol level was higher in the TS group after weaning, it decreased compared to the period before weaning and the behavioural signs of perturbation disappeared a few days after weaning.

In conclusion, weaning at 3 months, the age when the lamb–ewe bond is naturally weakening, did not appear very stressful whatever the method used. Indeed, if behavioural disturbance seemed higher after separation, especially in PS animals, it decreased rapidly a few days later. Even if the plasma cortisol level was higher after total separation than after partial separation, it did not increase in either method. Only a greater sensitivity to coccidiosis and a lower growth rate among female lambs was observed in the TS group.

These results might indicate that these methods of weaning have limited consequences on both partners.

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REFERENCES

[1] Bernard S., Guilloteau L., Buzoni-Gatel D., Bernard F., Zygmunt M., Bézard G., Lantier F., Mechanisms of acquired immunity induced by a live attenuated *Salmonella Abortusovis* (Rv6) vaccine, in: Cabello, Hormaeche, Mastroeni, Bonina (Eds.), Biology of Salmonella, Plenum Press, New York, 1993, pp. 437–446.

[2] Cockram M.S., Imlah P., Goddard P.J., Harkiss G.D., Waran N.K., The behavioural, endocrine and leucocyte response of ewes to repeated removal of lambs before the age of natural weaning, Appl. Anim. Behav. Sci. 38 (1993) 127–142.

[3] Gautier A., Lantier I., Lantier F., Mouse susceptibility in infection by *Salmonella abortusovis* strain Rv6 is controlled by hte ity/Nramp 1 gene and influence the antibody but not the complement responses, Microb. Pathog. 24 (1998) 47–55.

[4] Gonzalez R., Orgeur P., Signoret J.P., Luteinizing hormone, testosterone and cortisol responses in rams upon presentation of estrous females in the nonbreeding season, Theriogenology 30 (6) (1988) 1075–1086.

[5] Gregory M.W., Norton C.C., Catchpole J., Les coccidioses ovines, Point Vét. 19 (103) (1987) 29–40.

[6] Hargreaves A.L., Stress, handling and reproduction in sheep, in: Oldham C.M., Martin G.B., Purvis I.W. (Eds.), Reproduction Physiology in Merino Sheep: Concepts and Consequences, UWA Ed, Perth, 1990, pp. 253–264.

[7] Hartmann H., Bruer W., Herzog A., Meyer H., Rhode H., Schulze F., Sterinbach G., Allgemeines Adaptation syndrome (Seylié) beim Kalb, Arch. Exp. Vet. Med. 30 (1976) 553–566.

[8] Hennessy M.B., Moorman L., Factors influencing cortisol and behavioral response to maternal separation in Guinea pigs, Behav. Neurosci. 103 (2) (1989) 378–385.

[9] Hennessy M.B., Nigh C.K., Sims M.L., Long S.L., Plasma cortisol and vocalization responses of postweaning age Guinea pigs to maternal and sibling separation: evidence for filial attachment after weaning, Dev. Psychobiol. 28 (2) (1995) 103–115.

[10] Hinch G.N., Lynch J.J., Elwin R.L., Green G.C., Long-term associations between Merino ewes and their offspring, Appl. Anim. Behav. Sci. 27 (1990) 93–103.

[11] Kuhn C.M., Pauk J., Schanberg S.M., Endocrine responses to mother-infant separation in developing rats, Dev. Psychobiol. 23 (5) (1990) 395–410.

[12] Lantier F., Kinetics of experimental *Salmonella abortusovis* infection in ewes, Ann. Rech. Vet. 18 (1987) 393–396.

[13] Lefcourt A.M., Elsasser T.H., Adrenal responses of Angus × Hereford cattle to the stress of weaning, J. Anim. Sci. 73 (1995) 2669–2676.

[14] Levine S., Johnson D.F., Gonzalez C.A., Behavioral and hormonal responses to separation in infant Rhesus monkeys and mother, Behav. Neurosci. 99 (3) (1985) 399–410.

[15] Mc Call C.A., Potter G.D., Kreider J.L., Locomotor, vocal and other behavioural responses to varying methods of weaning foals, Appl. Anim. Behav. Sci. 14 (1985) 27–35.

[16] Mears G.J., Brown F.A., Cortisol and β-endorphin responses to physical and psychological stressors in lambs, Can. J. Anim. Sci. 77 (4) (1997) 689–694.

[17] Napolitano F., Marino V., De Rosa G., Capparelli R., Bordi A., Influence of artificial rearing on behavioural and immune response of lambs, Appl. Anim. Behav. Sci. 45 (1995) 245–253.

[18] Orgeur P., Mavric N., Yvoré P., Bernard S., Nowak R., Schaal B., Lévy F., Artificial weaning in sheep: consequences on behavioural, hormonal and immuno-pathological indicators of welfare, Appl. Anim. Behav. Sci. 58 (1998) 87–103.

[19] Pihoker C., Owens M.J., Kuhn C.M., Schanberg S.M., Nemeroff C.B., Maternal separation in neonatal rats elicits activation of the hypothalamic-pituitary-adrenocortical axis: a putative role for corticotropin-releasing factor, Psychoneuroendocrinology 18 (7) (1993) 485–493.

[20] Pollock J.M., Rowan T.G., Dixon J.B., Carter S.D., Spiller D., Warenius H., Alteration of cellulare immune responses by nutrition and weaning in calves, Res. Vet. Sci. 55 (1993) 298–305.

[21] Shaw K.L., Nolan J.V., Lynch J.J., Coverdale O.R., Gill H.S., Level of nutrition and age at weaning: effects on humoral immunity in young calves, Int. J. Parasitol. 25 (1995) 381–387.

[22] Shillito Walser E., Walters E., Ellison J., Observations on vocalisation of ewes and lambs in the field, Behaviour 91 (1984) 190–203.

[23] Tsuma V.T., Einarsson S., Madej A., Lundeheim N., Cortisol and β-endorphin levels in peripheral circulation around weaning in primiparous sows, Anim. Reprod. Sci. 37 (1995) 175–182.

[24] Yvoré P., Esnault A., Besnard J., Les coccidioses des petits ruminants: coccidioses ovines, Bull. Groupement Technique Vétérinaire 2 (1980) 15–19.