



Drug induced folic acid (vit B₉) deficiency in a hydrocephalus mini lop rabbit

Esther van Praag

The concurrent administration of the anticonvulsant drug levetiracetam and the antibiotic trimethoprim-sulfamethoxazole can lead to folic acid deficiency.

Dwarf rabbits and lops have become popular pets as they are easy to keep in an apartment. They are also valued for the roundness of their heads and their bodies, which is reminiscent of young animals (Figure 1). Their weight ranges between 1 kg (2.2 lbs.) and 1.25 kg (2.75 lbs.) for breeds with straight ears and between 1.4 (3.0 lbs.) and 1.7 kg (3.7 lbs.) for breeds with falling ears (lops).

The selection of small sized animals within the Polish rabbit breed has, nevertheless,

introduced hereditary defects such as the dwarfism lethal gene ('n') and the dental malocclusion gene (Figure 2). All dwarf rabbit breeds are affected: dwarves with straight ears, dwarf lops, Dutch dwarfs, mini-Rex, dwarf satin rabbit, dwarf Hotot, Polish rabbits, and dwarf Angora rabbit.

The dwarfism gene 'n' is dominant over the normal size gene 'N'. Thus, when mating two true dwarfs carrying the 'Nn' genes, their offspring will theoretically be composed of:



Figure 1: True dwarf rabbits with straight and hanging ears (lop), all heterozygote carriers of the lethal dwarfism gene. (Pictures: A. van Praag)

- 50% of 'Nn' heterozygotes. These are true dwarf rabbits that carry the dwarf characteristics and the lethal 'n' gene, which they can in turn transmit to part of their descendants.
- 25% of 'NN' homozygotes. They are false dwarfs or dwarf hybrids, their size is small, but their ears are longer and their bodies longer. Their weight can reach up to 2.5 kg (5.5 lbs.).
- 25% of 'nn' homozygotes. These newborn rabbits are called double-dwarfs (Figure 2). The double-dwarfs are smaller than other kits of the same litter and show a slow and difficult growth. They have abnormalities and/or deformities: transparent or no skin, swollen skull and hydrocephalus (Figure 3), tiny ears, deformed front limbs, poorly developed hips and malformation, crossing of hind

limbs or hind limb paralysis, abnormal protrusion of the eyeball from the orbit (exophthalmos). Most die soon after birth. Only a few double-dwarfs reach adulthood, but suffer from many health problems.

Neonatal hydrocephalus

Hydrocephalus is the result of an imbalance between the production of cerebrospinal fluid and its drainage from the brain. The accumulation of fluid in the cerebral ventricles and the subarachnoid space has several origins:

- Obstruction in case of non-communicating obstructive hydrocephalus;
- Inadequate absorption in case of non-obstructive hydrocephalus.

This severe neurological abnormality is



Figure 2: A homozygous dwarf 'nn' (double dwarf, left) has a smaller size and shows various deformations, a swollen skull, tiny ears, front legs deformities and underdeveloped hips. (Picture: Gilles Python)



Figure 3: Nest with newborn rabbits aged 4 days. The middle one (arrow) shows a raised skull characteristic of hydrocephalus. (Picture: Sabrina Martinez).

often observed in double-dwarf (Figure 3).

Another cause of neonatal hydrocephalus is an overdose of vitamin A in does during gestation. Neonatal hydrocephalus may also be acquired after infection of the fetus by the protozoan parasite *Toxoplasma gondii*, by an intra-placental injury or illness during gestation.

Etiologies of different types of epilepsies

Seizures observed in rabbits belong to the partial type with conscious perception of the environment or to the generalized type with the loss of conscious perception of the environment.

Their etiologies are multiple. In the case of hydrocephalus, accumulation of non-drained cerebrospinal fluid in the brain leads

to an increase of the intracranial pressure. In this case, administration of omeprazole can sometimes help to reduce the frequency of seizures.

Epileptic seizures may also be secondary to an evolutionary process in the brain: neoplasia (brain tumor), metabolic diseases or viral or bacterial infections. Other common causes of seizures in rabbits are parasitic infections of the nervous system by protozoa such as *Encephalitozoon cuniculi* or *Toxoplasma gondii* or infections by the nematode worm *Baylisascaris procyonis*.

Idiopathic or traumatic origin cannot be excluded.

Various drugs are administered to the rabbit in order to control seizures. Most lose efficiency over time and the doses need to be increased.

Interference with the metabolism of folic acid

Levetiracetam

Levetiracetam is an anticonvulsant drug belonging to the class of pyrrolidines. It is used in human medicine, but is also administered to dogs, cats and rabbits suffering from seizures that are refractory to other anti-epileptic drugs. It can be used alone or in combination with phenobarbital or potassium bromide.

In rabbits, levetiracetam diffuses rapidly into the tissue at concentrations similar to those found in blood, with the exception of the adipose tissue and the ocular lens. It is higher in the kidneys, which can be explained by the fact that these organs are involved in the elimination of this drug. In the brain, the absorption of levetiracetam is linear and dose-dependent. It spreads throughout the brain tissue.

Its action is fast and the drug seems well tolerated. Serious adverse reactions have, however, been observed in man:

- Loss of weight;
- Liver failure ;
- Pancreatitis;
- Decrease in the number of white blood cells (leucopenia), of neutrophil granulocytes and of platelets (pancytopenia), with possible bone marrow depression;
- Decreased number of red blood cells;
- Decreased levels of vitamin B₈ (biotin), B₉ (folic acid) and B₁₂.

When this drug is used to control seizures in animals, it is imperative to do blood tests at regular intervals.

Antibiotics

Few antibiotics like trimethoprim-sulfamethoxazole interfere with the metabolism of folic acid, which increases the

risk of anemia and, in severe cases, leukopenia. This antibiotic can also have nephrotoxic effects and affect kidney function. In this case, the elimination of other drugs administered to the patient may be delayed. As a result, their concentration increases in the blood with potential severe systemic toxic effects or death.

When trimethoprim-sulfamethoxazole is concurrently administered with other drugs acting on folic acid, it is important to do a blood test (CBC and tests of liver and kidney functions) regularly and analyze it carefully in order to quickly detect a deficiency in folic acid.

Folic acid deficiency

Folic acid deficiency is accompanied by various side effects like:

- Loss of appetite and weight;
- Irritation of the mucosal membranes;
- Inflammation of the tongue, causing a swelling, a change of color or a change of shape of the tongue (glossitis);
- Generalized weakness;
- Permanent thirst and need to drink;
- Digestive problems, gastritis, diarrhea;
- Intestinal malabsorption, nutritional deficiencies, e.g. iron, leading to iron deficiency even if the relationship between folic acid and the metabolism of iron is yet poorly understood;
- Cardiovascular problems.

When the deficiency is severe, the level of white blood cells and blood platelets may be affected too, and decreased.

Hydrocephalus mini-lop rabbit: history

The mini male lop is 4 years old. Its weight is 1.56 kg (3.4 lbs.) (Figure 4). Radiographs of the skull show the presence of fluids in the brain (hydrocephaly), a severe malocclusion of the incisors and

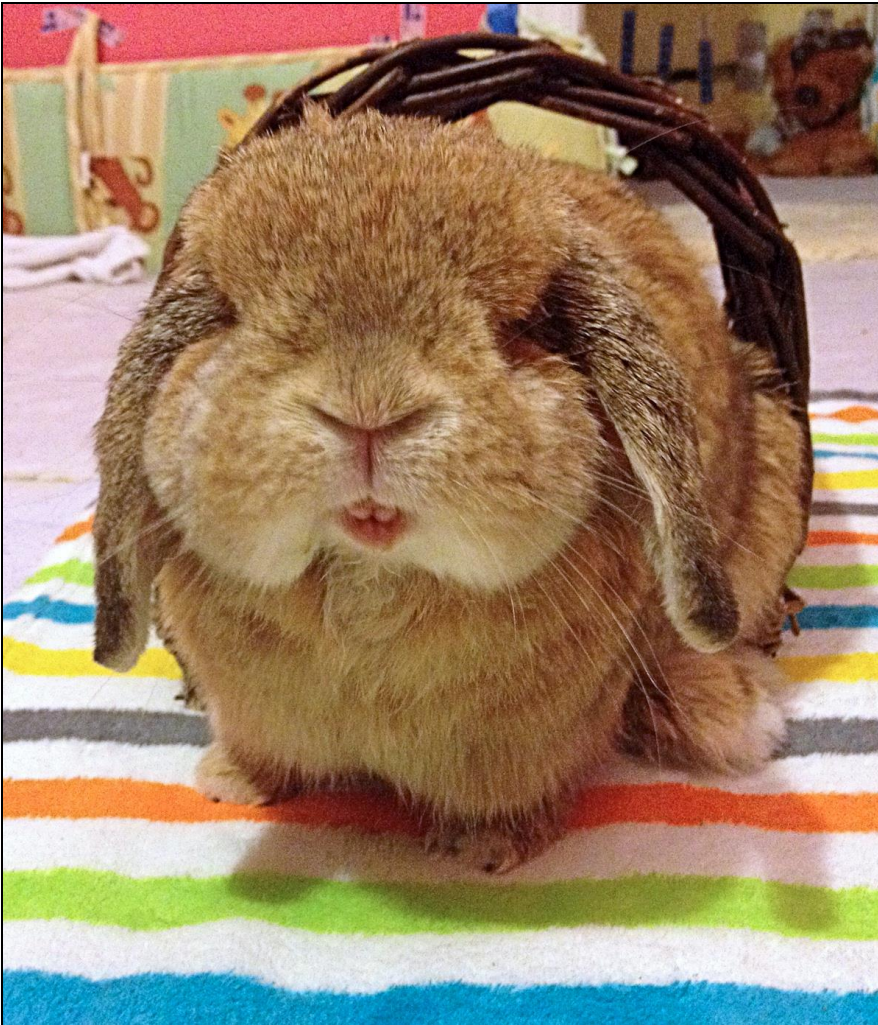


Figure 4: Male mini-lop suffering from hydrocephalus and various other health problems including regular seizures. (Picture: Delia Canas).

cheek teeth, as well as abscesses of the tooth roots. The mandibular incisors and few molars are missing. Coronary growth and wear of the cheek teeth (premolars and molars) is irregular and dental treatment is needed regularly to remove spurs and other elongation (Figure 5).

This lop also suffer from congenital agenesia of the punctum (small openings in the inner corner of the eyelids, by which the tear fluid is flowing) and from eye and nasal discharge, and sneezing. The ocular and nasal discharge is muco-purulent and has become worse over time. He developed an ear infection of the outer ear.

A deformity of the spine is observed on the chest radiograph; it is of lordosis type. It also suffers from digestive problems, and recurrent episodes of stasis. A link between these disorders and the administered drugs has not been studied.

The seizure episodes that affect this lop started a few months after the fracture of his left hind limb (3 years ago). They belong to the partial type; they are isolated or are repeated at one-hour intervals for 12 hours. The attacks last from 30 seconds to a few minutes, during which the rabbit seems to remain conscious and aware of its surroundings (Figure 6).

After an increase in the frequency of seizures, the rabbit was given a levetiracetam monotherapy. The initial dose of this anti-convulsing drug was 100 mg/ml. As efficiency decreased over time, the dose was readjusted and increased.

When the first blood test was done, the mini-lop received the following medications:

- Levetiracetam, 181 mg/ml, TID;
- Gabapentin, weaning - 0.8 mg/kg BID;
- Simethicone child, TID;
- Metacam, 0.45-0.6 mg/kg QD;
- Trimethoprim-sulfamethoxazole, 25 mg/kg sulfa and 5 mg/kg trimethoprim, BID;
- Cisapride, weaning - 0.35mg/kg once per week.

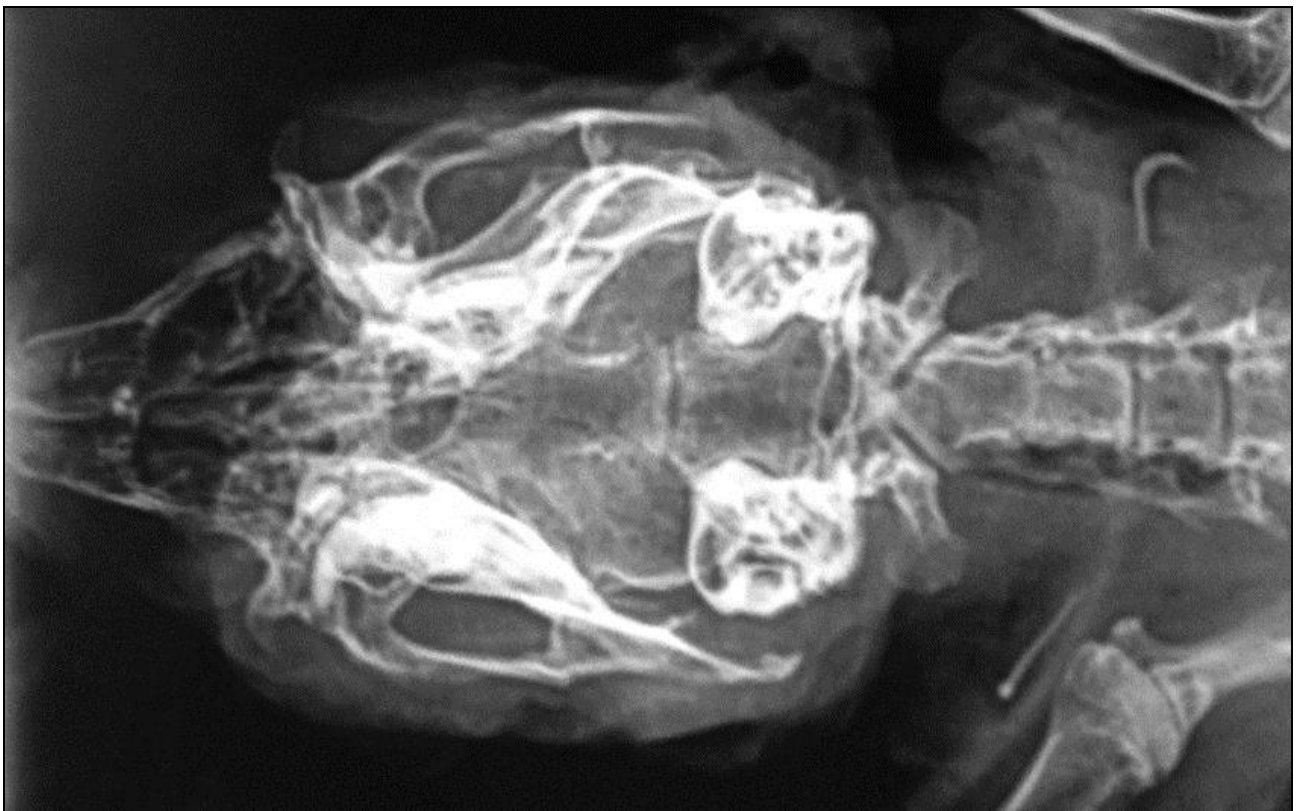
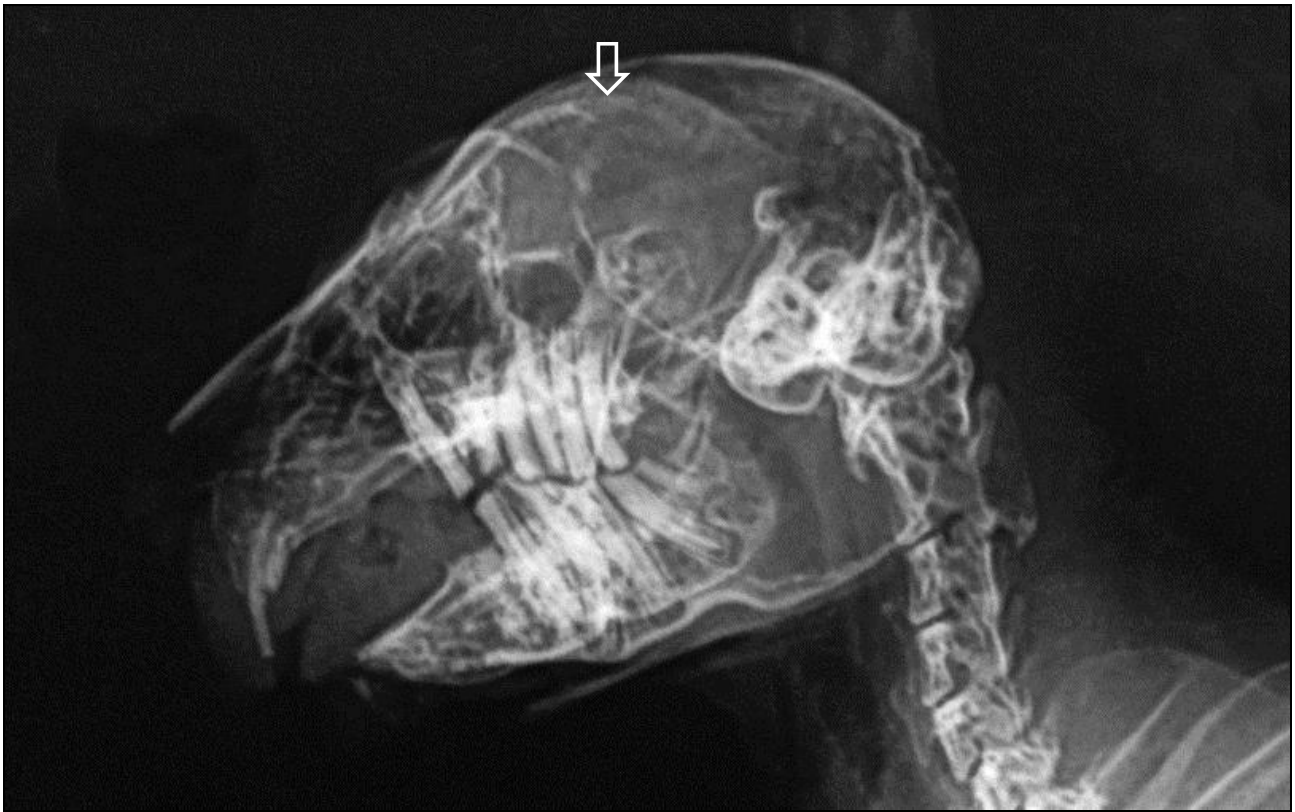


Figure 5: Lateral and dorso-ventral x-ray views of the skull of a mini lop rabbits that suffers from hydrocephalus and seizures.



Figure 6: Male mini-lop during seizure episodes. (Pictures from video's: Delia Canas).

The mini-lop received penicillin by injection during 5 days, 4 days before the blood test, followed chloramphenicol during 2 days, 2 days before blood test, and, finally, enrofloxacin. The first two antibiotic treatments were discontinued after observing a decrease in appetite.

First blood test

Biochemical parameters

The results obtained for the liver and kidney functions are within range. The trimethoprim-sulfa antibiotic does not cause liver or kidney failure.

The only abnormal value is that of phosphorus, which is too low (2.6 mg/dl, normal: 4-6 mg/dl). It is hard to find an explanation since the level of calcium is normal. It may be related to the prolonged use of laxatives, to endocrine problems or to digestive or respiratory issues.

Hemogram n° 1

Quantitative study of white blood cells

A hematological test shows changes in levels of specific white blood cells (Table 1):

Monocytosis: the number of monocytes is

high, which is indicative of bone marrow damage, but also of a chronic infection or heart disease. The latter is possible after e.g. a treatment of dental root abscesses in rabbits. Bacteria can enter the bloodstream during the surgery, invade the heart and cause heart disease.

Neutropenia: a low number of neutrophils is indicative of bone marrow problems or it may be secondary to the administration of penicillin, trimethoprim-sulfamethoxazole or of levetiracetam.

<u>Liver function</u>		
	Measure	Normal
ALK phosphatase	35	0-150 U/l
Alanine aminotransferase (ALT)	81	10-110 U/l
Aspartate aminotransferase (AST)	19	< 105 U/l
Albumin	4.2	3.5-5.0 g/dl
Globulin	2.6	1.5-2.7 g/dl
<u>Kidney function</u>		
Blood urea nitrogen (BUN)	18	6-20 mg/dl
Creatinine	0.9	0.7-2.0 mg/dl
BUN/Creatinine	20	

	CBC	Reference value	Units
Red blood cells	5.87	4.37-6.21	10 ⁶ /ul
MCHC	33.1	30.0-36.1	g/dl
Polychromasia	moderate		
White blood cells	4.8	5.0-11.8	10 ³ /ul
Monocytes	7	1-6	%
Neutrophils, segmented	2.7	3.28-6.22	10 ³ /ul
Lymphocytes	1.6	2.42-4.62	10 ³ /ul

Table 1 : Résultats du premier hémogramme réalisé 3 jours après l'administration de lévétiracétam chez un bélier hydrocéphale souffrant de crises épileptiques partielles.

Lymphopenia: a low count of lymphocytes is observed in case of an immune deficiency or bone marrow depression. It may also be drug induced, e.g., the administration of levetiracetam.

Basophilia: a low level of basophils is not indicative of health disorders and is, therefore, not considered as medical important.

The amount of platelets in the blood is normal.

Quantitative study of red blood cells

There are no changes in the level of erythrocytes.

Polychromasia: moderate. Red blood cells have a blue-grayish color, indicating that they are released prematurely from the bone marrow into the blood. In rabbits, polychromasia is, however, not indicative of a clinical problem. Indeed, the life span of erythrocytes is relatively short - 57 days, and cell renewal is regular. The presence of some nucleated cells and Howell-Jolly bodies is considered normal.

51 days later...

The rabbit has episodes of respiratory distress; it remains with its neck and head

extended upwards. During a clinical examination it appeared that the mucous membranes of the oral cavity and gums are cyanotic, the tongue is swollen and the larynx is inflamed.

Hemogram n° 2

A new CBC done 51 days after the first one. The quantitative study of white blood cell shows that levels returned to normal (Table 2). It also shows a reduction of the amount of hemoglobin and of the hemoglobin concentration in a given volume of packed red blood cells (MCHC). The mean corpuscular volume or hemoglobin average rate per erythrocyte (MCV) is high, but within normal range (Table 2).

The lower values of hemoglobin (HGB) and of the concentration of red blood cell hemoglobin (MCHC) may indicate maturation problems of the hematopoietic stem cells in the bone marrow. This type of anemia has been observed, *inter alia*, in cases of:

- Myelodysplastic syndrome (MDS);
- Internal bleeding in the digestive system (ulcers);
- Deficiencies of iron, folic acid (vitamin B9) and vitamin B12;

	CBC	Reference values	Units
Red blood cells	6.18	5-00-9.0	10 ¹² /l
Hemoglobin	11.4	12.7-16.3	g/dl
MCHC	26.2	30.0-38	g/dl
MCV	70	57-70	
White blood cells	5.02	5.0-11.8	10 ⁹ /l
Monocytes	0.14	0-0.5	10 ⁹ /l
Neutrophils	2.04	3.28-6.22	10 ³ /ul
Lymphocytes	56.6	0-100	%

Table 2 : Results of the second blood count done 54 days after the administration of levetiracetam in a hydrocephalic ram with partial epileptic seizures.

- Depression of bone marrow;
- Drugs.

Biochemical parameters show changes as compared to the first test. The level of alanine aminotransferase (ALT) is, thus, very high.

<i>Biochemical parameters of the blood test n° 2</i>		
ALK phosphatase	33	U/l
Alanine aminotransferase (ALT)	223	20-109 U/l
Aspartate aminotransferase (AST)	-	
Globulin	4.7	g/dl

As the ALK phosphatase marker is normal, liver failure is unlikely. The sole increase in alanine aminotransferase (ALT) can, however, be indicative of a folic acid deficiency

The trimethoprim-sulfamethoxazole treatment was stopped 14 days after the first blood test. However, 51 days after the first blood count, the lop rabbit was still

administered levetiracetam. These results, combined with observations during the clinical examination are typical of folic acid deficiency.

Conclusion

Clinical examination of the lop rabbit and analysis of the blood tests leads to the conclusion that it is likely suffering from **a severe deficiency in folic acid** caused by the anti-convulsing drug levetiracetam acid or the trimethoprim-sulfa antibiotic or an additive effect of both drugs.

This case shows the importance of knowing the side effects of the different medications administered to an animal, assessing their interactions and their toxic additive or even fatal effects.

And never administer simultaneously two drugs that lead to folic acid deficiency in the body.

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