

# Chapter 2

## Classical and Atypical Scrapie in Sheep and Goats

Christine Fast and Martin H. Groschup

**Abstract** Scrapie is a naturally occurring transmissible spongiform encephalopathy (TSE) in sheep, goat and muffs almost world-wide and is known for about 250 years. It is characterized by the accumulation of an abnormal isoform (PrP<sup>Sc</sup>) of host encoded prion protein (PrP<sup>C</sup>) in the central nervous system which leads to progressive neurodegeneration and death. Scrapie represents the prototype of the so-called prion diseases. It is observed to date as two types, classical and atypical scrapie. The susceptibility to both types is modulated by polymorphisms of the prion gene. Whereas classical scrapie is clearly a naturally occurring transmissible disease, atypical scrapie may also be caused by the spontaneous misfolding of prion protein. This review gives an overview on the current knowledge of classical and atypical scrapie in sheep and goats with special emphasis on epidemiology, clinical and pathological signs, genetic susceptibilities, diagnosis and the characteristics of the most common scrapie strains.

**Keywords** Atypical scrapie • Classical scrapie • Pathological prion protein • Prions • Scrapie • TSE

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## 2.1 Overview

Scrapie is the most common name for the transmissible spongiform encephalopathy (TSE), which affects sheep, goats and moufflons almost worldwide. Like all other prion diseases, scrapie is a neurodegenerative progressive and eventually fatal disease. Scrapie is associated with a number of clinical signs ranging from subtle behavioural abnormalities to more obvious neurological signs. The clinical diagnosis needs to be confirmed by the demonstration of pathognomonic spongiform lesions and the immunodetection of pathological prion protein (PrP<sup>Sc</sup>) depositions in the CNS primarily (OIE-Manual of Diagnostic Tests and Vaccines for Terrestrial Animals). PrP<sup>Sc</sup> depositions can be revealed by immunohistochemical and biochemical methods (see Chap. 13). To date, two distinct scrapie types are known: classical and atypical scrapie.

## 2.2 History

Scrapie is not only the prototype of TSEs but also the prion disease with the longest history of publication. The first authentic report on scrapie was written in Germany and dates back to year 1750 (Leopoldt 1750). However, a later publication (Comber 1772) even mentions cases in England that occurred already in 1732. Several authors at later times even referred to much earlier time periods, spanning from Roman times up to the seventeenth century, but without giving corresponding references (for a detailed review see Schneider et al. 2008). Moreover in former times, many sheep diseases were confused with scrapie. Other difficulties were the various names that were used to describe this disease throughout Europe: “Goggles”, “Ricketts”, “Rubbing Disease” and “Trotting Disease” in England, “Scratchie” and “Yeukie pine” in Scotland, “Basquilla Disease” in Spain, “La maladie convulsive”, “La Tremblante” and “Prurigo lumbaire” in France, “Rida” in Iceland, “Gnave-og travesjuka” in Norway and “Gnubberkrankheit”, “Petermännchen”, “Traber” or “Reiberkrankheit” in Germany. Altogether, at least 42 different names were used in Europe and India (Schneider et al. 2008) for this disease in small ruminants.

The infectious nature of scrapie was already reckoned in the eighteenth century (Leopoldt 1750). In the following decades and centuries, different transmission routes were discussed in which the sexual intercourse was the most suspected modus. However, among other causes like atmospheric disturbances, a few authors proposed a mere coexistence of infected and non-infected animals or a spontaneous origin of the diseases (Schneider et al. 2008). In addition, a broad consent existed already in the nineteenth century concerning the role of hereditary factors for scrapie. Initially, a hereditary predisposition and the transmission by asymptomatic animals were assumed (Thaer 1821; von Richthofen 1821) and even the existence of hereditary and non-hereditary scrapie forms was postulated (von Richthofen 1826).

A number of experimental transmission studies were subsequently carried out in order to clarify the origin and transmission routes of scrapie. These experiments

included contact studies with infected and non-infected sheep and subcutaneous and intravenous inoculation studies using different tissues and bodily fluids from infected animals. However, most of these studies were terminated prematurely and therefore failed due to the long incubation period of scrapie (for detailed review see Schneider et al. 2008). However, in 1936, the transmissibility of scrapie was first time proven by experimental inoculation of healthy animals with brain and spinal cord of diseased sheep. In this experiment, the inoculated animals were kept for longer periods of time and sheep could develop scrapie after incubation periods of up to 2 years (Cuille and Chelle 1936, 1938a, b).

Since the 1930s, scrapie research was intensified when substantial financial losses to the sheep industry were caused by increasing numbers of cases. These losses prompted also studies on the true nature of the infectious agent. Besides parasites (M'Gowan 1914) and bacteria (Bastian 1979) as causative agents, a virus infection was the most commonly proposed theory, already formulated in 1938 (Cuille and Chelle 1938a, b). In 1954, the term of a “slow virus infection” was first time introduced (Sigurdsson 1954). However, already in 1966, an alternative to the virus origin was postulated as the causative agent, i.e., polysaccharides (Alper et al. 1966, 1967; Field 1966) or lipids (Alper et al. 1978). In 1967, for the first time, a protein was assumed as infectious agent (Pattison and Jones 1967) and the first “protein-only-hypothesis” was enunciated (Griffith 1967) followed in the 1970s by the “virino” theory (Dickinson and Outram 1979). Finally, based on the resistance of the pathogen, in 1982, the term “proteinaceous infectious particle” (acronym: prion) was introduced (Prusiner 1982) and the conversion of a normal cellular protein (PrP<sup>c</sup>) into a pathological isoform (PrP<sup>Sc</sup>) as key event of TSE pathogenesis was postulated shortly after (Oesch et al. 1985). PrP<sup>Sc</sup> is currently considered to be the biochemical marker and the causative agent of TSEs. However, the prion theory is still debated since PrP<sup>Sc</sup> is not always infectious and the phenomenon of strains is still an enigma (Lasmezas et al. 1997; Piccardo et al. 2007).

In 1998, the atypical form of scrapie, termed Nor98, was first time discovered in Norwegian sheep (Benestad et al. 2003). However, retrospective studies revealed atypical scrapie cases in the UK already in the late 1980s. Therefore, this disease is not considered as new emerging form of TSE (Bruce et al. 2007). Atypical scrapie is distinguished from classical scrapie by clinical and epidemiological as well as by molecular and histopathological features. It is not rare compared to classical scrapie in most countries and found worldwide at a comparable incidence rate, which is indicative for a different, perhaps non-infectious aetiology (Fediaevsky et al. 2008).

Scrapie in goats was initially described after an experimental exposure in 1939 (Cuille and Chelle 1939) and the first natural case was reported a few years later (Chelle 1942). The first experimental challenge of goats with sheep scrapie showed 100% susceptibility suggesting that goats are highly susceptible (Pattison et al. 1959; Cuille and Chelle 1939). Like classical scrapie atypical scrapie cases were reported also in goats (Fediaevsky et al. 2008, for detailed review see Vaccari et al. 2009) but showed a lower prevalence as compared to sheep (EFSA 2010).

In Moufflons, only classical scrapie was reported in six natural cases so far (Wood et al. 1992a, b).

### 2.3 Geographical Distribution and Surveillance

Scrapie is endemic in almost all member states of the European Union (EU 27) as well as in Norway, Iceland and Switzerland. Brazil, Canada, Israel, Japan, Palestinian Autonomous Territories, Russia, Tajikistan and the USA reported scrapie cases (atypical and/or classical) in the last 6 years. Only individual atypical scrapie cases were documented on the Falkland Islands and New Zealand (Epstein et al. 2005, EU Commission, Kittelberger et al. 2010, World Animal Health Data Base (WAHID); [http://web.oie.int/wahis/public.php?page=disease\\_timelines](http://web.oie.int/wahis/public.php?page=disease_timelines)). According to the “World Livestock Disease Atlas 2011” (Anonymous 2011), scrapie ranks third worldwide as cause for sheep and goat losses.

An introduction of classical scrapie via imported sheep from the UK was suspected for countries like Australia and New Zealand (1952–1954), South Africa (1964–1972), Colombia (1968–1971) and Kenya (1970). After thorough eradication by slaughtering the imported sheep and their flock mates, Australia and New Zealand remained free of scrapie to date (Detwiler and Baylis 2003).

However, the true scrapie status of many countries remains unknown because there is usually only an inadequate passive surveillance system in place to detect infected animals. It is nearly impossible to establish freedom from infection without establishing an active surveillance system, which includes the examination of fallen stock and emergency slaughter (Detwiler and Baylis 2003, OIE Manual). This is exemplified by the introduction of a harmonised active surveillance program for scrapie in sheep and goats throughout the EU in 2003. In the context of this program, animals over 18 months of age (fallen stock, emergency slaughter, as well as healthy slaughtered animals) were examined for TSE.

### 2.4 Prion Protein Gene and Susceptibility

It has been shown in several epidemiological studies that the successful transmission of classical scrapie requires genetically susceptible sheep. In year 1968, the effect of a so-called *Sinc*-gene (scrapie incubation gene) on the length of the incubation period of experimentally infected mice and a synonymously so-called *Sip*-gene (scrapie incubation period gene) in sheep were proposed (Dickinson et al. 1968a, b). Eventually, different polymorphisms of the prion protein gene (*Prnp*) were matched in the 1980s and 1990s with the *Sip*-/*Sinc*-genes (Oesch et al. 1985; Westaway et al. 1987; Goldmann et al. 1991; Moore et al. 1998; Hunter et al. 1996).

The murine *Prnp* consists of two alleles, s7 and p7, which differ in their PrP amino acid sequence at codons 108 and 189 and are associated with short or prolonged incubation times after infection with particular (i.e., ME-7) experimental strains. However Infections with other strains (i.e., 22A) showed reversed results (Dickinson et al. 1968a). Similar results were obtained in sheep. The ovine *Prnp* consists of two alleles sA (short incubation period) and pA (prolonged incubation period), which are distinct primarily in the amino acid sequences encoded at codon

136 (Dickinson et al. 1968b; Hunter et al. 1996, 1997). Similar as in mice, the length of the incubation period is depending on the scrapie strain that is used (Foster and Dickinson 1988). Furthermore, in susceptible animals, effects on the incubation period can also result from polymorphisms at codons 154 and 171 (Hunter et al. 1996). Thus, the incubation period is determined at least by two factors: the genotype of the host and the agent strain.

The ovine PrP is located on chromosome 13 (Iannuzzi et al. 1998) and the functional length of the PrP gene is approximately 21 kb and is composed of three exons, from which exon III contains the complete uninterrupted open reading frame. The length of the unprocessed precursor protein is 256 amino acids. After post-translational modifications, about 210 amino acids remain in the mature protein (for detailed review see Goldmann 2008).

Ovine PrP polymorphisms influence not only the susceptibility to the disease but also modulate the progression including the incubation period and clinical signs. The vast majority of polymorphisms are due to single nucleotide polymorphisms (SNP) in the DNA, which often cause single amino acid changes. Of particular interest are polymorphisms at codons 136, 154 and 171 within the ORF, which are clearly linked to scrapie susceptibility in sheep (Goldmann 2008). Standard abbreviations describe the alleles in reference to the three codons:

- A136V in which Alanine (A) is associated with resistance and Valine (V) is associated to susceptibility (Goldmann et al. 1991; Hunter et al. 1994).
- Q171R in which Arginine (R) is associated with resistance and Glutamine (Q) is associated with susceptibility (Westaway et al. 1994; Clouscard et al. 1995; O'Rourke et al. 1997).
- R154H in which Histidine (H) is associated with resistance (Goldmann et al. 1991; Laplanche et al. 1993).

The polymorphisms mentioned above result in five different alleles (ARQ, VRQ, AHQ, ARR and ARH), leading to 15 different genotypes, which are the only alleles with significant distribution worldwide (Goldmann 2008). Some further genotypes, ARK and TRQ among others, are known (Gombojav et al. 2003; Guo et al. 2003; Billinis et al. 2004), but due to their low frequencies they are not included into a TSE genotype classification system (Dawson et al. 1998). This five group risk classification (Table 2.1) is the basis for breeding and scrapie eradication programs applied in the EU. The highest risk to develop scrapie carry VRQ/VRQ animals, the highest genetic resistance is associated to ARR/ARR sheep (Belt et al. 1995; Hunter et al. 1996; Hunter 1997). However, this classification is subject to restriction as, for example two ARR/ARR sheep from different flocks in France and Germany have been shown to be subclinical carriers of classical scrapie (Groschup et al. 2007). Additionally, ARQ/ARQ animals, classified in R3, can be at highest risk in flocks where the VRQ allele is absent for example due to breed (Goldmann 2008).

Furthermore, several polymorphisms are described at other positions, for example 25% of all ARQ alleles revealed additional polymorphisms (Goldmann 2008). However, it is unclear whether such polymorphisms have a profound effect on the disease. Some studies refer to resistance and/or prolonged incubation times in sheep

**Table 2.1** Ovine five group risk classification system

Risk group	Genotype	Susceptibility
1	ARR/ARR	Highest genetic resistance
2	ARR/AHQ ARR/ARH ARR/ARQ	Genetic resistance
3	AHQ/AHQ AHQ/ARH AHQ/ARQ ARH/ARH ARH/ARQ ARQ/ARQ	Low genetic resistance
4	ARR/VRQ	Genetic susceptibility
5	AHQ/VRQ ARH/VRQ ARQ/VRQ VRQ/VRQ	Highest genetic susceptibility

carrying for example AC151RQ, AT137RQ, or ARQK176 (Acin et al. 2004; Thorgeirdottir et al. 1999).

The classification system described above and in Table 2.1 does not work for atypical scrapie. In contrast to classical scrapie in most of the atypical cases, animals of PrP genotype risk groups R1-3 (Benestad et al. 2008) are affected. Most frequently found in such cases are haplotypes such as AHQ/AHQ, AHQ/ARQ and ARR/ARR, respectively. It has been shown that polymorphisms at codons 141 and 154 are linked to susceptibility. Genotype AF141RQ encoded for a higher susceptibility than the AL141RQ allele or even the AHQ genotype (Goldmann 2008).

Although the wild-type amino acid sequence of goat and sheep PrP are similar, the PrP genetics in goats is much more variable, yet without polymorphisms at codons 136 and 171 surprisingly. In goats 29 other polymorphisms of the caprine Prnp, resulting in amino acid changes, have been found in different countries and breeds (Vaccari et al. 2009; Goldmann et al. 2011). At least five of them seem to be associated with TSE susceptibility (for detailed review see Vaccari et al. 2009):

- I142M haplotypes have a lengthened incubation period after experimental inoculations and are associated with increased resistance to classical scrapie under natural conditions (Goldmann et al. 1996; Barillet et al. 2009).
- R154H haplotypes are associated with some resistance to classical scrapie in different breeds and countries (Barillet et al. 2009; Billinis et al. 2002; Papasavva-Stylianou et al. 2007; Vaccari et al. 2006) but have a comparable high risk associated with atypical scrapie (Moum et al. 2005; Arsac et al. 2007; Seuberlich et al. 2007).
- N146S/D polymorphisms encode low risk (some resistance) for scrapie infection but this genotype is confined to Damascus/Damascus crossbreed goats on Cyprus primarily (Papasavva-Stylianou et al. 2007).
- R211Q haplotypes have shown an increased resistance to classical scrapie in French case–control studies (Barillet et al. 2009).

- Q222K haplotype is associated with protection against classical scrapie in several breeds and countries, but heterozygous animals are reported to be infected (Acutis et al. 2006; Barillet et al. 2009; Vaccari et al. 2006).

At the time of writing, both haplotypes 146S/D and 222K are considered as candidate for TSE resistance breeding and eradication programs for goats.

In summary, the number of variables influencing the susceptibility to scrapie are high and depend not only on the genotype of the host and the infectious agent but also on individual flocks, breeds and geographical location, not to forget dose and route of inoculation effects.

## 2.5 Epidemiology of Scrapie

Summarising the prevalence of TSE infections in small ruminants worldwide is a difficult task in the face of the long incubation periods, the missing availability of a practical ante mortem tests (which prevents the detection of subclinical-infected animals), the variable clinical signs (which may result in unidentified animals), the potentially unknown host-encoded genetic components (which influence both the risk of infection and the incubation period) and the not yet fully understood routes of transmission (for detailed reviews concerning the epidemiology of scrapie see Hoinville 1996; Detwiler and Baylis 2003; Benestad et al. 2008).

### 2.5.1 Prevalence in the EU

A comprehensive overview on the prevalence of classical and atypical scrapie in the European Union is given by Fediaevsky et al. as well as by the European Commission (Fediaevsky et al. 2008, 2010; EFSA 2010).

Following the introduction of active surveillance programs for TSEs in sheep and goats in the EU in 2003, clearly defined epidemiological data were obtained for the first time. In this regard, the prevalence of classical and atypical scrapie showed different patterns with more variation seen in classical scrapie (Fediaevsky et al. 2008). In sheep, the overall prevalence of classical scrapie in the EU (excluding Cyprus) decreased from 2002 to 2009. The number of cases in fallen stock was significantly higher as compared to healthy slaughter animals (Fediaevsky et al. 2008). For example in 2009 in the EU, 27 1.158 sheep (of 331.027 tested) and 89 goats (of 117.868 tested) were TSE positive but only 14 cases of sheep and no goat scrapie case were detected in slaughter animals (EFSA 2010, European Commission). This data compilation excludes Cyprus and Slovenia which presented, at the time of writing, a very specific situation. It can be assumed that due to the genetic breeding programs for resistance to scrapie, the proportion of sheep carrying ARR alleles in the populations will increase and therefore the prevalence rates of classical scrapie will further

decrease (EFSA 2010). However, it has to be emphasised that the incidence rates of classical scrapie in geographical areas were non-uniform. Areas could be grouped into (1) countries with no cases detected at slaughterhouse, (2) countries with low prevalence rates and (3) countries with high prevalence rates at the slaughterhouse. This classification did not exclude areas with high rates in countries with low prevalence rates (EFSA 2010). Furthermore, due to the long incubation time and particular pathogenesis of classical scrapie, an underestimation of the real prevalence may apply and substantial numbers of undetected cases (up to 17%) were reported (Jeffrey et al. 2002; Ligios et al. 2006; Reckzeh et al. 2007; González et al. 2009).

The distribution of atypical scrapie cases is remarkably homogenous in space and time as compared to classical scrapie and no infection clusters were observed in positive flocks (Fediaevsky et al. 2008, 2010). In eight EU countries between 2007 and 2009, the incidence of atypical scrapie in healthy slaughtered sheep was similar or higher than the incidence of classical scrapie. These data suggest that atypical scrapie represents a significant proportion of TSE-infected small ruminants (EFSA 2010). The prevalence seems to be stable within the EU and due to the breeding programs favouring the susceptible ARR genotype, it can be assumed that atypical scrapie will not be eliminated (Lühken et al. 2007). Furthermore, the same limitations of the active surveillance associated with classical scrapie are true for atypical scrapie. Other problems in estimating the exact prevalence of atypical scrapie include the age-dependent variations and the inconstant detection of atypical scrapie by using brainstem samples (Benestad et al. 2008), the low sensitivity of some rapid tests for atypical scrapie (EFSA 2005) and the absence of detectable pathological prion protein in the lymphoreticular tissues. In this regard of particular interest are recent results, which show infectivity in the lymphoreticular system of sheep infected with atypical scrapie (Andreoletti et al. 2011).

### ***2.5.2 Transmission Routes in Scrapie***

In the last centuries, a lengthy discussion about the mode of transmissions of scrapie took place (Schneider et al. 2008) and even up to now the exact transmission routes are not resolved entirely. It is known that scrapie can transmit laterally between sheep under natural conditions. Such transmissions occur either via direct contact or through contamination of the environment. The oral route is most efficient (Jeffrey and Gonzalez 2007; van Keulen et al. 2008). Scrapie in goats is often found in mixed herds with sheep, but it has also been observed to spread from goat to goat (Wood et al. 1992b).

The main source of infection is the infectious placenta. Infectivity and PrP<sup>Sc</sup> have been detected in the foetal parts, depending on the genotype of the offspring (Pattison et al. 1972; Onodera et al. 1993; Race et al. 1998; Andreoletti et al. 2002; Alverson et al. 2006; Lacroux et al. 2007). However, results of different studies indicate that an in utero infection prior to parturition does not occur (Hadlow et al. 1982; Andreoletti et al. 2002). The placenta and the amniotic fluid are shed into the



environment during lambing and their ingestion by other sheep (and goats) is still assumed to be the most important infection mode within the flock (Pattison et al. 1972; Hoinville 1996). Moreover, it has been shown that scrapie agent remains infectious even after years in the environment (Brown and Gajdusek 1991; Seidel et al. 2007). Anecdotal data indicate even survival of infectivity for more than 16 years (Georgsson et al. 2006). Additional results indicate that released PrP<sup>Sc</sup> may be sequestered near the soil surface and bound on soil minerals, which may then be ingested during grazing of farm animals (Johnson et al. 2006). Besides the placenta, amniotic fluid (Hoinville 1996), faeces (Terry et al. 2011) and milk (Konold et al. 2008; Lacroux et al. 2008; Maddison et al. 2009) have been shown to contain PrP<sup>Sc</sup> and/or infectivity. Recent results revealed PrP<sup>Sc</sup> also in the oral cavity of scrapie-infected sheep (Maddison et al. 2010; Gough et al. 2011) and PrP<sup>Sc</sup> and/or infectivity in urine was demonstrated in experimental scrapie models in hamster and mice (Seeger et al. 2005; Gonzalez-Romero et al. 2008; Gregori et al. 2008). More artificial routes demonstrated in several experimental infections include transmissions via subcutaneous inoculation (Stamp et al. 1959; Kratzel et al. 2007), conjunctival exposure (Haralambiev et al. 1973), skin scarification (Taylor et al. 1996) and blood transfusions (Houston et al. 2008). Some scrapie infections were consequences of iatrogenic transmissions due to contaminated vaccines (Gordon 1946; Caramelli et al. 2001).

In most flocks, only a single case of atypical scrapie is found. The transmission mode of atypical scrapie under natural conditions is not understood at all and it is even questioned whether this disease is contagious under all circumstances. The intracerebral route of infection has been clearly established in both rodent and sheep models (Le Dur et al. 2005; Simmons et al. 2007, 2010). Under experimental conditions, an oral challenge of newborn lambs within 24-h post-partum was successful in AHQ homozygous sheep (Simmons et al. 2011). Epidemiological data obtained by active surveillance programs indicate that the capacity of atypical scrapie to transmit disease within the herd under field conditions is quite low and most probably non-existent (Fediaevsky et al. 2009, 2010). However, also cohort cases of atypical scrapie are reported in flocks and also coinfections with classical scrapie in some herds (Konold et al. 2007a, b; Onnasch et al. 2004; Orge et al. 2010). Taken together, these data support the theory of a spontaneous origin of the disease, which might be associated with a very low or absent natural transmissibility (Benestad et al. 2003; Moum et al. 2005; Hopp et al. 2006; Green et al. 2007). Retrospective studies indicate that large flock sizes (>1,000 sheep), overaverage animal exchanges within flocks and vitamin and mineral feed supplements may be risk factors for atypical scrapie (Hopp et al. 2006; Green et al. 2007).

### ***2.5.3 Incubation Period***

The incubation time of scrapie depends on the infection route and the animal's age at infection, its genotype, the involved agent strain and the infectious dose. There is a negative relationship between the genotype-encoded susceptibility in sheep and

the incubation period of the disease, i.e., sheep with VRQ homozygosity have shortest incubation periods (Detwiler and Baylis 2003; Ersdal et al. 2005; Ryder et al. 2004). Iatrogenic infections lead to slightly shorter incubation periods (Caramelli et al. 2001).

In classical scrapie, sheep come down with clinical disease usually between 2 and 5 years of age (average age 3.5 years). Although both sexes appear to be equally affected, disease manifestations in rams occur often at slightly younger age (Parry 1983; Wineland et al. 1998; Lühken et al. 2007; McIntyre et al. 2008). However, also shorter and longer incubation periods ranging between 1 and up to 11 years are reported (Parry 1983). Scrapie-diseased animals younger than 18 months are fairly rare (Dickinson and Stamp 1969). However, it is usually not possible to tell the time of infection in older scrapie-diseased sheep (Detwiler and Baylis 2003).

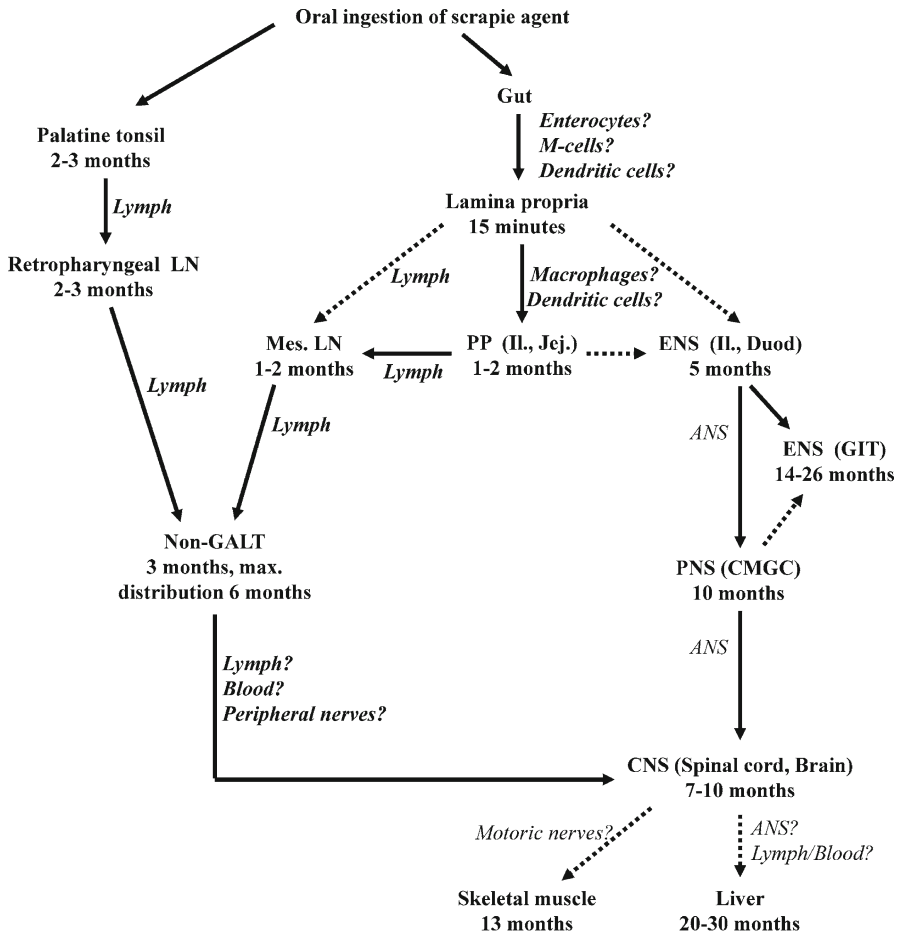
The frequency of atypical scrapie cases increases with the age of the animals. Atypical cases are on average 6.5 years old (Benestad et al. 2008). In a German (Lühken et al. 2007) and in a larger pan-European (20 countries) study, almost 60% or 70% of the atypical scrapie cases were 5 years or older, respectively (Fediaevsky et al. 2008).

As for sheep, the incubation period of goats is influenced by the genotype (Goldmann 2008). Data concerning the age distribution of TSE-infected goats are rare but indicate similarities to the distribution in sheep scrapie. Most of the goats affected by classical scrapie are between 2 and 5 years old; however, cases up to 10 years of age were also reported (Brotherston et al. 1968; Hourrigan et al. 1969; Harcourt and Anderson 1974; Wood et al. 1992b; Capucchio et al. 1998; Konold et al. 2007b; Papisavva-Stylianou et al. 2010; Fast and Groschup unpublished results). Atypical scrapie in goats was described in eight animals with an average age of 6.3 years (Colussi et al. 2008) and in two animals with 10, respectively, 12 years of age (Nentwig et al. 2007).

#### ***2.5.4 Pathogenesis and Tissue Distribution of PrP<sup>Sc</sup> and/or Infectivity***

The pathogenesis of TSEs is discussed separately in Chap. 4. Nevertheless, the most important facts are summarised here and in Fig. 2.1.

After oral uptake, it still remains enigma how the infectious agent overcomes the mucosal barrier of the gut (for detailed review see Mabbott and MacPherson 2006). First results indicate that the genotype does not affect this process (Jeffrey et al. 2006). M cells within the follicle-associated epithelium of the gut and specialised for the transport of macromolecules could be a possible route (Heppner et al. 2001). A transport across the villous enterocytes (Jeffrey et al. 2006; Akesson et al. 2011) and a direct uptake by processes of dendritic cells extending into the gut lumen (Rescigno et al. 2001) are another option. After crossing, the mucosal barrier PrP<sup>Sc</sup> was found within 15 min after inoculation in the lacteals of the villi (Jeffrey et al. 2006; Akesson et al. 2011). A first accumulation of PrP<sup>Sc</sup> was seen in the



**Fig. 2.1** Schematic illustration of the most common theories concerning the pathogenesis of scrapie (modified from van Keulen et al. 2002 and Sisó et al. 2010). The time periods stated are from different studies showing PrP<sup>Sc</sup> accumulation by immunohistochemistry (Andreoletti et al. 2000, 2002, 2004; van Keulen et al. 2000, 2002; Jeffrey et al. 2006; Everest et al. 2011), which mostly rely on VRQ/VRQ sheep. ARQ sheep and experimentally infected goats revealed (as far as known) similar distribution but delayed dynamics. In ARR sheep PrP<sup>Sc</sup> is mainly confined to the CNS. *Dotted arrows* indicate possible but not yet clarified routes of dissemination. *LN* lymphonodus; *GALT* gastrointestinal associated lymphoid tissue; *GIT* gastrointestinal tract; *PP* Peyer’s patches; *ENS* enteric nervous system; *ANS* autonomous nervous system; *PNS* peripheral nervous system; *CMGC* celiac and mesenteric ganglion complex; *IL* Ileum; *Duod* Duodenum

gut-associated lymphoid tissues (GALT) of the tonsil and Peyer’s patches in the intestines in lambs as early as 21-day post-partum (Andreoletti et al. 2000, 2002; van Keulen et al. 2002). Experimental infections indicate a rapid transport of inoculum into the GALT and corresponding lymph nodes, but a replication and accumulation of de novo PrP<sup>Sc</sup> was not seen before 1-month post-infection (Jeffrey et al. 2006).

As shown in naturally infected lambs, the accumulation of PrP<sup>Sc</sup> is restricted to the GALT and mesenteric lymph nodes for the first 2 months of age (Andreoletti et al. 2000, 2002; van Keulen et al. 2002). Subsequently in lambs older than 2 months, a spread to all lymph nodes of the lymphoreticular system (LRS) takes place and the amount of PrP<sup>Sc</sup> in the LRS increases with age up to a plateau level around 6 months (Andreoletti et al. 2000). At this time, after one-third of the incubation period, infectivity is found first time in blood with increasing tendency until to the clinical stage (Houston et al. 2008). The enteric nervous system (ENS) of the duodenum and the ileum are the first parts of the peripheral nervous system, which become affected after 5 months (van Keulen et al. 2000). The exact route of infection is not understood completely yet, especially whether a prion replication in the GALT is necessary for further neuroinvasion. For example, sheep of the VRQ/ARR genotype have no or only low amounts of PrP<sup>Sc</sup> in the lymphoid tissues but develop scrapie albeit only after longer incubation periods (Bossers et al. 1996; van Keulen et al. 1996). A direct infection via subepithelial nerve endings or an indirect infection via infected Peyer's patches and submucosal plexus of the ENS are conceivable (Jeffrey et al. 2006; van Keulen et al. 2008). With progression of the disease starting at 14 months, PrP<sup>Sc</sup> spreads within the ENS in all directions and other parts of the small intestine and at later stages (21–26 months) even the oesophagus, forestomach, large intestine and rectum become involved (van Keulen et al. 2000). Along parasympathetic and/or sympathetic nerve fibres, prions ascend after 10 months via the celiac and mesenteric ganglion complex to the spinal cord and/or brainstem (van Keulen et al. 2000). From these sites in the CNS a further ascending and descending spread of PrP<sup>Sc</sup> takes place (van Keulen et al. 2008).

Between 7 and 10 months of age, PrP<sup>Sc</sup> can be demonstrated first time in the brainstem and spinal cord of young VRQ sheep (Andreoletti et al. 2000; Jeffrey et al. 2001; van Keulen et al. 2002). At 13 months of age, PrP<sup>Sc</sup> is eventually identified in skeletal muscle (Andreoletti et al. 2004) and after 20–30 months in the liver of naturally infected sheep (Everest et al. 2011).

Most of the aforementioned data was obtained for VRQ/VRQ animals, which are considered to be most susceptible and having a comparatively fast dissemination dynamic. Only limited data are available for sheep of other genotypes (Jeffrey et al. 2001; Lacroux et al. 2008). However, these data indicate that the topology and timing of the PrP<sup>Sc</sup> dissemination in ARQ/ARQ and ARQ/VRQ sheep are quite similar, apart from a slightly delayed dynamic in ARQ carriers (EFSA 2010). There are only a few reports on classical scrapie in heterozygous ARR sheep, perhaps due the lower susceptibility of animals carrying this genotype. In such cases, PrP<sup>Sc</sup> is mainly confined to the CNS (van Keulen et al. 1996).

The dissemination dynamics of classical scrapie in goats is well documented but relies mostly on experimentally challenged wild-type goats. The spread of PrP<sup>Sc</sup> during the prion ascension seems to be quite similar to classical scrapie in sheep (EFSA 2009; González et al. 2009, 2010a, b). However, a French study shows that the time course may be prolonged as compared to scrapie in sheep. In goat kids infected around birth, PrP<sup>Sc</sup> was detectable in the GALT not before 4 months of age, peripheral lymphoid tissues turned PrP<sup>Sc</sup> positive after 6 months of age and the CNS

showed the first PrP<sup>Sc</sup> accumulations at 18 months of age. In skeletal muscle, PrP<sup>Sc</sup> was not detected before 21 months of age (EFSA 2010).

However, it should be noted that there is a high diversity of classical scrapie strains in sheep and goats. Their interaction with the particular host genotypes may result in different dissemination dynamics. Therefore, the tissue distribution described above cannot be considered as definitive (EFSA 2010). For example, several ARQ/VRQ and ARQ/ARQ sheep and some goats affected with classical scrapie were reported without any detectable PrP<sup>Sc</sup> in the LRS (Jeffrey et al. 2002; Ligios et al. 2006; Konold et al. 2007a, b; González et al. 2009). Additionally, first results from ongoing experiments of scrapie-infected I142M goats revealed that the dissemination of the TSE agent in peripheral tissues is delayed as compared to wild-type goats (EFSA 2010).

The limited data concerning the tissue distribution of atypical scrapie indicate that detectable amounts of PrP<sup>Sc</sup> seem to be confined to the CNS (Benestad et al. 2003; Simmons et al. 2007; Benestad et al. 2008; Vidal et al. 2008). However, in mouse bioassays, infectivity was shown in the absence of any detectable PrP<sup>Sc</sup> in peripheral tissues including the LRS (Andreoletti et al. 2011, Simmons et al. 2011). Data on the tissue distribution of atypical scrapie in goats are not available to date.

## 2.6 Clinical Signs

Clinical signs are quite variable in different breeds, flocks, regions and countries and are influenced by genotype, agent strain and stage of the disease (for detailed review see Parry 1983; Ulvund 2007, 2008).

The clinical phase mostly progresses slowly over several weeks and months, but acute onsets and durations up to 1 year with intermittent remission of the signs are also seen. Recumbent or sudden deaths of animals were recorded (Parry 1983; Clark et al. 1994; Capucchio et al. 2001; Healy et al. 2003; Humphrey et al. 2004).

Deficits in the disease recognition by shepherds/veterinarians, the subtle onset, the variability of signs as well as the slow clinical progression of the disease are reasons why the disease often remains unidentified. Isolation of animals from the flock is often the first clinical sign. More specific symptoms at the early stage are central nervous system deficits and loss of wool caused by pruritus. Affected animals may appear normal but stimulated by stress (i.e., sudden noise, excessive movement and handling) tremor becomes obvious. At later stages, the animal may even fall down in a convulsive state (Hörnlimann et al. 2007; Ulvund 2007). Clinical signs of scrapie fall into five different categories (Ulvund 2008):

- General signs: Depression, wool loss, regurgitation and cardiac arrhythmia
- Changes in behaviour: Head tremor, altered mental status, nibble response (reflex), teeth grinding, altered head carriage, hyperresponsive, anxious, apprehensive, salivation, aggressiveness and reluctance to be milked
- Changes in sensitivity: Pruritus, “cannibalism”, allotriophagia and biting

- Changes in locomotion: Hind limb ataxia, dysmetria, abnormal posture, hind limb weakness and circling
- Other signs: Weight loss, labial oedema, visual impairment, brief epileptiform attacks and hypogalactia

Not all symptoms are always present, but usually at least more than one is noticeable (Hörnlmann et al. 2007). Moreover, a nervous form may dominate in one flock, while the pruritic form prevails in another (Ulvund 2008). In general, head tremor, nibble response, hyperresponsiveness, salivation, pruritus and weight loss are the most often reported symptoms in different flocks and countries (Healy et al. 2003; Capucchio et al. 2001; Ulvund 2007; Vargas et al. 2005). The final stages are characterised by massive weight losses often despite of unchanged appetite and recumbency due to severe ataxia (Hörnlmann et al. 2007; Ulvund 2007).

Data on clinical signs in goats are rare and most authors refer to scrapie symptoms as described for sheep. Disease durations from 1 up to 3 months are described (Capucchio et al. 1998; Foster et al. 2001; Konold et al. 2007b). The most frequent signs described are weight loss despite of remaining appetite, ataxia and progression to recumbency and pruritus. Behavioural changes include apathy, nervousness or aggressiveness. Less frequently found symptoms are sometimes confined to single animals and include lateralisation of neurological signs such as circling, biting, ptyalism, hyperaesthesia, dribbling/regurgitation, visual impairment, difficulties to milk and tremor (Brotherston et al. 1968; Hourrigan et al. 1969; Harcourt and Anderson 1974; Wood et al. 1992a, b; Capucchio et al. 1998; Foster et al. 2001; Konold et al. 2007a, b).

Only few reports are describing clinical signs of atypical scrapie in sheep and goats. This could be interpreted as if there was a less pronounced clinical phase. However, since normally only singleton animals are affected, they are recognised not quite well by veterinary professionals (Benestad et al. 2008). The overall clinical signs of atypical scrapie are ataxia and weight loss and behavioural changes such as nervousness and anxiety. Circling movements of the sheep may also occur. Tremor was hardly seen and— with exception of two British cases— alopecia due to pruritus is not occurring. Animals die unexpectedly or after a very acute progression phase. One goat was described with blindness, stiff gait and apathy (Benestad et al. 2003; Gavier-Widen et al. 2004; Onnasch et al. 2004; Epstein et al. 2005; Nentwig et al. 2007; Simmons et al. 2007; Dagleish et al. 2008).

None of the clinical signs described above, in combination or alone, are pathognomonic for scrapie. Therefore, the clinical diagnosis must always be confirmed by laboratory investigations (Ulvund 2007, 2008).

## 2.7 Diagnosis of Scrapie

The diagnosis of TSEs is discussed separately in Chap. 13. Nevertheless, the most important facts are summarised here.

The TSE surveillance in small ruminants is based on rapid tests using brainstem material. To diagnose atypical scrapie, it is recommended to include samples of the cerebellum as well (OIE). All samples with a reactive result in one of the rapid tests must be retested in the national reference laboratory using one of the OIE approved confirmatory methods (Matthews et al. 2004). These are histopathology, immunohistochemistry (IHC), electron microscopy and scrapie-associated fibrils (SAF) immunoblot. For practical reasons, mainly the IHC and SAF immunoblot are of relevance today.

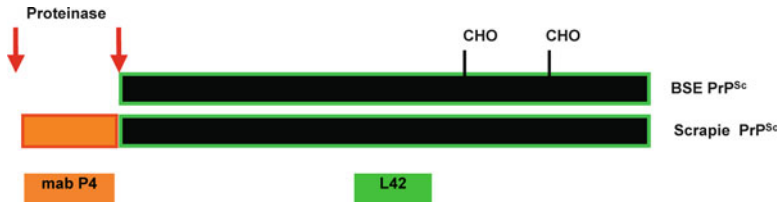
### **2.7.1 Discriminatory Immunoblot**

According to the EU-legislation (January 2005, EC regulation 36/2005), all confirmed TSE cases in small ruminants should be examined by a discriminatory testing to reveal BSE infections in sheep and goats. This includes discriminatory immunoblots (by defined immunoblot protocols) and mouse bioassay (strain typing) of any isolate with a BSE-like immunoblotting pattern (EFSA 2007).

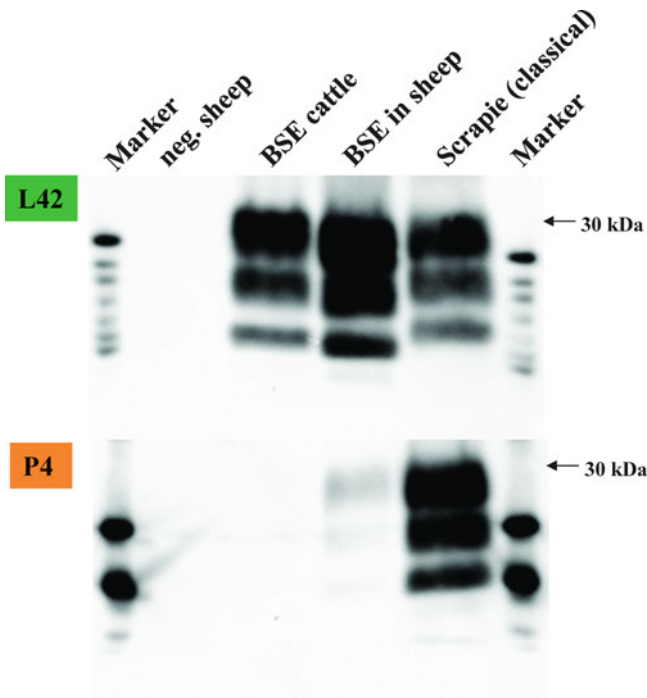
Size differences of proteinase K (PK)-treated, non-glycosylated PrP<sup>Sc</sup> can be shown by high-resolution sodium dodecyl sulphate polyacrylamide-gel electrophoresis (SDS-PAGE) followed by immunoblotting or by using monoclonal antibodies binding to an epitope located on the ragged end of PK-cleaved PrP<sup>Sc</sup>. One of these antibodies is, for example, mab P4, whose epitope WGQGGSH remains detectable after PK digestion of scrapie PrP<sup>Sc</sup>, in contrast to BSE PrP<sup>Sc</sup>, from which this epitope is trimmed off by this enzyme. Antibodies that recognise an epitope in the core region of PrP<sup>Sc</sup>, mab L42 for example, detect scrapie as well as BSE PrP<sup>Sc</sup> after PK digestion, because this treatment has no influence on epitopes of the protein's core region (Figs. 2.2 and 2.3). In the last years, several biochemical strain typing techniques were developed, which utilise these differences in the PK cleavage site of PrP<sup>Sc</sup> (Stack et al. 2002; Lezmi et al. 2004; Nonno et al. 2003; Thuring et al. 2004; Gretzschel et al. 2005). In Germany, the so-called FLI test is applied (Gretzschel et al. 2005), which is a biochemical BSE/scrapie typing strategy that utilises the differences in the glycosylation and PK cleavage site of PK treated and immunoblotted ovine BSE and scrapie PrP<sup>Sc</sup>. Detection antibodies are mabs L42 and P4. According to the FLI test, PrP<sup>Sc</sup> in a sample will be judged BSE-like and subjected to further mouse bioassaying (strain typing), if the sample is conform to the following three biochemical attributes (1) the glycoform ratio for the diglycosylated form is above 50%; (2) the antibody binding ratio P4/L42 has a lower value than 0.4 and (3) the molecular mass is by >0.5 kDa lower than that of the internal scrapie standard.

### **2.7.2 Histopathology**

Gross lesions are not visible and the histomorphological alterations are confined to the central nervous system. The first description of typical scrapie lesions dates back to the nineteenth century (Besnoit and Morel 1898). Scrapie is a neurodegenerative



**Fig. 2.2** Lack of detection of ovine and bovine BSE PrP<sup>Sc</sup> by mab P4. As the PK cleavage sites vary between BSE and scrapie, mab P4 can be used to discriminate between these two TSE types. While BSE-related PrP<sup>Sc</sup> is trimmed approximately to the amino acid 100 and the P4 epitope is therefore destroyed, the trimming of scrapie-related PrP<sup>Sc</sup> stops 10-15 amino acid positions further N terminally. Therefore, the P4 epitope remains intact and the PK-digested PrP<sup>Sc</sup> is easily detected by the antibody

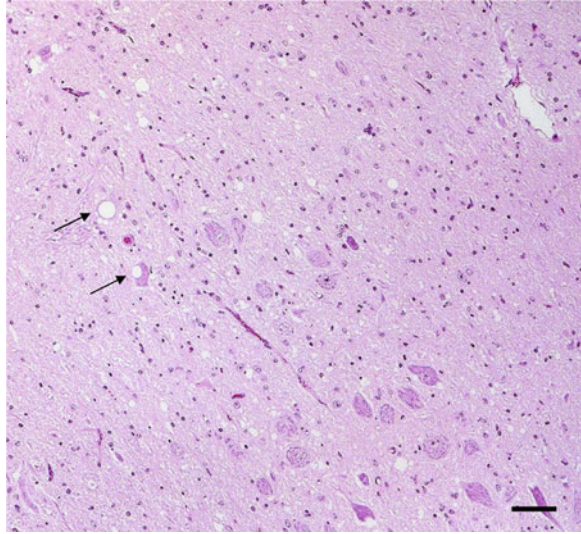


**Fig. 2.3** Comparison of electrophoretic profiles and antibody labelling of PrP<sup>Sc</sup> after proteinase K digestion, PTA precipitation and immunoblotting using mab L42 or mab P4. Both blots are loaded with the same quantities of precipitated PrP<sup>Sc</sup> of each sample

disease with vacuolation of the grey matter as a hallmark, often accompanied by astrocytosis but without signs of inflammation. Neuronal loss is present, but significant cell losses are not evident on routine examination (Jeffrey and Gonzalez 2004; Wells et al. 2007). The development of clinical signs is not necessarily reflected by the severity of the pathology changes (Jeffrey and Gonzalez 2004, 2007).



**Fig. 2.4** Scrapie-infected goat with clear signs of spongiform encephalopathy in the brainstem at the level of the obex (dorsal motor nucleus of the vagus nerve). Vacuolation is detectable in the neuronal perikarya (arrow) and in the neuropil, H&E staining, bar 50  $\mu$ m



Lesions are usually bilaterally symmetrical (Fraser 1993), especially at the brainstem at the level of the obex (Fig. 2.4) and the dorsal motor nucleus of the vagus nerve is the most commonly affected site (Wood et al. 1997). However, a considerable variation in the neuroanatomical distribution of the spongiform lesions is obvious, especially in more rostral areas of the brain. The formation of lesions depends not only on the prion strain but also on the genotype of the host, breed and presumably also other individual factors (Ligios et al. 2002; Begara-McGorum et al. 2002). Additionally, the magnitude of vacuolation is influenced by the age at onset of clinical disease (Ligios et al. 2002).

In classical scrapie vacuolation is detectable in the neuronal perikarya and in the neuropil but can be rare in some naturally occurring and experimental scrapie cases (Zlotnik 1960; Dickinson 1976; Fraser 1976; Chaplin et al. 1998; Begara-McGorum et al. 2002). These membrane-bound vacuoles are found within the neuronal perikarya as single or multiple vacuoles distending the cell body, and/or within processes leading to the typical spongiform appearance in the grey matter neuropil (Jeffrey et al. 1995; Jeffrey and Gonzalez 2004). The proportion of perikaryonal to neuropil vacuolation differs in respect of the disease and agent strain. In murine scrapie models, dendrites are most frequently affected, neuronal perikarya, axons and axon terminals to a lesser extent (for detailed review see Jeffrey et al. 1995). Additional findings might be other signs of neuronal degeneration like chromatolysis, neuronophagia and dark shrunken neurons. Astrocytosis is also an inconsistent finding seen in some scrapie cases (Wood et al. 1997; Jeffrey and Gonzalez 2004; Wells et al. 2007).

In atypical scrapie, the vacuolation is most prominent in the molecular layer of the cerebellar cortex, neocortex hippocampus, basal nuclei and nucleus accumbens. The brainstem is, in contrast to classical scrapie, affected to a much lesser degree

**Table 2.2** Morphological types of PrP<sup>Sc</sup> accumulation (Jeffrey and Gonzalez 2007)

Intracellular	Intraneuronal, Intramicroglial, Intra-astrocytic	
Extracellular/cell membrane associated	Neuropil associated	Linear
		Fine punctuate
		Coarse particulate
		Coalescing
		Perineuronal
	Glial cell associated	Stellate
		Perivacuolar
		Subpial
		Subependymal
		Perivascular
Ependymal cell associated	Supraependymal	
Endothelial cell associated	Vascular plaques	

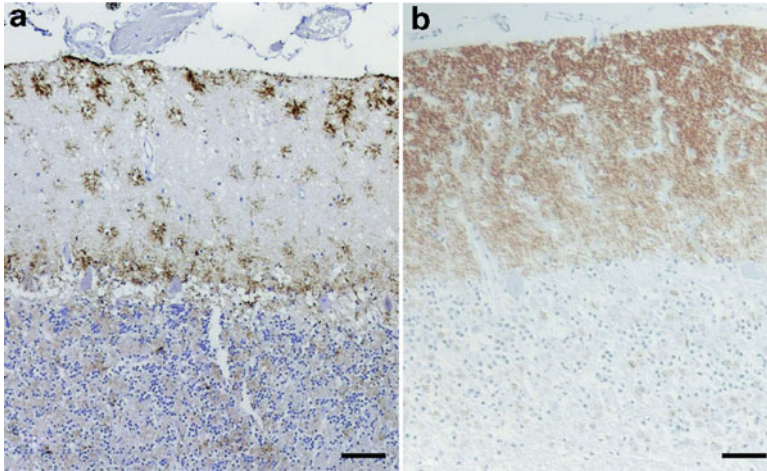
and no lesions are observed at the level of the obex (Benestad et al. 2003; Moore et al. 2008). Intraneuronal vacuolation is not (Moore et al. 2008) or only infrequently seen (Benestad et al. 2003).

### 2.7.3 Immunohistochemistry

The second hallmark of TSEs is the accumulation of PrP<sup>Sc</sup> in the brain, which precedes morphological alterations (DeArmond 1993; Jeffrey et al. 2000). Previous studies (van Keulen et al. 2000) demonstrated that the brainstem at the level of the obex, in particular the dorsal motor nucleus of the vagus nerve, is the first area in the CNS to become affected in advance of any morphological alterations. With progression of the disease, the PrP<sup>Sc</sup> accumulation becomes more widespread and spreads in ascending and descending directions to finally involve at clinical endpoint the entire neuraxis.

It is possible to differentiate several morphological types of PrP<sup>Sc</sup> accumulation (Table 2.2). These PrP<sup>Sc</sup> profiles provide strain and source-specific information on the cell types, which sustain the infection (cellular tropism) and the cellular processing of PrP<sup>Sc</sup>. Not all these types and patterns are found in all scrapie cases. Furthermore, in immunohistochemistry (IHC), a differentiation between some sheep and caprine TSEs (including ovine/caprine BSE) is possible by using the immunoreactivity of antibodies recognising different epitopes of PrP<sup>Sc</sup> (epitope mapping). This method relies on the different protease cleavage sites for PrP<sup>Sc</sup> in different cell types (the same principle as shown in Fig. 2.2). Both approaches may allow the definition of an immunohistochemical phenotype and the subsequent identification of the host and agent strain (for detailed review see Jeffrey and Gonzalez 2007).

Atypical scrapie cases are characterised by a distinctly different PrP<sup>Sc</sup> distribution pattern as compared to classical scrapie. The brainstem at the level of the obex is only inconstantly involved. In contrast to classical scrapie, a PrP<sup>Sc</sup> accumulation at



**Fig. 2.5** Distinct differences in the PrP<sup>Sc</sup> distribution pattern in the cerebellar cortices of sheep infected with (a) classical scrapie and (b) atypical scrapie, immunohistochemistry mab 6C2, bar 50  $\mu$ m

the DMNV was never seen (Nentwig et al. 2007; Benestad et al. 2008). PrP<sup>Sc</sup> accumulations found at the obex are mainly confined to the spinal tract nucleus of the trigeminal nerve with primary involvement of the white matter, formatio reticularis, ventrolateral solitary tract and ambiguous nucleus (for detailed review see Benestad et al. 2008). The most pronounced immunostaining is usually detectable in the cerebellar (Fig. 2.5) and cerebral cortices (Benestad et al. 2008) as well as in the substantia nigra, thalamus and basal nuclei (Moore et al. 2008). However, cases without any cerebellar accumulation were also described (Nentwig et al. 2007). PrP<sup>Sc</sup> accumulations are generally mild to moderate and only few morphological types (including fine granular, aggregates, plaque-like, linear and perineuronal) can be seen. An intraneuronal deposition staining has never been reported (Benestad et al. 2008; Moore et al. 2008).

## 2.8 Scrapie Agent Strains

The first reports on the existence of different scrapie strains date back to the 1960s (Fraser and Dickinson 1968, 1973) and more than 20 experimental TSE strains of scrapie were found to date (Bruce 2003). TSE strains are distinguished by their incubation period in a panel of inbred mouse lines, their vacuolation pattern (so-called “lesion-profile”) and the PrP<sup>Sc</sup> deposition pattern (Bruce and Fraser 1993; Bruce 2003) in the brains of these mice. Although this method allows the discrimination between BSE and scrapie as well as the differentiation between natural and experimental scrapie strains, not all scrapie isolates transmit to wild-type mice.

A panel of transgenic mice overexpressing ovine PrP<sup>C</sup> as well as bank voles have been established as alternative models for strain characterisation. However, it has to be borne in mind that murine scrapie strains may also result from inter-species interaction and do not necessarily reflect the original ovine/caprine strain. Therefore, an improved characterisation of isolates from natural hosts (sheep and goats) was attempted in most recent studies (see Sect. 2.7.1 and 2.7.3). Investigations included the use of biochemical parameters of PrP<sup>Sc</sup>, revealing differences in molecular masses, antibody binding affinities, glycosylation patterns and degree of resistance to trypsin digestion. This way ovine/caprine BSE, CH1641, CH1641-like strains/isolates and atypical scrapie isolates could be distinguished. Additionally, differences seen in some classical scrapie isolates were indicative for the existence of more scrapie strains in sheep and goats (Stack et al. 2002; Buschmann et al. 2004, 2006; Gretzschel et al. 2005, 2006; Baron and Biacabe 2007; Benestad et al. 2008; Fragkiadaki et al. 2011; Fast and Groschup unpublished results).

Vacuolation profiles in the brain of the natural hosts, as revealed by histopathology, showed a high individual variability and can therefore not be used as strain typing method (Ligios et al. 2002; Begara-McGorum et al. 2002; Gonzalez et al. 2010a, b). The differences seen by IHC PrP<sup>Sc</sup> deposition pattern allow the discrimination clearly between an infection with classical and atypical scrapie, CH1641 and ovine/caprine BSE. Moreover, the variations of the PrP<sup>Sc</sup> depositions in the brain may reflect the strain diversity and perhaps even allow a discrimination of classical scrapie strain types (Jeffrey and Gonzalez 2007). Five different IHC phenotypes were found in a recent study on wild-type sheep from different flocks throughout Europe irrespective of the genotype and geographical origin (Gonzalez et al. 2010b). However, neither the influence of other genotypes nor further factors which might influence the IHC phenotype are completely understood to date.

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