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ETIO PATHOGENESIS OF AUTOIMMUNITY

Autoimmune/autoinflammatory syndrome induced by adjuvants (ASIA syndrome) in commercial sheep

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Abstract We describe a form of the autoimmune/autoinflammatory syndrome induced by adjuvants (ASIA syndrome) in commercial sheep, linked to the repetitive inoculation of aluminum-containing adjuvants through vaccination. The syndrome shows an acute phase that affects less than 0.5 % of animals in a given herd, it appears 2–6 days after an adjuvant-containing inoculation and it is characterized by an acute neurological episode with low response to external stimuli and acute meningoencephalitis, most animals apparently recovering afterward. The chronic phase is seen in a higher proportion of flocks, it can follow the acute phase, and it is triggered by external stimuli, mostly low temperatures. The chronic phase begins with an excitatory phase, followed by weakness, extreme cachexia, tetraplegia and death. Gross lesions are related to a cachectic process with muscular atrophy, and microscopic lesions are mostly linked to a neurodegenerative process in both dorsal and ventral column of the gray matter of the spinal cord. Experimental reproduction of ovine ASIA in a small group of repeatedly vaccinated animals was successful. Detection of Al(III) in tissues indicated the presence of aluminum in the nervous tissue of experimental animals. The present report is the first description of a new sheep syndrome (ovine ASIA syndrome) linked to multiple, repetitive vaccination and that can have devastating consequences as it happened after the compulsory vaccination against bluetongue in 2008. The ovine ASIA syndrome can be used as a model of other similar diseases affecting both human and animals. A major research effort is needed in order to understand its complex pathogenesis.

Keywords Sheep · Aluminum · Vaccines · Adjuvant · Autoimmunity · Cachexia · Intoxication

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Introduction

The so-called autoimmune/inflammatory syndrome induced by adjuvants (ASIA syndrome) is a newly recognized entity that includes at least four apparently unrelated syndromes, such as postvaccination phenomena, the macrophagic myofasciitis syndrome (MMF), siliconosis and the Gulf war syndrome (GWS) [1–4]. All these syndromes share hyperactive immune responses as their common denominator and a similar complex of signs and symptoms [3]. The ASIA syndrome is a multifactorial process triggered by exposure to certain environmental factors, such as infections, toxins, drugs, vaccine adjuvants, and that is influenced by forms of stress [3]. The use of adjuvants has been documented in some of the above-mentioned medical conditions, such as postvaccination phenomena, MMF and GWS. The most widely used adjuvant in medicine is aluminum that is used

in both human and veterinary vaccines under the form of aluminum salts (alum). Whereas alum is an excellent adjuvant for antigen recognition and it stimulates a strong immune response to a specific agent [5], its toxic effects are well but only partially known [6–8]. The precise mode of action of alum is not completely understood although basic mechanisms have been investigated [9–11]. The safety of alum as a vaccine adjuvant is currently being questioned [8].

In veterinary medicine, the use of alum-adjuvanted vaccines is generalized and the number of vaccines inoculated to several animal species is increasing over the years. In Spain, sheep production is an important economic field as the country holds about 20 millions of animals. One of the most common production systems imply several type of stresses such as quality of pastures, exposure to weather conditions and an increased pressure for lambing using systems for maximizing lamb production. Adjuvanted vaccines against a variety of sheep pathogens are used on a routine and systematic basis and they are administrated at almost any age. Antiparasitic treatments that were previously given orally are now inoculated subcutaneously and carry the same type of adjuvants. As a result, a single animal can receive a mean of 4 alum-adjuvanted inoculations under the form of aluminum hydroxide salts every single year (range 2–9, depending on the flock), along the 7- to 8-year animals are considered economically productive.

In 2008, the reemergence of bluetongue, a viral disease of sheep, made the European Union to decide to vaccine all animals in Europe, this decision implying the widest vaccination campaign in ovine history, as the European census is about 90 million of animals. In Spain, animals received vaccines against two strains of the virus (mostly strains 1 and 8) with prime inoculation and boosting, thus allowing sheep to receive four inoculations in less than a month. Vaccines used were always inactivated and they were produced by several manufacturers, both national and multinational. A previously undescribed syndrome with an acute nervous phase normally followed by a chronic cachectic phase (see description below) appeared shortly after across the country with devastating consequences for sheep industry. The syndrome was more severe during winter cold and seemed to partially regress under favorable weather conditions. However, in Spain, similar but sporadic syndromes had been observed long before this episode of massive vaccination against bluetongue. The objectives of this work are: (1) to summarize the main features of this new ovine syndrome based on the work done in natural cases since 2007, including cases studied before and after the vaccination against bluetongue and (2) to describe its experimental reproduction in a group of lambs. This disease can be considered the ovine version of the ASIA syndrome, linked to the repetitive inoculation of adjuvants over the years, the influence of several stresses and the hyperstimulation of the immune system.

Materials and methods

Natural cases: animals, samples and type of studies

This work is based on the study of 64 animals selected since 2007 from 23 flocks either before or after the compulsory vaccination against bluetongue in 2008. Flocks were located in the province of Zaragoza (northeast of the Iberian Peninsula) or in nearby areas. Fifty-five sheep (out of 64) were alive at the time of being referred for post-mortem studies. If possible, a blood sample with and without anticoagulant and a urine sample were taken to undergo hematologic and a variety of analyses. Post-mortem studies included gross investigation of tissues, and microscopic stainings used were hematoxylin–eosin, PAS, Masson and Gallego's trichrome, Grocott, Congo Red, Pearl' staining for iron and Morin staining for aluminum. Electron microscopy was undertaken in acute phase cases. A comprehensive array of samples and techniques were used to study the presence of the most compatible and/or common ovine diseases that could be present in the affected flocks.

Experimental reproduction of the syndrome

A 120 sheep flock without previous records of vaccination, integrated in an ecological farming system, was located and six castrated male, 3-month-old lambs, were selected for the study. Three animals formed the vaccinated group and the other three the control group. The experiment started on the June 28, 2010 and ended on March 18, 2011, thus occupying a complete winter season. During this period of time, animals were kept within the original flock under the same management and weather conditions and sharing the same habitat. The three vaccinated lambs received a total of 14 inoculations of commercial vaccines, distributed along the experiment (Table 1). These vaccines were selected for sharing the same declared amount of adjuvants: 4 mg Al(III) under the form of aluminum salts and 0.2 mg thiomersal per dose. Therefore, vaccinated lambs received, during the experiment, a total amount of 56 mg of Al(III) and 2.8 mg of thiomersal (Table 1). Vaccines were against a variety of ovine pathogens, such as bluetongue (Zulvac 1® and Zulvac 8®, Fort Dodge; $n = 10$ inoculations), clostridial bacteria (Cubolac® CZ Veterinaria; $n = 2$ inoculations) and abortive bacteria: *Chlamydophila* spp and *Salmonella* spp (Syvabort®, Syva; $n = 2$ inoculations). The control lambs were inoculated with the same amount of PBS at the same inoculation days. Established legal periods

between vaccinations were always fulfilled, thus mimicking a decision of a veterinarian in practice. Blood samples were taken weekly and weight was also recorded weekly. Behavioral observations were done along the study. Pairs of control and vaccinated lambs were studied at 177, 254 and 261 days by postmortem in a similar manner that those described above for the natural cases.

Presence of adjuvants in blood/tissue samples

Blood samples in EDTA and heparin together with samples of nervous tissue taken at postmortem were studied for the presence of Al(III) or Hg by inductively coupled plasma–mass spectrometry (ICPMS), X-ray photoelectron spectroscopy (XPS) and energy dispersive X-ray spectroscopy (EDAX). Blood samples studied by ICPMS included animals ($n = 5$) severely affected by the chronic phase of the disease and sex- and age-matched controls ($n = 2$). The surface composition of spinal cord tissue from naturally chronic phase affected sheep ($n = 6$) and experimentally vaccinated lambs killed at days 254 and 261 was analyzed by XPS with an Axis Ultra DLD (Kratos Tech.). Spinal cord tissue from the two pairs of experimental animals killed at days 254 and 261 (one control and one vaccinated at each time point) were also studied by scanning electron microscopy–EDAX (FESEM, Inspect form, FEI).

Results

Clinicopathological data in natural cases and differential diagnosis

In natural cases, most bio-pathological data were within normal values. The only significant change was the presence of a non-regenerative anemia with an erythrocyte mean value of 7.92 (normal range = 9.0–14.0 10^{12}/l) and hematocrit mean value of 23.14 (normal range = 27–50 %). Variable hipoproteinemia and different degrees of proteinuria were also detected. Melatonin values were always within range.

Pathological, microbiological, parasitological and many other specific techniques disregarded the occurrence of the following well-known ovine diseases in both the acute and chronic phase: Visna/maedi (small ruminant lentivirus), pseudorabies or Aujeszky's disease (herpesvirus suis), enterotoxaemia (*Clostridium* spp.), listeriosis (*Listeria monocytogenes*), louping ill (flavivirus), rabies (lyssavirus), border disease (pestivirus), West Nile fever (flavivirus), paratuberculosis or Johne's disease (*Mycobacterium avium* subsp.*paratuberculosis*), ovine pulmonary adenocarcinoma (SPA), bluetongue (orbivirus), coenurosis (*Taenia* spp.), borna disease (bornavirus), scrapie (ovine spongiform encephalopathy), neosporosis (*Neospora caninum*), toxoplasmosis (*Toxoplasma gondii*) and mycotoxicosis. Generalized parasitic diseases were sometimes detected, but they never could explain the massive appearance of cachectic animals.

Table 1 Number of vaccine inoculations given to experimental animals at a specific date

Days	Inoculations (n)	Al(III) (mg)	Thiomersal (mg)
0	2	8	0.4
21	2	8	0.4
42	1	4	0.2
63	1	4	0.2
84	1	4	0.2
105	1	4	0.2
126	1	4	0.2
149	1	4	0.2
177	2	8	0.4
198	2	8	0.4
Total	14	56	2.8

Quantity of Al(III) and thiomersal (per day and total) given to each experimental lamb

Description of the ovine ASIA syndrome in natural cases

The ovine ASIA syndrome can be divided in two phases: acute and chronic.

The acute phase is observed in approximately 25 % of flocks and affects less than 0.5 % of animals within a given flock. This phase appears a few days (range = 2–6) after an adjuvant-containing inoculation that can be a prime or a boosting vaccine or an injectable antiparasitic treatment. This acute phase is characterized by an array of acute nervous clinical signs that include lethargy, reluctance to movement, bruxism, transient blindness, nystagmus, stupor, abnormal behavior, disorientation and a low response to external stimuli. In the most severely affected cases, animals show prostration and seizures involving extremities and head, followed by death. However, direct losses due to the acute phase are low and most of the animals make an apparent recover after a 2–3-day clinical period. At postmortem examination, gross changes are either not consistent or irrelevant. Histopathologic lesions consist in a severe, acute meningoencephalitis that is characterized by inflammation of the meninges at the cerebral lobes and especially at cerebral sulcus and, to a much lesser degree, the spinal cord. This inflammatory reaction is composed by a cellular pool that includes macrophages, lymphocytes, neutrophils, eosinophils and few plasma cells (Fig. 1). Remarkably, scattered, large cells compatible with activated macrophages, showing granular and eosinophilic,

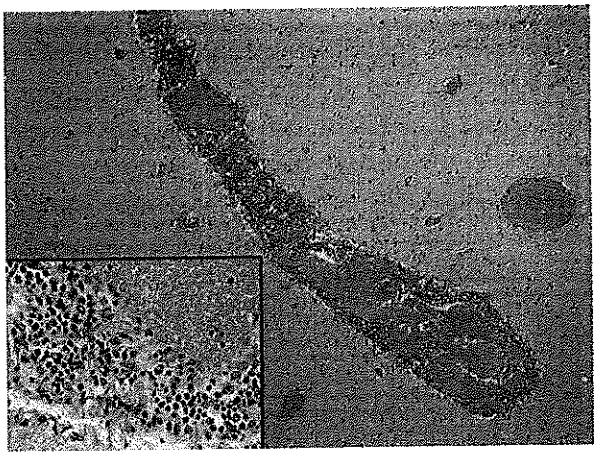

Fig. 1 Adult sheep. Acute phase of the ovine ASIA syndrome. Severe meningitis is observed in a cerebral sulcus together with intense hyperemia of blood vessels. Perivascular cuffs are also distinguished in the cerebral gray matter. Hematoxylin–eosin, ×40. *Inset* Detail of the infiltrate showing mostly macrophages, lymphocytes and neutrophils. Hematoxylin–eosin, ×200

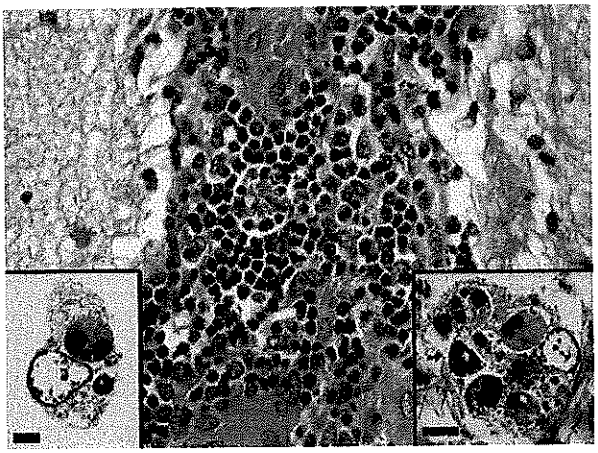

Fig. 2 Adult sheep. Detail of the meningitis in the acute phase of the ovine ASIA syndrome. A large, bizarre macrophage is observed at the center of the image, showing multiple, intracytoplasmic eosinophilic deposits together with intracytoplasmic and peripheral inflammatory cells. Hematoxylin–eosin, ×400. *Inset* Emperipolesis: presence of intracytoplasmic inflammatory cells (degraded or not) within a macrophage. TEM, *bars* 10 μm

PAS-positive deposits in the cytoplasm, are observed within the inflammatory infiltration. By electron microscopy, these cells show the presence of other cells within their cytoplasm, either intact or degraded, an image that is compatible with emperipolesis (Fig. 2). Inflammatory perivascular cuffs are observed in vessels of the gray matter in both encephalon and spinal cord, and they are composed by the same cellular pool as in the meninges but with less histopathologic severity. Other features are mild gliosis, subpial glial foci, scattered neuronal death in encephalon and spinal cord, choroiditis, multifocal white matter demyelination and severe hyperemia of blood vessels with multifocal intraparenchymal hemorrhages.

The chronic phase of the ovine ASIA syndrome is seen in a higher proportion of flocks, as it was the case during the vaccinations against bluetongue in Spain, but clinical incidence of the chronic phase can vary greatly. It can affect 50–70 % of flocks in a specific area and the number of affected animals in a given flock can reach almost 100 %. The chronic phase of the ovine ASIA syndrome can appear without the previous concurrence of the acute phase in a given herd. Flocks that have shown the acute phase most normally evolve toward the chronic phase. This phase is independent of age, and it is triggered by external stimuli/stresses that influence the clinical appearance of the disease, the most important of them being low (winter) temperatures. The chronic phase starts with an excitatory period where affected animals show constant movement, abnormal behavior, restlessness and compulsive wool biting, resulting in animals with a very poor wool coat, a diffuse redness of the skin and thinning of the affected sheep. However, during this first part of the process, there is normal food consumption or even polyphagia. Immediately after, sheep develop generalized weakness, light but constant head tilt, muscle tremors and weight loss leading to extreme cachexia (Fig. 3). Finally, the animal enters into a terminal phase with lack of response to stimuli, ataxia and tetraplegia, the sheep being unable to stand up. This is followed by stupor, coma and death, but no seizures are observed in this phase. Affected flocks have also increased rates of spontaneous abortion of unknown etiology. The most relevant lesions are extreme depletion of fat deposits and serous atrophy of fat, ascites, hydrotorax, hydropericardium and generalized skeletal muscular atrophy. Remarkably, most animals showed a marked thickening of the peripheral nerves, a lesion easy to see on the subcutaneous tissue covering the abdomen and thoracic cavity (Fig. 4) but also seen in main nerves such as sciatic. The main histopathologic change of the chronic phase of the ovine ASIA syndrome is located at the spinal cord and consists in multifocal neuronal necrosis and neuron loss in both dorsal and ventral column of the gray matter (Fig. 5). Multifocal meningoencephalitis was also present in most animals and shared similar characteristics to those described in the acute phase but with a lesser severity. The lesion affecting the peripheral nerves was characterized as a severe perineural edema with formation of myxoid tissue, but not endoneural edema or inflammation (Fig. 6). Light, multifocal and scattered, lymphoplasmacytic perineuritis was also seen in almost all animals (Fig. 6). Other histopathologic lesions in the central nervous system (CNS) were less evident and consisted in neuronal loss, light patchy demyelination and presence of glial foci. Presence

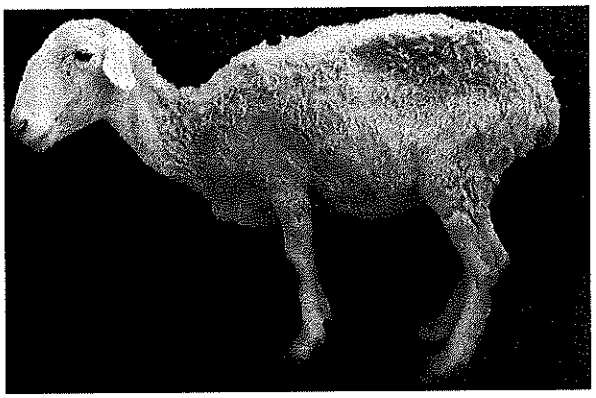

Fig. 3 Adult sheep affected by the chronic phase of the ASIA syndrome. Note extreme cachexia, poor wool coat, redness of the skin, atrophy of muscular masses and generalized weakness. The animal also shows abnormal posture

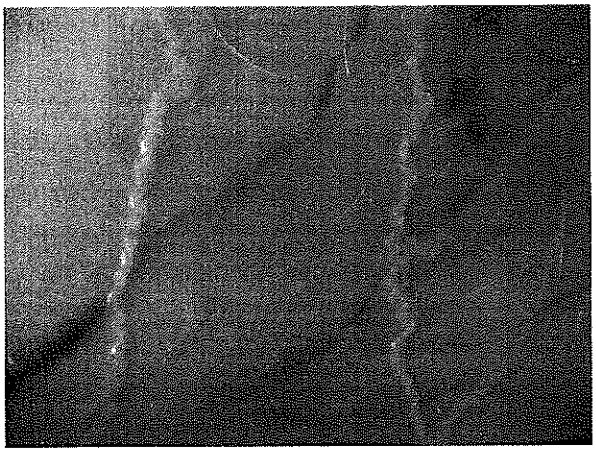

Fig. 4 Adult sheep affected by the chronic phase of the ASIA syndrome: subcutaneous tissue of the abdominal wall. Two parallel, thickened peripheral nerves. Normal peripheral nerves are much thinner and difficult to see

of membranous glomerulonephritis was seen in approximately 50 % of sheep studied.

If they occur in the same flock, the chronic phase can immediately follow the acute phase or it can also be detected after some months. In any case, external factors, such as low temperatures, forced lambing in winter and previous poor nutritional status (or a combination of), are key features that can trigger its development.

Experimental reproduction of the syndrome

The evolution of lambs' weight per group is depicted in Fig. 7. During the first months, the difference in weight between groups evolved in parallel and reflected normal variations linked to climatology, the availability of pastures or the supplementation with grain. By day 164 (8 December), together with the arrival of low temperatures (mean 4.7 C in Dec 2010), the mean weight of the vaccinated group descended and it started to diverge from the control, the difference becoming significant ($p < 0.01$) by day 177 (January 2011). This significant result was maintained during the rest of the experiment, the difference in weight between groups reaching 8.5 % at the end of the challenge (day 261), coinciding with the end of the winter (18 March).

Vaccinated animals showed an array of nervous clinical symptoms that could be more easily seen by the end of the experiment. These included periods of depression with reluctance to move, light stupor and tendency to collapse when handling. However, these periods alternated in the same animals with periods of excitement and abnormally stimulated behavior.

In vaccinated animals, postmortem studies demonstrated accentuated but not complete depletion of fat deposits, light hydroperitoneum and hydropericardium, together with slight increase in the diameter of some peripheral nerves. Histopathology was mostly not remarkable with the exception of the spinal cord of the CNS, mainly the lumbar medulla, where neuronal lesions similar but less intense to those observed in the chronic phase of the natural ASIA syndrome could be observed.

Fig. 5 Spinal cord in a sheep affected by the chronic phase of the ASIA syndrome. Note diffuse and widespread necrosis of the neurons and gray matter at the dorsal column. Hematoxylin–eosin, ×40. *Inset* Severe neuron necrosis and loss. Hematoxylin–eosin, ×400

Detection of Al and Hg in blood and tissues

Detection of Al and Hg in blood was studied by ICPMS in a group of five chronic-phase, severely affected sheep and two control animals, showing values of Al ranging between 266.75 and 289.76 ng/mL and negative results for Hg, whereas controls showed traces of Al(III) and negative results for Hg. XPS analysis in nervous tissue from the spinal

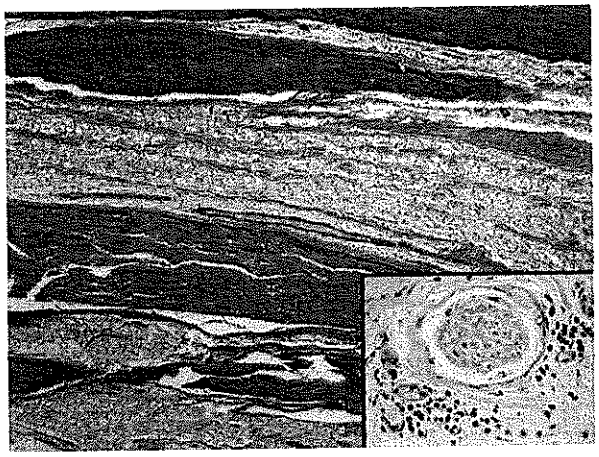

Fig. 6 Subcutaneous peripheral nerve from an adult sheep affected by the chronic phase of the ASIA syndrome. Note the severe perineural edema with formation of myxoid tissue. Hematoxylin–eosin, ×40. *Inset* Light lymphoplasmacytic infiltration surrounding a sectioned peripheral nerve

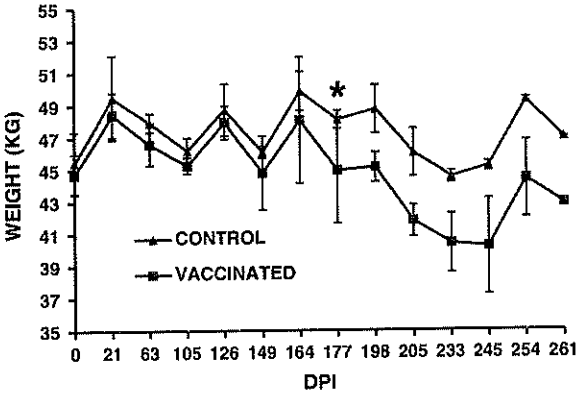

Fig. 7 Mean weight of the vaccinated and control lamb groups. Differences start to be observed at day 164 and become significant by day 177 (*). Significant results were maintained until the end of the challenge (day 261)

cord in six chronic-phase, severely affected sheep and the experimentally vaccinated lambs killed at days 254 and 261 showed no positive results with the exception of the experimentally vaccinated animal killed at day 261 that showed traces of Al in nervous tissue. Finally, spinal cord samples from the two pairs of experimental animals killed at days 254 and 261 were studied by scanning electron microscopy–EDAX, and mean values were 8.445 Al wt% for experimentally vaccinated animals and 3.825 Al wt% for controls.

Discussion

This is the first description of the ASIA syndrome in an animal species which importantly relies on: (1) the impact on ovine health. This is an non-described problem that has been affecting sheep production for decades and that can produce devastating problems under certain circumstances, as it happened in Spain after the massive vaccination against bluetongue; (2) the impact on other animal species, as some disease of unknown etiology have many common aspects with the ASIA syndrome in sheep and might share similar pathogenic mechanisms; and (3) the impact on human health. The ovine ASIA syndrome is similar to some human diseases also linked to the effect of multiple vaccinations, and it could serve as a very useful animal model as the ovine disease appears spontaneously under routine production conditions and it is also experimentally reproducible.

The acute phase of the ovine ASIA syndrome occurs a few days after a vaccination, only affects a low number of animals, and most of them apparently recover after a brief period of time, results that coincide with previous works [12]. This phase is characterized by a severe meningoencephalitis, similar to postvaccine reactions seen in humans [13, 14]. The reason for this inflammation is unclear, but it might be due to the direct transport of Al(III) by macrophages to the CNS, a route described in rodents [15]. It is known that aluminum salts-loaded macrophages can release pro-inflammatory cytokines such as IL-1beta, IL-18 and IL-33, thus leading a severe perivascular inflammatory reaction with the presence of macrophages, lymphocytes, neutrophils and eosinophils [10, 11]. The cellular pool observed in our cases fully coincides with this description, and it is not characteristic of any known ovine disease. The observation of a phenomenon similar to emperipolesis in those macrophages can be consistent with this hypothesis as this phenomenon is seen in very active cell populations that target diseased cells, although it is mostly seen in tumor pathology [16]. Therefore, the acute phase might just be a toxic event depending on the arrival of alum at the CNS, whereas the low number of cases observed might relate to a certain specific genetic background [17] and maybe the concurrence of other factors. However, the transportation of alum into the CNS might happen in all animals, irrespectively of the appearance of acute clinical symptoms. The presence of Al and/or Hg in these large, bizarre macrophages is currently being investigated by energy dispersive X-ray spectroscopy (EDAX) in cases showing the clinical acute phase.

The chronic phase of the ovine ASIA syndrome is a more frequent event in our local conditions of ovine production and causes a neurological and cachectic process that has no parallel in ovine pathology, and it is a serious but unexplained concern for farmers and veterinarians. The chronic phase does not necessarily follow an acute episode, and it is triggered by the combination of multiple alum adjuvant-containing inoculations over the years and external stimuli.

Among the external stimuli/stress that influences the clinical appearance of the disease, the most important is low temperatures. This effect was clearly seen in winter 2008–2009 when the chronic phase of the ovine ASIA syndrome caused a devastating disease across Spain, linked to the vaccination against bluetongue (four inoculations in less than 30 days) on a previously over vaccinated sheep population. However, the influence of low temperatures had already been observed before that massive vaccination and it has kept being seen to date. Other stresses that might favor the ovine ASIA syndrome, alone or in combination with others, are poor body condition/poor nutritional status and high level of meat or milk production. This description is similar to a vaccine reaction described before in sheep [12], but in this paper, authors only related reaction with bluetongue vaccines. Spontaneous abortion and glomerulonephritis leading to renal failure described in the present work in animals suffering the chronic phase of the ASIA syndrome was also observed in the same report [12]. Similar chronic conditions of unknown etiology are seen in other animal species. For instance, the disease known as equine motor neuron disease shares many clinicopathological aspects with the ovine ASIA syndrome in horses, specie also frequently vaccinated [18]. Chronic neurological disorders are also seen in human beings after vaccination and long-term persistence of Al. For instance, in MMF patients, neurological disorders mainly correspond to cognitive impairment, without focal deficits or visible lesions at brain MRI [19]. In other cases, motor neuron syndromes have been suspected to be induced by aluminum vaccines [4], and interestingly, motor neuron degeneration and motor deficits have been described in mice after aluminum hydroxide injections [20]. In the present work, presence of Al in CNS has not been studied in a representative number of animals and samples, mostly due to the lack of funding. Moreover, methodology was not totally setup for biological samples in some of the analysis performed something that could have affected our results. However, there are indications that Al might be present in some areas of the CNS, and thus, Al might be linked to the lesions observed in our sheep. Further and more complete studies are needed to define the presence of Al in CNS and other locations in vaccinated sheep.

The pathogenesis of the chronic phase of the ovine ASIA syndrome is not known, but it might rely on persistence of Al in CNS and the chronic hyperstimulation of the immune system caused by routine vaccination of sheep against a variety of pathogens. The strong and unspecific stimulation of the immune system caused by adjuvants such as alum might induce a chronically hyperactivated status that facilitates the production of autoantibodies leading to the development of a very complex autoimmune disease. Actually, deposition of the C3 fraction of complement in CNS blood vessels has already been described in sheep double vaccinated with bluetongue vaccines and affected by the acute phase of the disease [12]. Moreover, Al(III) given subcutaneously might also be retained within macrophages [15] or other cell types, thus leading to an even more chronic stimulation of the immune system, without the need of further vaccinations and triggering an autoimmune disease in sheep. The clinical chronic phase of the ovine ASIA syndrome might only be the iceberg tip of a much more common disease that goes mostly underdiagnosed.

One important fact is the influence of low temperatures on the clinical detection of the chronic phase of the ovine ASIA syndrome, something that could be explained by the immunostimulatory properties of alum that activates an intracellular innate immune response system called the Nalp3 inflammasome or cryopyrin [9]. Nalp3 is a protein involved in neurodegenerative processes [21, 22] and that is encoded in humans by the NLRP3 (NOD-like receptor family, pryin domain containing 3) gene. This gene is associated with a certain group of human diseases that are seen under cold weather conditions such as familial cold autoinflammatory syndrome or Muckle–Wells syndrome [23]. If Al(III) is retained in vivo in the sheep body, it might induce severe immunostimulation under cold weather conditions, leading to acute neurodegeneration maybe through an autoimmune process and to the clinical appearance of the chronic phase of the ASIA syndrome.

In conclusion, the present work describes a newly recognized entity in sheep that is similar to the ASIA syndrome in humans. The sheep disease parallels many aspects of the human affections under this name, and it could serve as a reliable experimental animal as sheep develops the syndrome under normal production conditions and it is able to reproduce it under experimental conditions. A huge research effort is needed in this field to help understanding this process, something that will be of great benefit for both human and animals.

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Conflict of interest None.

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