CHRONIC FATIGUE SYNDROME (C.F.S.) ASSOCIATED WITH *STAPHYLOCOCCUS SPP.* BACTEREMIA, RESPONSIVE TO THIACETARSAMIDE SODIUM IN 7 DOGS.

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SUMMARY
Chronic Fatigue Syndrome (CFS) in human patients remains a controversial and perplexing condition with emerging zoonotic aspects. Recent advances in human medicine seem to indicate a bacterial etiology and the condition has already been described in horses, dogs, cats and birds of prey in association with micrococci-like organisms in the blood and *Staph*-positive blood cultures. To evaluate the possibility of an underlying chronic bacterial infection, seven dogs diagnosed with CFS were submitted to rapid blood cultures and fresh blood smears investigations. Blood cultures proved *Staph*-positive and the isolates were identified as *S. epidermidis* (1), *S. intermedius* (2), *S. cohnii* (1), *S. chromogenes* (2) and *S. lugdunensis* (1). The presence of micrococci-like organisms in the blood was a constant observation in these subjects, in association with fatigue/pain-related symptoms and biochemical abnormalities (high creatine kinase levels) suggestive of an underlying neuro-muscular dysfunction. Following treatment with a low dosage arsenical drug (thiacetarsamide sodium, *Caparsolate*, iv., 0.1 ml/kg/day, for 2 days) all patients experienced complete lasting remission. In all cases, micrococci had disappeared from the blood at post-treatment controls made 10-30 days later. The outcome, compared with those of 4 healthy animals in the control group, were strongly suggestive of a *Staphylococcus spp.* bacteremia related to chronic fatigue/pain symptoms.

KEY-WORDS: Chronic Fatigue Syndrome – CFS – micrococci – *Staphylococcus* – zoonosis – dog – thiacetarsamide sodium – arsenic.

RESUME’
L’étiologie du Syndrome de Fatigue Chronique (SFC) chez l’homme reste entourée de nombreuses spéculations et perplexités. Apparemment il s’agit d’une zoonose émergente qui a déjà été décrite chez les chevaux. Récentes publications humaines semblent indiquer une étiologie bactérienne. Afin d’évaluer la possible association avec une infection chronique à bactéries, 7 chiens avec un diagnostic de SFC ont été soumis à hémocultures et à la recherche microscopique directe des germes dans le sang. Les hémocultures furent positives pour *Staphylococcus spp.* et les isolations ont été identifiées pour *S. epidermidis* (1), *S. intermedius* (2), *S. cohnii* (1), *S. chromogenes* (2) et *S. lugdunensis* (1). Chez tous les sujets examinés, des bactéries types micrococciques ont été trouvées sur les globules rouges, et leur présence était associée aux symptomes de fatigue/douleur et à des anomalies biochimiques (élévation de l’enzyme musculaire creatine kinase) suggérant un dysfonctionnement neuro-musculaire. Ces microrganismes ont disparu après le traitement avec un médicament arsenical, le thiacetarsamide sodium (*Caparsolate*, iv., 0.1 ml/kg/jour), utilisé pendant 2 jours, et qui eut comme résultat la complète et durable guérison du syndrome chez tous les chiens. Les résultats, comparés avec ceux d’un groupe contrôle de 4 chiens sains, étaient fortement suggestifs d’une bactériémie à *Staphylococcus spp.* liée à des symptomes de fatigue et douleurs chroniques.

MOTS-CLE’ : Syndrome de Fatigue Chronique – CFS – micrococci – *Staphylococcus* – zoonose – chien – thiacetarsamide sodium – arsenic.

INTRODUCTION

The challenges of Chronic Fatigue Syndrome (CFS), often called Myalgic Encephalomyelitis (ME), to analytical and medical approaches are connected to the fact that its diagnosis is largely based on subjective complaints in the absence of reliable tests [3, 11, 25]. The pathogenesis of CFS/ME is unknown [7] and no consistent cellular or biochemical alteration has been found which could be used to differentiate the condition from similar fatigue-related diseases [9].

CFS/ME in people is characterized by highly variable patterns of symptoms including prolonged debilitating fatigue, muscle cramps, multi-joint pain, sore throat, headaches, cervical lymphadenopathy, low-grade fever, loss of libido, irritable bowel and neurocognitive disorders [25]. Some physicians accept the existence of CFS, while others are convinced that it exist only in the minds of the sufferers [17].

Nevertheless, during the past decade, substantial evidence has been generated to support the existence of a CFS-like illness among animals [8, 12, 13, 27]. The condition has already been described in horses [35], dogs, cats [34,37] and birds of prey [38] in association with long-term exhaustion and resistance to standard therapies.

Prevalence surveys indicate that a remarkable number (97%) of patients with CFS had animal contact, particularly with dogs and cats [12], and that 75% of these pets appeared sick, with signs and symptoms which mimicked CFS in humans, strongly suggesting a zoonotic transmission [13]. Additionally, 2.9% and 7.5% of veterinary surgeons, respectively younger and older than 40 years, have suffered from chronic fatigue in Switzerland [4], a percentage significantly higher than the 0.2% estimated prevalence of CFS in the entire population [19]. An increased carriage of coagulase-negative staphylococcal toxins was found in humans with chronic pain/fatigue symptoms [6] and CFS [9, 10, 22]. The staphylococci recovered from 89% of these patients (*S. xilosus, S. epidermidis, S. warneri, S. hominis, S. lugdunensis, S. haemolyticus)* produced a significant association of membrane-damaging toxins, and/or 'horse-'haemolysins, whereas the control subjects did not [6]. Similarly, a recent study on animals [37] showed that 9 out of 15 dogs and cats diagnosed with CFS and carrying micrococci-like organisms in the blood, produced *Staph*-positive blood cultures. Their CFS-resembling lethargy had a complete remission after a treatment with thiacetarsamide sodium, an arsenical drug, given intravenously in low dosage (0,1 ml/kg/day) for 2-3 days. The clinical response was associated with disappearance of micrococci from the blood in tests made subsequently. The primary purpose of this study was to describe the frequency of certain haematological and microbiological findings that in association with clinical features led to a diagnosis of canine CFS. An additional objective was to report how the syndrome was responsive to an arsenical drug, thiacetarsamide sodium (*Caparsolate,* Abbott Laboratories, IL) given intravenously in low dosage, which had previously been used with success in other animals [34, 35, 37, 38] .

MATERIALS AND METHODS

Animal investigation

The medical records of 7 dogs from Aosta Valley (north-west Italy) diagnosed with Chronic Fatigue Syndrome between August and December 1993, were studied retrospectively. Dogs included in this study were characterized by clinically evaluated chronic fatigue/weakness lasting >1 month, high creatine kinase (CK) values and detection of bacteria-like organisms in fresh blood smears. All animals had to meet these criteria, in order to be diagnosed with CFS, before performing blood cultures, bacteria identifications and specific low-dosage arsenical therapies.

Symptoms and signs were organized into 8 groups (Table I). 'Asthenia/lethargy' included prolonged fatigue, difficulty in rising, lack of liveliness, lack of interest in playing and in performing normal activities, post-exertional malaise and an unusual marked tendency to lay down and to sleep. 'Anorexia/poor appetite' included any diminution of appetite, from slight (+) to complete (+++), lasting more than 3 days. 'Pharyngitis/sore throat' included any upper respiratory tract dysfunction, sore throat, difficulty in swallowing and/or breathing and partial to complete weakening of the voice. 'Weight loss' was defined on the basis of spontaneous owner's report and/or thorough physical examination. 'Muscular pain' included evident lamentations during walking or jumping and soft-tissue pain on moderate palpation, particularly in the lumbar region and hind legs. 'Joint pain' included lameness, stiff gait and staggering. 'Neurologic dysfunctions' included somnolence, photophobia, shivering, seizures, depression, timidity and fear. 'Cervical lymph-adenopathy' was defined on the basis of a macroscopic enlargement and tenderness of cervical lymph nodes.

Hematology

Two fresh blood smears, stained with the May-Grunwald-Giemsa technique were prepared each time. A Knott test for microfilariae was also performed at each visit.

Biochemistry

Serum values of creatine kinase (CK) were determined at rest, on the basis of the muscular pain and fatigue symptoms. These values are summarized in Table I. In all subjects, the examination of other parameters (Total Bilirubin, Creatinine, BUN, GOT, GPT, Glucose, LDH, Total Protein, Albumin) constantly gave results falling within the normal ranges, as well as the White Blood Cells count, and consequently considered unremarkable and not included in the present study.

Microbiology

Sterile equipment (gloves, needles) and conditions (asepsis of the skin) were required each time for drawing blood from the brachial vein. Immediately after sampling, and under laminar-flux hood (*Mini-Securitas PBI*), 1-2 ml of blood were cultured on agar Columbia 5% ram's blood plates (*bioMérieux*) for 2-3 days at 37°C, in CO2 enriched atmosphere. Representative colonies were then submitted to speciation (*API-Staph, bioMérieux*). All samples were subject to Gram and catalase test. In order to assess the risk of contamination, 4 healthy dogs owned by the author were submitted to similar blood-cultures, using the same procedure. These animals were all greyhounds, showing no fatigue-related symptoms, CK values below 100 IU/L at rest and normal blood smear appearance.

Therapy

Thiacetarsamide sodium was administered intravenously at low dosages (0.1 ml/Kg/day) for 2 days. No other medication was given.

RESULTS

The clinical signs and physical examination findings are listed in Table I. 'Asthenia/lethargy', 'anorexia/poo appetite' and 'neurologic dysfunctions' were mostly reported by their owners. 'Pharyngitis/sore throat', 'weight loss', muscular pain' and 'lymphadenopathy' were recorded usually on examination. All animals showed positivity to at least three of the eight symptom and sign categories, with prevalence of asthenia & lethargy (7/7), anorexia/poor appetite (7/7), and neurologic dysfunctions (5/7), followed by pharingitis/sore throat (4/7), muscular pain (3/7), multijoint pain (2/7), weight loss (2/7) and lymph-adenopathy (2/7). Rectal temperature was higher than 39°C in three dogs (#211, #220 and #241). All Knott tests for microfilariae were negative and these dogs had no history of travel out of the region (Aosta Valley) which is free of indigenous cases of heartworm disease [33, 36].

The CBC was unremarkable and the white blood cells count was always within the ranges, including the differential counts of lymphocytes and granulocytes. Serum values of creatine kinase (CK) were higher at rest (Table I) then the normal reference values (52-100 IU/L) [43].

Examination of fresh blood smears led to the exclusion of babesiosis and ehrlichiosis. However small micrococci-like organisms 0.3-0.5 m in diameter, were found attached to the external surface of the red blood cells in percentages varying from 5 to 35% (Fig. 1 and 2).

Based upon the previous criteria, a diagnosis of CFS was made in all dogs. Rapid blood cultures were performed on Columbia plates and generated bacterial growth in 2-3 days. Gram positive and catalase positive cocci were identified in all plates. Representative colonies from the *Staphylococcus*-positive Columbia plates, submitted to speciation, gave the following results:

1 – Dog #173: acceptable identification (84.8%) of *Staphylococcus epidermidis* (API-Staph code 6706052),

2 – Dog #175: acceptable identification (85.8%) of *Staphylococcus intermedius* (API-Staph code 6716153),

3 – Dog #186: low discrimination (26.7%) of *Staphylococcus intermedius* (API-Staph code 6726111). This strain produced acid from mannitol.

4 – Dog #211: good identification (98.1%) of *Staphylococcus cohnii* (API-Staph code 6314042),

5 – Dog #219: acceptable identification (82.5%) of *Staphylococcus chromogenes* (API-Staph code 6716052),

6 – Dog #220: good identification (94.5%) of *Staphylococcus chromogenes* (API-Staph code 6716053),

7 – Dog # 241: good identification (91.4%) of *Staphylococcus lugdunensis* (API-Staph code 6316150).

Blood cultures from the 4 healthy dogs of the control group showed negative results after 5 days of incubation.

In accordance with previous observations [35], a therapy with thiacetarsamide sodium (0.1 ml/Kg/day) given intravenously for two days, was performed in all 'chronically fatigued' dogs. In a few days weakness decreased, appetite and exercise tolerance improved markedly in all subjects.

Physical and haematological controls made 10-30 days after therapy showed complete clinical remission from the condition and fresh blood smears revealed absence of micrococci in all cases. Response to therapy appeared satisfactory as fatigue, sore throat , muscular pain and resistance to physical activity had disappeared. Somnolence, depression, shivering, seizure and other neurologic dysfunctions previously observed in dogs #173, #175, #186, #211 and #241, were no longer present. Lymph node dimensions were decreasing in dogs #211 and #241 and the hair-coat was brighter in all subjects.

DISCUSSION

A canine disease characterized by severe fatigue, the presence of micrococci in the blood and *Staph*-positive blood cultures, went into complete remission after an intravenous treatment with a low dosage of thiacetarsamide sodium (*Caparsolate,* 0.1 ml/kg/day for 2 days), a trivalent organic arsenical, used as single drug.

In the absence of a specific test, a diagnosis of Chronic Fatigue Syndrome (CFS) in human medicine is currently carried out by exclusion of other known fatigue-related diseases and by compliance with a clinical definition [9,25]. Consequently, the differential diagnosis of CFS is potentially vast and should include malnutrition, cancer, senescence, hypothyroidism, cardiomyopathy, myasthenia gravis, diabetes mellitus, glucocorticoid deficiency, Lyme disease, potassium deficiency, kidney failure, adrenal under-stimulation, Chiari malformation, *Coxiella burnetii* infection, growth hormone deficiency, hypercoagulability , lupus, mercury poisoning and many other causes that in human medicine [39], as well as in veterinary practice can rarely be fully explored. In this report, exclusion of some alternative and common problems which may produce chronic fatigue/pain disorders in dogs (heartworm disease, malnutrition, senescence, diabetes mellitus, liver and kidney diseases, babesiosis and ehrlichiosis) was carefully carried out in all patients referred as having CFS, based on normal serum results for Glucose, Creatinine, BUN, Total Bilirubin, GOT. GPT, LDH, Total Protein and Albumin, normal White Blood Cells counts, negative Knott tests and absence of *Babesia spp., Haemobartonella canis, Hepatozoon canis* and *Ehrlichia spp*. from the fresh blood smears.

Diagnosis of CFS was based on high CK levels, presence of micrococci on the erythrocytes, *Staph*-positive blood cultures and response to an arsenical drug in low dosages. Similar observations and outcomes have been recently described in two persons affected by CFS and meeting the CDC criteria for this condition [39].

On blood smear examination, differential diagnosis should also include *Haemobartonella canis*, a canine haemoparasite transmitted by *Rhipicephalus sanguineus ,* which produces no or slight pathogenic effects under naturally occurring conditions [14,15]. Its effective therapy is based on tetracyclines and chloramphenicol [1]. These rickettsial pleomorphic organisms have typical a shape (short rod or coccus, sometimes associated in small chains of 2-4 elements) , colour (blue or purple-red) and size (coccus: 0.1-0.8 m; rod: 0.2-0.5 m x 0.9-1.5 m)[37], which apparently differ from those of micrococci reported here.

The 7 canine cases described here apparently matched the clinical picture of CFS in humans [3,17], and are similar to other canine cases previously reported [37] and found unresponsive to extensive prior therapy [34], in combination with biochemical results, high CK activity at rest, suggestive of a neuro-muscular dysfunction. CK is the most sensitive indicator of muscle damage available and causes of increased plasma CK activity are primarily associated in pets with skeletal muscle damage and Central Nervous System disorders, and seldom with myocardial infarction [5]. Elevated CK values are also observed occasionally in humans patients with CFS, particularly in the acute phase of the illness and in the persons most affected [2, 26, 39].

The micrococci-like organisms seen in the blood smears before treatment were strikingly similar to those previously reported in other animals with CFS [34,35,37,38] and no other concurrent

blood parasites were detected in the cases here reported. There is no direct evidence that these bacteria cause the disease but, apparently their presence in the blood was linked to the condition, being the only remarkable haematological difference between:

a) pre- and post-thiacetarsamide treatment in subjects with CFS, and
b) healthy and 'chronically fatigued' dogs.

In fact, all fresh blood smears taken from 4 healthy dogs of the control group, were micrococci free and their blood cultures were negative, using same procedure. Their CK values were also normal (<100 IU/L). In contrast with these findings, all dogs diagnosed with CFS, produced *Staph*-positive blood cultures and the identified bacteria were both coagulase negative (*S. chromogenes, S. epidermidis, S. lugdunensis, S. cohnii)* and coagulase positive (*S. intermedius*) staphylococci. The hallmark of this group was the notable presence of micrococci in the blood, in association with pain/fatigue symptoms and high CK activity. Taken together, these results apparently exclude a risk of contamination of the Columbia plates during procedure and seem to confirm the bacterial nature of the micrococci-like organisms observed in the blood of the reported cases (Fig. 1). It could, therefore, be suggested that their presence may be used as a diagnostic tool in the diagnosis of CFS in dogs and cats. These findings seem to confirm the report of a newly-identified human blood bacterium (HBB) which is claimed to be present in low numbers in the blood of healthy people [23] and in high numbers in the blood of persons who have CFS [35,37,39] or Multiple Sclerosis [18]: a reduction in these bacteria is associated with clinical improvement and an increase corresponds with an increment of symptoms.

Review from human literature [41] shows increasing incidence and severity of bacteremias due to CNS during the last 20 years. Currently, the definition of 'true bacteremia' is given by one positive blood culture in association with a clinical picture compatible with infection, both in human [41] and in veterinary [24] medicine. The isolation reported here of different *Staphylococcus spp.* in the same condition, is not incompatible with the recent observation that true bacteremias due to coagulase-negative staphylococci are frequently the result of multiple strains [29]. A microbiology research article has showed that 89% of patients with chronic muscle pain had multiple carriage of coagulase-negative *Staphylococcus* strains which produced membrane damaging toxins, meanwhile the healthy control group had none [6]. The carriage of membrane-damaging toxins was strongly correlated with increases in symptom severity, palpitations, fatigue, pain and weakness. As in the present animal study, *S. epidermidis, S. intermedius (S. aureus* if of human origin) and *S. lugdunensis* were among the species identified in human patients with chronic muscle pain [6]. The recovery of *S. xilosus* have also been reported both in humans with chronic pain [6] and in dogs and cats diagnosed with CFS [34,37]. An increased carriage of toxin-producing coagulase negative staphylococci appears evident today in CFS human patients [22], strongly correlated with the catabolic response and pain severity [10].

In the present study, recovery from a CFS-resembling illness in dogs carrying several *Staph* strains was associated with disappearance of micrococci in the blood. These findings are compatible with an underlying staphylococcal infection responsive to sodium thiacetarsamide, an arsenic-based medicament. This drug was used as the first choice of treatment in all patients diagnosed with CFS, following previous personal observations of *in vivo* multiple antibiotic-resistance [35,38]. The antibacterial action of arsenic is acknowledged in both human [21,28] and veterinary [16,30] medicine. Organic arsenicals were still used in human medicine in the mid-1950 against certain nutritional disturbances, rheumatisms, anemia and asthma [39], ailments partially overlapping the current description of CFS. A recent human study correlate low serum arsenic with Central Nervous System disorders, vascular diseases and cancer [42].

The anticancer effect of arsenic trioxide and melarsoprol, an organic trivalent arsenical like thiacetarsamide, has been recently rediscovered particularly against acute promyelocitic leukaemia [31] and other lymphoproliferative malignancies [32]. In the case of lymphoma and leukaemia, fatigue is the most common symptom. Surprisingly, a noticeable rate of lymphoproliferative disorders and leukaemia have already been described in cats with CFS-resembling illness owned by CFS patients [13] and in people with CFS [20].

In summary, seven dogs diagnosed with chronic fatigue syndrome produced *Staph*-positive blood cultures with good or acceptable identification of several coagulase- negative and positive staphylococci. The CFS-related symptoms were associated with the presence of micrococci-like organisms in the blood and high CK values at rest, suggestive of a neuromuscular dysfunction. All animals experienced complete clinical and haematological remission 10-30 days after treatment with thiacetarsamide sodium, an organic trivalent arsenical given intravenously in low dosages (0.1

ml/kg/day) for 2 days. It seems worthwhile to suggest that the presence of micrococci in the blood could be used as a coadjutor tool in the diagnosis of this particular kind of arsenic-responsive 'general debility' in dogs, because they apparently are the main haematological difference between healthy and chronically fatigued animals. The association between blood anomalies and microbiological findings is strongly suggestive of a chronic bacterial infection. Superposable results recently came from research in human medicine, where similar coagulase-negative staphylococci (*S. epidermidis, S. lugdunensis, S. xilosus*) were isolated in people with chronic muscle pain [6] as well as in CFS patients [9,10,22].

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FIGURES

Figure 1. Fresh blood smear from a dog diagnosed with CFS: Some red blood cells are carrying micrococci-like organisms 0.3-0.5 m in size (x100).

Figure 2. Dog with Chronic Fatigue Syndrome: micrococci can be observed on the external surface of some erythrocytes (x100).