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Hip Dysplasia in Rabbits: Association with Nest Box Flooring

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Purpose: **To study etiologic aspects of hip dysplasia in a colony of Dutch-belted rabbits.**

Methods: **Rabbits used in the study were part of a reproductive toxicologic study. Incidence of hip dysplasia among 296 Dutch-Belted rabbit kits raised on waxed cardboard, smooth Plexiglas, or Plexiglas covered with textured adhesive strips was recorded. All animals were examined at 2 to 4 weeks of age for inability to adduct one or more limbs, then were classified as normal or dysplastic. A subset of 16 juvenile male rabbits (4 normal, 12 affected) raised on Plexiglas flooring were given a physical examination at 12 weeks of age followed by complete necropsy. In four animals (one normal, three affected), pelvic radiography and neurologic examination were performed.**

Results: **Seven percent of the rabbits kits reared on waxed cardboard flooring and 22% of those reared on smooth Plexiglas flooring developed hip dysplasia. Animals reared on Plexiglas floor with traction strips did not have evidence of hip dysplasia. Among the animals selected for detailed analysis, body weight was similar between rabbits with or without splay leg. Affected animals had splaying of one or both hind limbs, various degrees of flattening and reduction of the size of the femoral head, subluxation of the hip, valgus deformity, and patellar luxation. Histologically, there was marked thickening of the hip joint capsule with fibrocartilage formation, mild trabecular bone loss, and bony sclerosis of the proximal portion of the femur and adductor muscle hypoplasia.**

Conclusions: **Provision of non-slippery flooring during the postnatal period is critical in preventing development of hip dysplasia in rabbits. Hip dysplasia resulted in significant musculoskeletal changes, but not abnormal neurologic development.**

Hip dysplasia or splay leg is a condition occasionally seen in pigs, dogs, humans, and rabbits. A genetic predisposition to the condition is suspected in several species (1-4). In pigs a major contributory factor is myofibrillar hypoplasia (5, 6). Exposure of neonatal piglets to slippery floor has been documented to induce splay leg (7). Children that position themselves in the 'TV-sitting' posture may have femurs with abnormal neck anteversion, similar to that of splay-legged rabbits (1). Results of a study of Dutch rabbits with splay leg indicated that the femurs had endtorsion of the shaft and anteversion of the neck, together with exotorsion of the tibia. Dislocation of the hip and abnormal laxity of the joint capsule or ligamentum teres were not observed. In this group of rabbits, the underlying genetic mechanism was thought to be composed either of one recessive gene with reduced expression or of more genes with probable involvement of environmental factors (1). Although environmental factors have been implicated in the pathogenesis in rabbits, specific factors that may be causative have not been reported. We describe impact of the type of flooring and the clinical and pathologic findings associated with this syndrome in a colony of Dutch-Belted rabbits. In addition, the effect of exposure to dichlorodiphenyltrichloroethane (DDT) and heredity on the incidence of the condition was examined.

Materials and Methods

Animals: Animals of the study reported here were part of a group of juvenile Dutch-Belted rabbits involved in an animal care and use committee-approved reproductive toxicologic study of testicular development. Dams and sires were purchased from a facility free of *Pasteurella multocida*, *Bordetella bronchiseptica*, *Encephalitozoon cuniculi*, and *Eimeria stiedae* (Myrtles Rabbitry, Thompson Station, Tenn). Animals were housed in an AAALAC International-accredited facility in stainless steel cages with slatted floors and drop pans containing aspen chips (Northeastern Products Corp., Warrensburg, N.Y.). Cages were cleaned and sanitized every two weeks, and drop pans were cleaned and sanitized twice a week. Temperature in the animal rooms was maintained at 18 to 20°C, with approximately 40% relative humidity. The light cycle was maintained at 14/10 hours (light/dark), and air circulation was 10 to 12 air changes/h. Dams were fed a breeder diet (Prolab breeder rabbit, 5P29, PMI Nutrition International, Inc., Brentwood, Mo.) and provided water ad libitum from a hanging bottle. After weaning, kits were switched to a high fiber diet (LabDiet, Prolab hi-fiber rabbit, 5P25, PMI Feeds Inc., St Louis, Mo.).

Breeding: Does were bred by artificial insemination. Semen was collected from eight to twelve bucks, using an artificial vagina. On microscopic examination, good-quality seminal ejaculates were pooled and extended in egg yolk citrate, then 200 μl of extended semen containing approximately 10 million spermatozoa was inseminated. Ovulation was induced by administering 10 μg of gonadotropin-releasing hormone (Calbiochem, San Diego, Calif.) intramuscularly. Pregnant does were then exposed to either corn syrup (vehicle only control) or to DDT (25 or 250 μmol/kg) in corn syrup, daily from mid-gestation through four weeks after kindling. Each of the three breeding trials involved use of

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pooled semen collected from 8 to12 sires. New dams were added in each trial since animals that were treated with DDT could not be re-examined due to the design of the primary toxicologic study. All kits were examined at 2 to 4 weeks of age for inability to adduct one or more limbs and were classified as normal or dysplastic.

Nest Boxes: Does were supplied with nest boxes containing autoclaved aspen wood shavings (Northeastern Products Corp., Warrensburg, N.Y.) on gestation day 27. Waxed cardboard nest boxes (Universal Sani-Nest, Bass Equipment Company, Monett, Mo.) were used for the first 95 kits, data from which were recorded in this study (Table 1). Due to concerns about inability to sanitize the paper nest boxes, we switched to metal nest boxes (Solid Steel Nest Box, Bass Equipment Company, Monett, Mo.). Ninety-five kits were raised in these nest boxes (Table 1). In the first group of rabbits reared in the new nest boxes, 22% of the offspring developed the splay leg condition. This was a marked increase over the 7% incidence associated with use of the previous types of nest boxes. Because of this seeming association with the change in flooring, textured non-slippery plastic strips (3M 370 Safety-Walk, Medium Resilient Treads, 3M, St. Paul, Minn.) were placed over the Plexiglas. One-hundred twenty-nine kits were raised on this flooring (Table 1).

Clinical Examination: To examine the clinical and pathologic character of the syndrome, 16 animals (12 affected, 4 normal) raised on the Plexiglas flooring underwent complete physical examination. Eight of these kits received vehicle and eight received DDT. In four animals (three affected, one normal) complete neurologic examination, including nerve conduction velocities of the hind limbs, was performed, followed by pelvic radiography. All rabbits were euthanized by administration of an overdose of barbiturate (Euthasol, Diamond Animal Health Inc., Des Moines, Iowa.). Complete necropsy was performed on all 16 rabbits.

Pathologic Examination: The hip joints and adductor muscles were examined grossly and histologically; stifle joints were examined grossly. Changes in the splayed rabbits were compared with those of unaffected (control) animals. Lesions classified as 'mild' included acetabula more visibly shallow than controls, subluxation of the femoral head, and thickening of the joint capsule. 'Severe' lesions consisted of markedly shallow to completely flattened acetabula, thickening and deformity of the femoral head and neck, and prominent thickening of the coxofemoral joint capsule. Tissues for histologic examination were fixed in neutral-buffered 10% formalin, embedded in paraffin, and sectioned at 5-μm thickness. Muscle specimens were attached to cardboard during fixation to prevent contraction. Sections were stained with hematoxylin and eosin, and skeletal muscle was stained with phosphotungstic acid hematoxylin as well to evaluate striations. Rabbits from the initial group (raised on waxed cardboard) were not examined because we did not consider the 7% incidence of dysplasia to be out of line with previous observations. No animals in the third group (reared on traction strips) were clinically affected, and thus, this group was not subjected to more thorough clinical or pathologic analysis.

Table 1. Incidence of hip dysplasia among 296 rabbit kits raised on various nest box floors

	Waxed cardboard		Plexiglas		Safety-Walk	
Treatment	No. born	No. splays	No. born	No. splays	No. born	No. splays
Control	27	2	36	3	40	0
Low DDT	39	4	24	9	51	0
High DDT	23	0	18	5	38	0
Total	89	6	78	17	129	0

Statistical Analysis: Statistical analysis was performed by use of an SAS statistical package (SAS Institute Inc, Cary, N.C.), with the Logistat procedure for logistical regression, the Freq procedure for χ^2- analysis, Cochran-Mantel-Haenszel statistics based on table scores, and a two-tailed *t*-test of unequal variance for comparison of weights. A value of $P < 0.05$ was considered significant.

Results

A total of 66 litters producing 296 kits were examined during the course of the breeding program. The breakdown of the animals with hip dysplasia is shown in Table 1. The most striking result is the variation of splay leg incidence relative to flooring substrate. Offspring from does provided with waxed cardboard nest box flooring had an overall incidence of 6.7% splay leg (6/89), kits raised on the smooth Plexiglas floor had an incidence of 21.8% (17/78), and none of the 129 kits (0%) reared on textured flooring developed the condition. The results were statistically significant for floor effect ($P = 0.002$, Cochran-Mantel-Haenszel comparison of χ^2- analysis, controlling for treatment group). Dosing with DDT was significantly correlated with incidence of splay leg in the Plexiglas group ($P = 0.0215$, χ^2-analysis), but not in the other groups; logistical regression analysis did not reveal significant interaction between dosing and floor use on splay leg incidence. Physical examination did not reveal abnormalities other than splaying of the limbs. Neurologic examination also did not reveal abnormalities other than gait deficits associated with inability to adduct the limbs. Nerve conduction velocities (300 to 400 milliseconds) were similar between affected and normal limbs as well as among various rabbits (n = 4). Affected and unaffected rabbits had similar appetite and body weights; the latter ranged from 1,334 to 1,540 g (1,470 ± 92 g [mean ± SD], n = 4) among unaffected animals, 1,267 to 1,665 g (1,391 ± 139 g, n = 7) among unilaterally affected, and 1,157 to 1,548 g (1,350 ± 143 g, n = 5) among bilaterally affected kits. The DDT-treated rabbits had body weights similar to those of the untreated animals (1,455 ± 120 g vs. 1,340 ± 121 g, n = 8 in each group. Comparison of body weight between normal and affected, and unilateral versus bilateral by use of a two-tailed *t*-test failed to reveal significant difference between any of the groups.

Radiography: Lateral and ventrodorsal radiographic views of the pelvis and hind limbs were taken from four rabbits, chosen to represent variations between affected and unaffected individuals. The single control animal had normal hind limb and pelvic images (Fig.1A). The three affected rabbits examined had characteristic radiographic changes (Fig.1B), varying only in severity. Most severe changes observed were right sided. Subluxation to luxation of the coxofemoral joint was noted in all three rabbits. Two of the animals radiographed had right- sided subluxation; one had luxation (Fig.1B). Evidence of bony proliferation and remodeling was observed around affected acetabula. Three of the six acetabula from affected animals were widened and shallow, measuring a third to a half the depth of those from the control rabbit.

Gross findings: Four animals used as negative controls did not have gross lesions. Appreciable musculoskeletal lesions were found in the remaining 12 rabbits, whereas other organ systems were grossly normal. Affected animals had coxofemoral subluxation or luxation, with one or both hind limbs extended laterad or caudad. Flexion of these limbs appeared to be markedly restricted. Acetabula were shallow, and the coxofemoral

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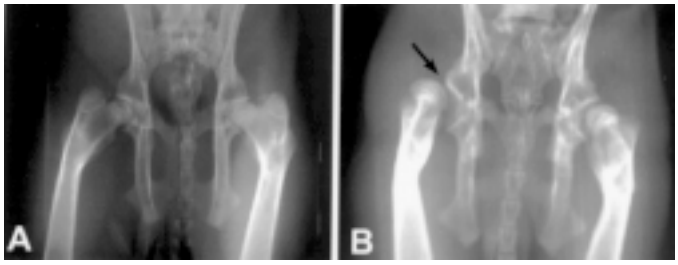

Figure 1. Photographs of a normal rabbit (left) and a rabbit with hip dysplasia (right). Notice subluxation of the left hip, luxation of the right hip (arrow), and shallow acetabula in the affected rabbit.

joint capsules were often thickened. Blunt femoral heads, varying from mildly affected to severely misshapen and decreased in size, were present. Eight animals also had valgus deformity and/or lateral patellar luxation and slight bowing of the tibia (Fig. 2). The vertebral column appeared normal and intact. Evidence of muscle atrophy or hypoplasia was not observed. The gross findings were in agreement with those seen radiographically.

Histopathologic changes: Right and left adductor muscles of all animals were examined. Of the unilaterally affected, four had changes in right adductor muscles, with three of the four having bilateral lesions, two had changes in the grossly unaffected limb only, and one did not have muscle changes. Among the bilaterally affected animals, three had histopathologic muscle changes in both hind limbs and two in one limb only. Changes were mild in all cases and consisted of plump nuclei in skeletal muscle, increased in number and often arranged end to end in a tandem arrangement. This was interpreted as regeneration or active formation indicative of muscle hypoplasia. This "rowing" of nuclei was seen in 25% (1/4) of control animals and 92% (11/12) affected animals. Scattered areas of increased vascularity were observed in several sections, often in conjunction with rowing of nuclei. Mild myofibril fragmentation was infrequently seen in the muscles of control and affected animals. Sections of peripheral nerves associated with the muscle appeared normal in all animals.

Decalcified sections of the proximal portion of the femur were examined in two control and three affected rabbits. Bony lesions were present in all three of the affected limbs examined, but not in the control animals. In affected animals, the coxofemoral joint capsules were thickened and contained multiple areas of fibrocartilage formation. There was often unilateral scalloping of the diaphyseal cortex, with Howship's lacunae and osteoclasts present. The opposite side of the bone exhibited periosteal fibroplasia and basophilic cement lines (glycosaminoglycan deposition at sites of intermittent bone formation) in layers of new bone. Decreased number and thickness of trabeculae in the epiphysis and metaphysis also were observed.

Discussion

The marked increase in the incidence of splayleg condition concomitant with the change in flooring from cardboard to smooth plastic strongly suggests traction as an important environmental factor in development of hip dysplasia in rabbits. This notion is further supported by resolution of the condition once textured plastic strips were applied to improve traction of the floor. These findings are similar to the observation that neonatal piglets kept on a slippery floor for 18 hours developed splay leg condition partially reversible by placing the animals on non-slippery straw bedding (7).

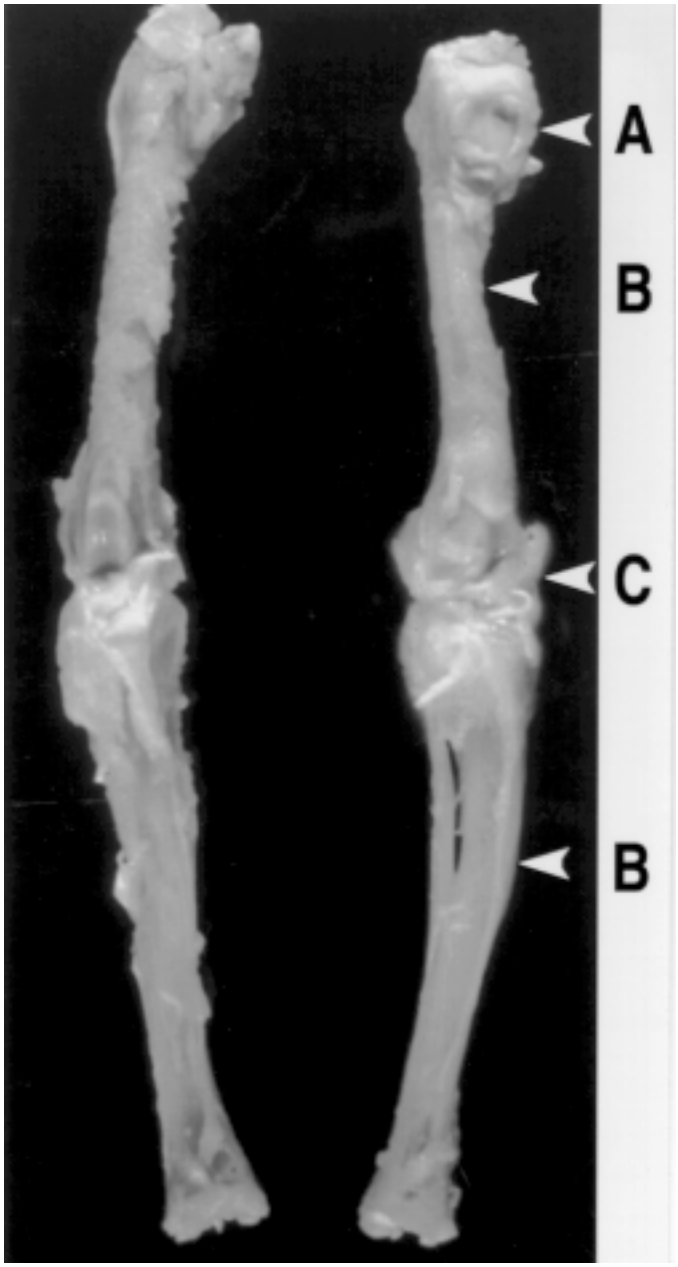

Figure 2. Photographs of a control hind limb (left) and an affected hind limb (right). Gross lesions on affected limb include: (A) anteversion of femoral head and neck, with blunting of head and thickened joint capsule; (B) 'bowed' femoral and tibial shafts, resulting in valgus deformity; and (C) lateral patellar luxation.

The compound DDT is known to affect neuromuscular function (8). A significant correlation with DDT treatment and increased incidence of splay leg was noted in rabbits of the Plexiglas group, which by far, had the highest number of affected animals. However, continued use of a variety of pesticides in these studies has not resulted in hip dysplasia since the nest box flooring was altered. It is, therefore, unlikely that DDT exposure played a primary role in development of dysplasia. It is possible that kits exposed to DDT may have been weaker or slower to develop, thus enhancing the consequences of an unstable floor surface on musculoskeletal development.

Genetic factors have been suggested to play an important role

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in this condition (1-4, 9). Arendar and Milch (2) provided strong evidence of splay leg in Lop rabbit inherited in a simple autosomal recessive pattern traced to a single male. Jooesten and colleagues (1) performed pedigree analysis and genetic mating tests and concluded that the underlying genetic system is composed of one recessive gene with reduced expression or of more genes, with probable involvement of environmental factors. These authors state that their does had nest boxes with straw, and do not provide further description of the flooring or the role of environmental factors. In another study (3), two males sired five litters, each of which produced at least one splay-legged kit. Here the evidence for a genetic trait is less clear since both males died and further offspring were not produced. The flooring conditions for the kits in these reports were not discussed, so the role substrate may have played in development of splay leg in these reports cannot be determined. In our study, the rabbits were from unrelated parents, and in three subsequent breeding trials using pooled semen from at least half the sires and dams in the original outbreak of splay leg, new case of splay leg have not been observed. In more than 300 kits born to date after application of traction materials to the nest box floor, no cases of hip dysplasia have been observed, supporting the association between floor substrate and development of splay leg. Although the role of a genetic component to this condition cannot be ruled out by the experimental design of this study, the likelihood of randomly eliminating all carriers of a recessive trait would seem small. We also cannot rule out other temporal factors that might have played a role in the development of splay leg because a randomized block study design was not used due to the nature of the primary toxicologic study. Our desire to avoid production of developmentally impaired kits that would have to be excluded from the primary study also limited the scope of the secondary study. The strong association of flooring substrate with development of this condition is, therefore, likely to be an important contributory factor for development of musculoskeletal anomalies, even though other contributory factors cannot be conclusively ruled out by this observational study.

The morphologic changes we noted are very similar to those previously reported (2, 3), and included coxofemoral subluxation, shallow acetabula, thickening of the hip joint and stifle joint capsules, lateral patellar luxation, valgus deformity, and slight bowing of the tibia. Marked difference in bone and joint morphology with minimal damage to muscle in the two groups suggests that rabbit splay leg is unlike the porcine form, which presents as myofibrillar hypoplasia. Lindsey and Fox (4) surmise that splay leg remains a descriptive clinical term without clearly established pathologic meanings. They suggest that it is a manifestation of several disease entities including hereditary conditions, such as syringomelia, hypoplasia pelvis, femoral luxation, and hereditary distal foreleg curvature.

Our results illustrate that the condition of hip dysplasia in rabbits is apparently closely associated with housing on smooth flooring during the early postnatal period. Provision of traction strips on standard nest box floors can greatly reduce the incidence of this condition. In this group of experimental animals, hip dysplasia appears to have been a developmental anomaly secondary to environmental factors. Our study did not completely resolve the hereditary component of this condition, which can only be resolved by extensive breeding trials.

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References

1. **Joosten, H. F. P., P. Wirtz, H. O. F. Verbeek, and A. Hoekstra.** 1981. Splayleg: a spontaneous limb defect in rabbits. Genetics, gross anatomy, and microscopy. Teratology **24:**87-104.
2. **Arendar, G. M., and R. A. Milch.** 1966. Splay-leg-A recessively inherited form of femoral neck anteversion, femoral shaft torsion and subluxation of the hip in the laboratory lop rabbit: its possible relationship to factors involved in so-called 'Congenital dislocation' of the hip. Clin. Orthop. Relat. Res. **44:**221-229.
3. **Innes, J. R. M., and W. K. O'Steen.** 1957. Splayleg in rabbits. An inherited disease analogous to joint dysplasia in children and dogs. Lab. Invest. **6:**171-186.
4. **Lindsey, J. R., and R. R. Fox.** 1994. Inherited diseases and variation, p. 293-319. *In* P. J. Manning, D. H. Ringler, and C. E. Newcomer (ed.), The biology of the laboratory rabbit, Academic Press, Inc., San Diego, Calif.
5. **Curvers, P., R. Ducatelle, P. Vanderkereckhove, W. de Coster, A. Calus, and J. Hoorens.** 1989. Morphometric evaluation of myofibrillar hypoplasia in splayleg piglets. Duetsche Tierarztliche Wochenschrift **96:**189-191.
6. **Hnik, P., and R. Vejsada.** 1979. Neuromuscular transmission in some hindlimb muscles of piglets with congenital myofibrillar hypoplasia (splayleg). Physiologia Bohemoslovaca **28:**385-392.
7. **Kohler, E. M., R. F. Cross, and L. C. Ferguson.** 1969. Experimental induction of spraddled-legs in newborn pigs. J. Am. Vet. Med. Assoc. **155:**139-142.
8. **Hong, J. S., D. W. Herr, P. M. Hudson, and H. A. Tilson.** 1986. Neurochemical effects of DDT in rat brain in vivo. Arch. Toxicol. Suppl. **9:**14-26.
9. **Reed, A. L., G. G. Keller, D. W. Vogt, M. R. Ellersieck, and E. A. Corley.** 2000. Effect of dam and sire qualitative hip conformation scores on progeny hip conformation. J. Am. Vet. Med. Assoc. **217:**675-680.

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