

# ARF Investigator Report

By

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## Investigation of the Multiple-Malformation Syndrome in Llamas and Alpacas Associated with Choanal Atresia

It is one thing to read about choanal atresia (CA) or calmly discuss details surrounding the defect with Anibal G. Armién, DVM, MSc, Dr. Vet. Med. over the telephone. It's quite another to see the anguish on the face of a breeder who experienced two occurrences of CA on her farm, and to listen to her voice shake with mingled emotions of horror and sadness.

"The first two crias born on our farm had choanal atresia," the woman told me while both of us stood next to the coffee urn in a conference room. "It was awful. Of course, we had them euthanized. There was other stuff, too." "What other stuff?" I asked gently. "The crias also had . . . I'm sorry . . ." The woman's eyes welled with tears. She shook her head, "I just can't talk about it."

Years later, I was to remember her unfinished sentence after reading Dr. Armién's investigator proposal prepared for the Morris Animal Foundation (MAF). While MAF is administering the study, it is fully funded by the Alpaca Research Foundation (ARF).

A research proposal is always very technical. I need to read and study each one carefully before formulating pertinent questions for the investigator. This proved to be true for Dr. Armién's proposal. Initially, however, my eyes did a preliminary, quick scan of several pages crowded with scientific information.

My attention was immediately drawn to a word spelled in capital letters: CHARGE. It's the acronym for Coloboma, Heart malformation, choanal Atresia, Retardation of growth and/or development, Genital anomalies, and Ear anomalies.

The association between CA and other anomalies in humans was first noted by B. D. Hall in 1979. Why is Dr. Hall's research in humans mentioned in Dr. Armién's proposal? What is its relevance to llamas and alpacas?

All mammalian species “share” considerable stretches of *deoxyribonucleic acid* (DNA) with each other. For example, the code of certain mouse genes is nearly identical to that of humans. Research results from one species can therefore be very helpful in pinpointing the genetic source of disease in another species.

In 1994, Dr. Horst Leipold et al already documented in roughly one third of the llama CA cases he studied multiple anomalies resembling those characterized by Dr. Armién as being found in CHARGE syndrome. In all likelihood, one or several of these defects were the “other stuff” the breeder I met at the conference had been referring to.

Previous research into the inheritance of CA in camelids proved inconclusive. To the great disappointment of breeders, the results of test breedings did not confirm CA to be inherited through a single, recessive gene. Had that been the case, both sire and dam would have to be carriers of the defective gene to produce CA in their cria. The offspring who inherits a double “dose”, so to speak, is born with the defect. A breeding of two (surgically corrected) CA parents should therefore have always resulted in a CA cria. That did not happen.

No clear cut mode of inheritance has ever been determined. Some breeders interpret this to mean that CA is not an inherited defect in llamas and alpacas. Such an assumption was and remains totally premature and without scientific validity. The inheritance of genetic traits, including anomalies, is often much more complicated than the simple carrier model. Like all other defects in camelids, we can presently accurately describe CA only as a congenital defect, meaning it is present at birth.

What is choanal atresia? “Choanal atresia is a congenital defect wherein there is either a membranous or osseous separation of the nasal and pharyngeal cavities at the level of the choanae” (M. Fowler, DVM, *Medicine and Surgery of South American Camelids*).

In lay language: A camelid is designed to breathe through its nose. Air enters the nostrils, passes into the nasal cavity, into the back of the nose, through the caudal nares (choanae) and eventually down the trachea. In a cria with choanal atresia, the passage into the back of the throat is blocked off, either by a membrane or bone. The cria must breathe in air through its mouth. It cannot do that and nurse at the same time.

How common is the defect? Dr. Armién’s proposal states that “CA may account for 10% of all the congenital defects in llamas and is a significant problem in South American alpacas.”

Of course, we know that CA occurs with alarming frequency in North American herds as well. Why is that, considering the fact that much of the imported seed stock was screened for defects by carefully trained professionals? In *Medicine and Surgery of South American Camelids*, Dr. Fowler answers that question: “The condition may be unilateral, bilateral, partial, or complete.” Dr. Fowler describes complete bilateral choanal atresia as “a life-threatening condition.” In contrast, a camelid with unilateral, partial CA would have slipped through the screening process. Minimal expression of a genetic trait, including a defect, is not uncommon.

Although it is cold comfort to camelid breeders who experience the birth of a CA cria, we must remember that no species is free of defects. *The Merck Veterinary Manual*, for example, discusses CA in horses. According to Dr. Armién, one human baby in seven thousand is born with CA. The research objectives formulated by Dr. Armién use the research into the human CHARGE malformations as their base and starting point.

They read as follows:

*Objective 1:* To morphologically identify and characterize the multiple-organ malformations associated with choanal atresia in llamas and alpacas.

*Objective 2:* To determine the most frequent congenital abnormalities associations in the multiple-malformation syndrome in llamas and alpacas.

*Objective 3:* To examine candidate genes (CHD7 and SEMA3E) in llamas and alpacas to identify mutations associated with choanal atresia.

My first question concerned the term *candidate genes* mentioned in the third study objective. “Candidate genes are genes that have been already identified in other species. We know that mutations in CHD7 and SEMA3E cause CHARGE syndrome in humans and mice,” Dr. Armién explained. “In other words, these two genes are the candidates for the “job” of causing identical or similar defect in camelids,” I translated the information into layman’s terms. “Yes, that’s correct,” Dr. Armién confirmed. “We are presently concentrating on CHD7,” he explained further, adding, “If we don’t find mutations in CHD7, we’ll look at the other candidate gene.”

According to Dr. Armién, the gene CHD7 has already been sequenced in normal camelids. This was one of the major goals of the project. Sequencing was successfully completed in the laboratory of Dr. Armién’s collaborator, Dr. Kent M. Reed, Associate Professor in the Department of Veterinary and Biomedical Sciences at the University of Minnesota. It represents a major achievement of the ARF funded study. Normal copies of the gene ensure normal development and function of the airway connection between the nose and the mouth. The mutated form of the gene spells trouble for the cria.

What are genetic mutations, and how or why do they occur? Mutations are random events that happen when *deoxyribonucleic acid* (DNA) is copied as the cells divide. The DNA of each gene is unique and passes on very specific instructions to the cells. When the “words” of this instructional “language” change from the original code (for example, letters are dropped or their sequence changes), the cells faithfully continue to copy the mutated version. Cells don’t question the genetic message. They simply perform according to instructions.

It is important that breeders understand an additional complexity regarding mutations. To do so, we must grasp and appreciate the key differences between germ line cells and body (somatic) cells. Germ line cells produce sperm and eggs, somatic cells are cells that become differentiated into organs, muscle, hair or fiber, skin, and all the other components that make up a body. If a genetic code mutates in a somatic cell, it affects only the individual in whose body the mutation occurred. A resulting defect is not passed on to future generations. In germ line cells, however,

mutations coding for disease can have major negative repercussions for the individual as well as future generations. Spontaneous mutations in germ line cells account for the huge diversity among the species on earth, so not all mutations are harmful.

“What about CA in camelids?” I ask Dr. Armién. “Which model applies? Germ line cell or somatic cell mutation?”

“It’s possible that we’re dealing with both,” Dr. Armién responded. When I sighed with frustration, he added sympathetically, “Yes, we may be dealing with a very complex situation here. This is very complicated.” The majority of mutations in CHD7 in cases of human CHARGE are *de novo*, that is they are present in offspring of parents that lack the mutation. The same could be true in camelids.

This possible cause of CA in camelids may explain the outcome of previous test breedings with the already mentioned, often puzzling results.

Unfortunately, there’s another complication to muddy the waters of CA inheritance in camelids. I have already discussed that the defect may be “expressed” to various degrees in individual llamas or alpacas. There is, for example, unilateral expression, where only one of the choanae blocks air flow to the trachea. Animals with minimal expression of the defect may be able to function sufficiently for the defect to go undetected. It’s financially not feasible to perform extensive testing for minimal expression of CA on every single cria. However, breeders can be observant. A cria that never prunks or races around a pasture for extended periods of time should arouse suspicion.

In humans, researchers established that CHD7 often affects a large number of developmental pathways. Will this prove true for camelids?

“Have you actually examined alpacas that support your hypothesis of a genetic mutation in one of the genes that have been identified to be responsible for CHARGE syndrome in humans ie CHD7 and SEMA3E? Have you seen CA crias where defects parallel those of the human CHARGE syndrome?” I asked Dr. Armién.

“Yes,” he replied. “We’ve seen various malformations. Defects of the brain and heart appear frequently along with CA. Along with the abnormal development of the nasal passages, we’ve also observed defective reproductive organs.” “Would you please give us concrete numbers?” I requested. “Sure.” Dr. Armién answered and continued, “I have necropsied twenty-three crias, both alpacas and llamas, with a clinical diagnosis of CA. One third of those met the criteria of CA with multiple malformations. On those animals, molecular genetic testing for the CDH7 gene is being performed.”

“Is it possible that environmental conditions are responsible for mutations resulting in CA?” was my next question.

“Exposure to certain chemicals or a virus can cause defects in animals,” Dr. Armién explained, “but it’s way too early to rule out a genetic component for CA in llamas and alpacas.” I’d like to

add that defects resulting from the consumption of certain plants are well documented in other livestock species.

Genetic tests exist to identify defective genes in humans, livestock species and animals bred as pets. Breeders have the option to pay for a test before selecting breeding stock. This is already a common practice in many species.

Obviously, we cannot prevent a spontaneous mutation occurring in a cria's somatic cells by testing sire and dam prior to mating. Selecting only breeding stock with normal copies of the gene that causes CA may not guarantee a cria free of CA. But how about a genetic test if Dr. Armién finds a mutation for CA in the germ cells?

"Is a genetic test on the horizon for the camelid community . . . anytime soon, I hope?" I inquired.

Dr. Armién laughed good naturedly, but I am sure he thought, "Whoa, not so fast."

He answered: "Our present research will be completed in October 2010. It's only a first step in helping breeders deal with CA. If we can make a link between the CA found in camelids and the candidate genes we're testing, then yes, we can talk about the possibilities of developing a diagnostic test."

The CA study is an excellent example of how research programs designed for one species can help another. While lay people often only see the differences between humans and animals, scientists have long come to recognize the genetic properties we share to various degrees.

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The interview was a pleasure as well as an educational opportunity. Dr. Armién had returned my initial phone call on the Friday evening before a major holiday weekend. "Please," I guiltily urged Dr. Armién after he identified himself, "we can do this another time if you have weekend plans." "I don't mind at all," he insisted and patiently answered all my questions with obvious enthusiasm for his work. A future article will report on the results of his research.