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CAMEL MILK AGAINST AUTISM – A PRELIMINARY REPORT

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ABSTRACT

The described trial substantiated the observation of physicians and parents, that after consuming pasteurised camel milk on a regular basis, a positive effect on impairments of various nature and proportions appeared to be apparent amongst a group of children with Autism Spectrum Disorder (ASD) symptoms or related neurological pathogenesis. Fourteen days after the consumption of 500 ml of pasteurised camel milk, the probands exhibited regular bowel movements and five of eight probands developed a normal sleep pattern. The overall observation revealed also a decreased hyperactivity, increased alertness, better social interaction and many parents observed a newly expressed effort of their children to obey instructions. However, there was no difference in the level of β-casomorphin-7 excretion in the urine of probands and controls using a non-commercial ELISA kit. The reason for this unexpected result is explained.

Key words: Autism, BCM 7, camel milk, casomorphin ELISA

Autism is a collection of behavioral symptoms characterised by dysfunction in social interaction and communication in affected children. It is typically associated with restrictive, repetitive, and stereotypic behavior and manifests within the first 3 years of life. The cause of this disorder is not known (Horvath and Perman, 2002) and should be investigated as a variant of behaviour (Mottron, 2011).

The aetiology of Autism is unknown. Several factors have been implicated in its pathogenesis, including genetic, environmental, immunological and neurological elements (Parcell, 2011).

Recent clinical studies have revealed a high prevalence of gastrointestinal symptoms, inflammation, and dysfunction in children with autism. Mild to moderate degrees of inflammation were found in both the upper and lower intestinal tract (Horvath and Perman, 2002).

Panksepp (1979) theorised that components of Autism might be due to excessive opiate activity.

The 'opioid peptide excess' hypothesis described by Dettmer *et al* (2007) postulates, that excessive amounts of endogenous or exogenous opioid peptides, derived from dietary (cow milk) proteins, may be pathophysiologically important in Autism. Casein proteins are incompletely metabolised in the intestine of Autism Spectrum Disorder (ASD) subjects, due to deficient enzyme activity. As a result, short neuroactive peptides – such as B-casomorphins, derived from casein, are formed. B-casomorphin-7 (BCM 7) has long been considered a risk factor for Autism, but the hypothesis remains controversial. Many children with Autism suffer from digestive disorders, which may make them susceptible to BCM 7 absorption (Woodford, 2011).

BCM-7 has also been suggested as a possible cause of sudden infant death syndrome. In addition, neurological disorders, such as autism and schizophrenia, seem to be associated with milk consumption and a higher level of BCM-7. Therefore, careful attention should be paid to that protein polymorphism, and deeper research is needed to verify the range and nature of its interactions with the human gastrointestinal tract and whole organism (Kamiński *et al*, 2007).

Components of camel milk have been described in various publications by different authors, defining the bacteriostatic and virucidal activities as further outstanding attributes, which contributed to the activities of protective proteins like lysozymes, immunoglobulins, lactoferrin and lactoperoxidase

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(Kappeler, 1998). The presence and high amount of these proteins help explain some of the natural healing properties of camel milk.

Gluten or casein elimination diets are beneficial in children with ASDs, which is discussed in numerous publications, books, and parent/lay Autism conferences. Such elimination diets make them less vulnerable to the neuro-active peptides derived from casein/gluten protein.

Camel milk does not contain beta - lactoglobulin found in cow milk, and a different beta-casein (Shabo *et al*, 2005), the two components in cow milk that are responsible for allergies. The whey proteins of camel milk show a different electrophoretic behaviour than those of other species. Differences also exist in the amino acid sequence of camel and cow milk ß-casein, altogether making camel milk beneficial to subjects with various physical and mental disorders. A group of researchers at the Central Veterinary Research Laboratory investigated the medicinal powers of camel milk in a controlled study amongst a group of children, which were believed to suffer from Autism Spectrum Disorders (ASD).

Materials and Methods

Control group and Probands

Fourteen children were selected for this study, of which 6 formed a control group and 8 children were the chosen probands.

The control group consisted of 3 girls and 3 boys. Their age varied between 2 and 7 years and they were personally known to the CVRL researchers and 8 probands group comprised of 2 girls and 6 boys, varying in age between 5 to 21 years. According to the director, all of the 8 probands were known as suffering from symptoms of ASD. But only during the study it became apparent, that two participants (proband G and proband H) could not be clearly described as being ASD sufferers.

The medical details of each proband were as follows:

A - 8 year-old girl

Bowel movement usually only 1 time in 2-3 days, hyperactive, seizures, no social interaction, and difficulties in constructing sentences. There were conflicting statements in regard to the medical condition, which varied between brain abnormality and mild Autism.

B - 13 year-old boy

Bowel movement usually only 1 time in 2-3 days, suffered from serious constipation, extremely hyperactive, little social interaction, speech incoherent, cravings for chocolate. The child was diagnosed with high functioning mild Autism, and was on a casein-free (CF) diet before the start of the trial.

C - 11 year-old boy

Bowel movement normal, hyperactive, does not speak, only gestures, no concentration, can only perform one task at the time, very irritable, frequent outbursts of anger. The child did not meet the usual criteria for Autism. His impairments might be due to a fall of his mother at 8 month in her pregnancy.

D - 5 year-old boy

Bowel movement only one time in 3 days, constipation, irregular sleep pattern, increased hyperactivity, no interaction with people around him unless addressed directly, very irritable, suffered under regular flu-like symptoms, craving for bread. The child was diagnosed with Pervasive Development Disorder (PDD).

E - 11 year-old boy

Attention Deficit Hyperactivity Disorder (ADHD) symptoms prominent, seizures, craving for sweets.

F - 12 year-old boy

Bowel movement one time in 2 days, constipation, irregular sleep pattern, words are repetitive expressed, difficulties in understanding meaning of words. The child was diagnosed with Mild Autistic Syndrome.

G - 14 year-old boy

The child was diagnosed with Hyper Adeno Corticotropic Hormone Deficiency, because of being hyperactive, impaired in social interaction and communication and his repetitive behaviour. He was potassium deficient, his mental growth was slow and he suffers under epileptic seizures.

H - 21 year-old female

Wheel chair bound because of progressive nerve degeneration.

Trial Design

Control group

Urine samples from the 6 control children were collected for a period of 7 days. The samples were immediately frozen at -80° C at CVRL. The urine of the control children were analysed with the aim of establishing the base line values of BCM 7. No special dietry intervention was assigned to this group and all the children were on cow milk products.

Proband group

The duration of the study for the probands was 9 weeks and conducted in 2 phases.

Phase 1 was the 1st week of the trial (week-0). During this period no special dietary intervention was assigned to the probands. During this period, the morning urine was collected daily by their mothers in sterile containers.

Phase-2 was the remaining 8 weeks (week 1 to week 8), during which camel milk was consumed. All 8 probands were provided with 500ml pasteurised camel milk per day, which was consumed without any problems and tolerated well. Parents were notified to refrain from giving their children cow milk products. The collection of morning urine samples of all 8 probands (week 1 – week 8) was conducted every alternative day, including weekends.

All urine samples were transported to CVRL within 2 hours after collection in cool condition. At CVRL, the urine samples were frozen at -80°C until tested.

The effects of camel milk in children with ASD in this trial were measured in two ways.

The excretion of BCM 7 in urine samples was determined using the casomorphin ELISA kit from Immundiagnostik, Bensheim, Germany.

From the start until the end of the experiment after 8 weeks, behavioural and physiological changes of the children participating were daily monitored by their parents and reported weekly to the CVRL researchers.

Two urine samples from each child in the control group and 4 urine samples from each proband in Phase-1 were tested. Six urine samples from each proband in Phase-2 (week 3,6,8) were selected, which comprised of 2 urine samples of different days from week 3, 6 and 8. Samples were analysed for BCM 7 with casomorphin ELISA kit.

The β-casomorphin ELISA test is a competitive enzyme linked immunoassay (cELISA) and the test procedure was carried out according to the manufacturer's recommendation, which is described in brief.

Diluted urine samples and a polyclonal casomorphin antiserum were incubated in microtitre plate wells, coated with a casomorphin derivative (tracer). During the incubation, the target casomorphin of the sample competes with the tracer for the binding of the polyclonal antibodies. The bound components were detected by peroxidase-conjugated antibody and the tetramethylbenzidine (TMB) is used as the secondary reagent. The enzymatic reaction was terminated by an acidic stop solution. The intensity of the yellow colour was inversely proportional to the casomorphin concentration in the sample.

A dose response curve of absorbance unit (optical density, OD at 450nm) vs. concentration was generated using the values obtained from standards. The ELISA results were normalised to the creatinine concentration of the urine sample and the result was expressed in ng/µmol creatinine.

Results

Table 1 shows the BCM 7 values in the control group and Table 2 summarises the BCM 7 values of all 8 probands in Phase 1 (week-0) and Phase 2 (week 3, 6 and 8). The figures in both tables show, that the casomorphin levels in the control and proband groups were within the normal range of 0 - 0,8 ng/µmol creatinine provided by the manufacturer.

The comparitive study of the BCM 7 results in Table 1 and Table 2 indicate that there is no statistical difference between BCM 7 levels of the probands before consuming camel milk and after consumption, as well as between probands and controls.

Although all BCM 7 values were in the normal range of 0 - 0,8 ng/µmol creatinine, we found a significant difference ($P < 0.01$) between the mean values of proband urine samples before and after camel milk consumption. No significant difference (P

Table 1. BCM 7 ELISA results of 6 controls with mean values and standard deviation (SD).

Controls	Sample	BCM 7 concentration (ng/µmol creatinine)
Control 1	Sample 1	0.35
	Sample 2	0.27
Control 2	Sample 1	0.35
	Sample 2	0.26
Control 3	Sample 1	0.31
	Sample 2	0.34
Control 4	Sample 1	0.37
	Sample 2	0.29
Control 5	Sample 1	0.29
	Sample 2	0.50
Control 6	Sample 1	0.24
	Sample 2	0.50
	Mean ± SD	**0.34 ± 0.08**

Table 2. BCM 7 ELISA results of 8 probands before and after camel milk consumption with mean values and standard deviation (SD).

	Week	Sample	BCM 7 concentration (ng/µmol creatinine)							
			Proband 1	Proband 2	Proband 3	Proband 4	Proband 5	Proband 6	Proband 7	Proband 8
Before camel milk consumption	Week 0	Sample 1	0.25	0.33	0.31	0.43	0.12	0.59	0.29	0.20
		Sample 2	0.26	0.34	0.33	0.37	0.26	0.49	0.04	0.10
		Sample 3	0.21	0.23	0.17	0.17	0.29	0.50	0.12	<0
		Sample 4	0.74	0.33	0.26	0.29	0.39	0.67	0.18	0.15
		Mean±SD	0.37±0.25	0.31±0.05	0.27±0.07	0.31±0.11	0.27±0.11	0.56±0.08	0.16±0.11	0.15±0.05
	Week	Sample	BCM 7 concentration (ng/µmol creatinine)							
			Proband 1	Proband 2	Proband 3	Proband 4	Proband 5	Proband 6	Proband 7	Proband 8
Aftre camel milk consumption	Week 3	Sample 1	0.42	0.29	0.11	0.40	0.19	0.23	0.22	0.05
		Sample 2	0.18	0.07	0.08	0.13	0.23	0.39	0.12	0.09
	Week 6	Sample 1	0.23	0.09	ND	0.18	0.13	0.67	0.17	ND
		Sample 2	0.16	0.29	ND	0.15	0.08	0.40	0.18	ND
	Week 8	Sample 1	0.17	<0 *	ND	0.21	<0	<0	0.05	0.13
		Sample 2	0.20	0.42	ND	0.13	0.21	0.18	0.25	0.15
		Mean±SD	0.23±0.1	0.23±0.15	0.09±0.02	0.2±0.1	0.17±0.06	0.37±0.19	0.17±0.07	0.11±0.04

* Not detectable ND: Not done

>0.01) was detected in the mean urine BCM 7 values between control group and proband urine before camel milk consumption.

Review of behavioural and physiological changes

From the second week onwards after the introduction of camel milk to the diets of the probands, the parents claimed, that their children exhibited regular bowel movements. This fact represented already a big improvement of the overall health condition of children, who normally show a discomforting prevalence of gastrointestinal problems. Furthermore, they overall revealed a decreased hyperactivity, increased alertness, grasping power and curiosity, better social interaction and many parents commented on the newly expressed effort of their children to listen and obey instructions.

One of the probands was regularly struck by bouts of flu, which resulted in the need of constant medication. During the 8th week trial period that child showed no recurrence of flu symptoms, even though other family members suffered under the disease. Proband developed a mild discomfort, only once which disappeared quickly without causing the need for medication.

Discussion

Over the last years, a significant increase of Autism cases has been observed in children worldwide. Only anecdotal evidence exists that camel milk may have a healing effect on ASD children.

The release of BCM 7 through enzymatic digestion of bovine β-casein is dictated by different amino acids sequences of this protein. The amino acid present in position 67 of the sequence in β-casein appears to be critical for the release of BCM 7. In the A2 variant of β-casein a proline residue occurs at position 67, whereas the A1 and B variants of β-casein have a histidine residue at this position (De Noni, 2008).

BCM-7 may play a role in the aetiology of human diseases (Kamiński *et al*, 2007).

Cass *et al* (2008) studied whether peptides from wheat or milk were leaking from the gut and making their way into the urine of children with autism and did not find any of these small proteins (peptides) in the urine of the boys with autism or Asperger syndrome.

In our study, no difference in the urine BCM 7 levels of both the control and proband groups was detected. This result was unexpected, since it was reported, that children with ASD, whilst drinking cow milk, have increased levels of BCM 7 in their urine. However, no increased BCM 7 levels were found in the urine of our control as well as in our proband group before drinking camel milk. We therefore could not substantiate the result of other researchers, that children with ASD on cow milk diet excrete elevated BCM 7.

Several reasons could be responsible for this discrepancy:

- For the selection of probands, the CVRL researchers depended on the judgement of the director of the centre for children of special needs and some probands included in the trial might not have suffered from classical ASD
- Prior to this trial, the probands were not assessed for the excretion of casomorphin in their urine, because no prior knowledge of biochemical consequences of dietary influence was known
- The ELISA test is yet not commercially available and most probably it's specificity and sensitivity has to be further evaluated for this kind of experiment
- Autism is a complex disease and other neuroactive peptides as well as various undisclosed factors may be responsible for this disorder

Our preliminary trial clearly has shown, that the consumption of camel milk has a positive effect on behavioural and pathophysiological disorders.

However, the level of BCM 7 excretion in the urine of probands and controls using a non-commercial ELISA kit were in the normal range.

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Treatment of digestive problems appears to have positive effects on autistic behavior. These new observations represent only a piece of the unsolved autism "puzzle" and should stimulate more research into the brain-gut connection (Horvath and Perman, 2002).

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